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Vacuum-enhanced stripping allows the extractive acidogenic fermentation at thermophilic conditions

Cristian Torri, Federica Zimbardi, Andrea Facchin*

Università di Bologna, Dipartimento di Chimica "Giacomo Ciamician", Laboratori "R. Sartori", Via Sant'Alberto 163, 48123 Ravenna, Italy. **cristian.torri@unibo.it*

Keywords: Volatile Fatty Acids, Butyric Acid, Extraction, Carboxylates, Calcium Butyrate, Vacuum.

Abstract

Acetic (A), propionic (P), isobutyric (IB), butyric (B), isovaleric (IV), and valeric (V) acids, known as Volatile Fatty Acids (VFAs), can be selectively produced by microbial mixed culture (MMC) through fermentation of waste materials with methane-arrested processes. Extracting VFAs during acidogenesis can overcome product inhibition while obtaining high-purity VFAs. Being volatile and acidic, VFAs can be recovered by stripping from the fermentation broth and contacting the stripping flow with an alkaline trap (e.g. $CaCO_3$). The stripping performance depends on concentration, pH, actual Henry solubility (H_s) of VFAs and stripping kinetics. Experimental results showed that, within actual conditions of acetogenesis, H_s is lower than expected, namely 1047±314, 529±93, 179±16, 127±10 and 154±20 (M/atm) respectively for A, P, IB, B, IV, and V. Low absolute pressure (0.15 bar) enhances the stripping rate at 50-55°C. With 30 $g_{VFA}\ L^{-1}$ at pH≈pK_a, vacuum enhanced stripping allows the extraction of 2.5 $gCOD_{VFA}\ L^{-1}\ h^{-1}$ from a 10 g/L solution of A, P, and B, with an extraction rate for B>P>A. An extractive fermentation process was finally tested by using food waste analogue (i.e. chickpea flour and vegetable oil) and a newly developed laboratory-scale

device that combines a standard anaerobic digester with a vacuum stripping mechanism; maintaining the pH at 5.2, a 74% yield ($gCOD_{VFA}$/gCOD) was achieved within 15 days, demonstrating the efficacy of the stripping method for the extraction of VFAs from fermentation systems.

1. Introduction

Volatile fatty acids (VFAs) are renewable building blocks that promise a new type of multiproduct biorefinery based on anaerobic digestion (AD) technology [1,2]. VFAs are used in the food, feed, and chemical industries in massive amounts (i.e. Mton/y), with economic values ranging from 0.6 €/kg (acetic acid) to 2.5 €/kg (propionic acid) [1]. Beyond the actual uses focused on fossil-based acetic acid, biobased carboxylic acids can be used as renewable intermediates for the synthesis of drop-in chemicals and fuels, oleochemicals**,** next-generation bioplastics like polyhydroxyalkanoates (PHA), single cell proteins and artificial meat [1,2]. Acidogenic fermentation yields six main VFAs with 2-5 carbon atoms: acetic (A), propionic (P), isobutyric (IB), butyric (B), isovaleric (IV) and valeric acids (V) [3]. The conversion of biodegradable organic matter into VFAs can be made with microbial mixed culture (MMC) through the inhibition of methane production in the AD, exploiting the fact that VFAs are the most stable fermentation products of the AD [4]. Methanogenesis, which is performed exclusively by *Archaea*, can be avoided using specific methanogen inhibitors or thermal treatments, which impact the methanogen more adversely, possessing different phospholipid membranes and a different enzyme set than bacteria.[5] Also, some selective production of VFA can rely just on the microbial ecology evolution that occurs with short residence time, as the faster-growing acidogenic bacteria are more favoured than slower-growing methanogenic *Archaea* [4,6]. Considering the Chemical Oxygen Demand (COD) balance rules [7], it is possible to obtain 1 g of COD as VFAs starting from 1gCOD of biodegradable organic matter (experimentally measured as Biological Oxygen Demand, BOD). This means a maximum yield equal to 0.93, 0.66, 0.55, and 0.49 g/gCOD for A, P, B, and V, respectively. Food waste and other fermentable materials are rich in biodegradable organic matter (>100 gCOD/L). They can be converted into VFAs with

44 higher volumetric productivity (VP) [[8] than that of AD [8], namely 4-40 gCOD L^{-1} d^{-1} at mesophilic
45 conditions [[9,10]. Once VFAs start to accumulate in the broth, the acidification slows down due to the
46 product inhibition exerted by VFAs and is enhanced by pH drop (i.e. IC_{90} (B)= 35 g L^{-1} at pH 5.8 [11]). This
47 phenomenon limits the actual VP of acidogenic fermentation and the maximum VFA concentration to
48 values below 5 gCOD L^{-1} d^{-1} [8] 40 g L^{-1}, respectively. Such a low maximum VFA concentration limits the
49 yields obtainable from concentrated feedstocks (>40 gBOD L^{-1}) and hampers the obtainment of a clean
50 VFAs stream from diluted feedstocks (<40 gBOD L^{-1}) [12]. This is one of the reasons why using renewable
51 VFAs as chemicals is still not viable or economical [[13,14].
52 Slip-stream approach or extractive fermentation (EF) can be a straightforward way to improve yields and
53 productivity of organic acids, including VFAs, meanwhile allowing continuous long-term fermentation
54 [15]. Most of the extraction methods (e.g., membrane separations, electrodialysis, solvent extraction, or
55 pertraction) can suit the EF concept [16]. Several authors demonstrated that continuous extraction of
56 VFAs provides higher VP than the end-of-pipe approach, and allows a complete conversion of the
57 substrate [17–22]. Even if the advantages of EF are clear, the application to a real fermentation
58 environment is quite challenging. Noticeably, most proposed methodologies require contact with a
59 complex fermentation broth with high surface materials or mixing it with a solvent. Such operations are
60 quite cumbersome when working with real substrates and slip-stream approaches, where the process
61 must be repeated many times and not just once, like in end-of-pipe processing.
62 The relative volatility of VFAs at the fermentation temperature can be exploited in stripping-based
63 solventless methods (Figure 1).

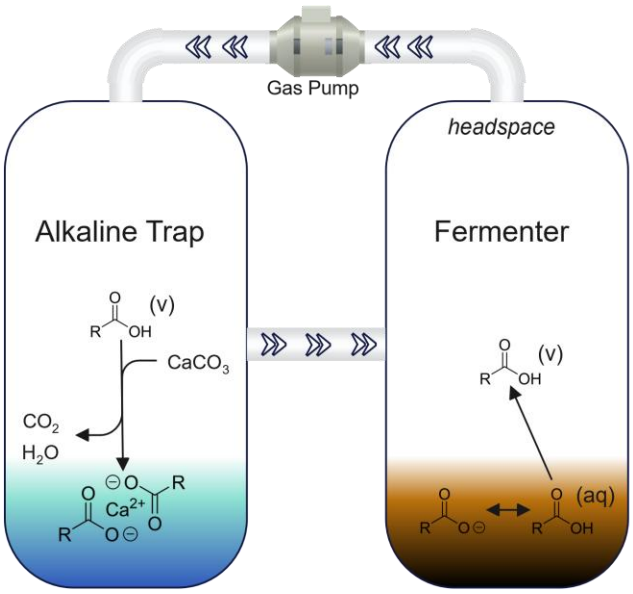


Figure 1: Scheme of VFA recovery through stripping.

Stripping has several analogies with pertraction, which has already been successfully tested for EF [19]. Both approaches can be applied to slightly acidic fermentation broths to obtain clean carboxylate streams, and both require a high mass transfer coefficient (K_La) to achieve acceptable performance. To the best of our knowledge, only Li et *al.* demonstrated this approach for recovering calcium carboxylates from the fermentation of glucose through the circulation and bubbling of biogas between a fermenter and a $CaCO_3$ slurry [23], obtaining carboxylate salts and maintaining broth pH at 5. This approach was solventless, safe, straightforward and reliable, but surprisingly was not furtherly investigated. This results in a significant knowledge gap about some fundamental details of VFA stripping, such as VP and energy consumption.

The VP and stripping energy consumption are functions of the Henry solubility (H_s, M/Atm) and the volumetric mass transfer coefficient (K_La, h^{-1}) within the anaerobic digestion conditions. The Hs of each VFA depend on the temperature and pH, while the extraction kinetics depend on the stripping gas flow and reactor design. Applying absolute pressure close to the vapour pressure enhances the mass transfer, increases the diffusion rate, and decreases the friction during gas displacement.[24–26] Such features suggest that stripping-based EF can improve performance by working at reduced pressure and under

thermophilic temperatures to achieve acceptable Hs. Given this hypothesis, the present work aims to evaluate the actual Hs of VFAs in fermentation systems and then investigate new approaches for reliable vacuum-enhanced EF using MMC fed with waste materials.

2. Materials and Methods

2.1 Analytical methods and H_s determination

VFAs in liquid samples (mock-up broth, trap and EF experiments) were quantified via solvent extraction and GC–MS on 0.1 mL samples as detailed in [27]. H_s values for each VFA were obtained from gas/liquid partition according to the method described by Kim and Kim [27], with some adjustments to mimic the AD environment, namely higher sample temperatures (35, 45, 55, and 65°C) and a higher concentration of VFAs (3.5 g/L). The effect of absolute pressure on Hs was evaluated using an approach similar to vacSPME, as proposed by Psillakis et al. [28]. Headspace analysis was performed using the automatic sampler PAL RSI 85 Series 2 (CTC Analytics AG, Switzerland) equipped with a PAL3-TH-HC2500 syringe for gas with a 2.5 mL volume and a controlled heated PAL3-agitator. The external calibration of VFAs was made with a mixture of pure compounds (>99% from Sigma-Aldrich) by adding 0.5-2 µl of an equivolume mix of 6 VFAs (A,P,IB,B,IV and V) within 20 ml headspace vials and performing headspace analysis at 110°C to vaporise the VFAs entirely. Then, VFAs spiked solutions or suspensions were subjected to headspace analysis at the target temperature. Headspace equilibration was made through 10 minutes of mixing at 250 rpm before headspace sampling. Sampling was performed with a syringe kept at 110°C to prevent condensation on the syringe wall. Before and after each injection, the syringe was purged for 5 seconds with helium to remove any gas collected between analyses. Samples were injected in split mode (5:1 split ratio, injector port temperature set to 200°C) with the following thermal program: 38°C for 8 minutes, then 170°C at 40°C·min^{-1} and 170°C held for 1.7 minutes. The column was a DB-FFAP polar column (Agilent, 30 m length, 0.25 mm i.d., 0.25 µm film thickness). Mass Selective Detector (MSD)

transfer line was kept at 230°C, and the detector operated under electron ionisation at 70 eV with a total ion count of 1 scan s^{-1} in the 5-300 m/Z range. Quantitation of H_2, CO_2 and CH_4 in extractive fermentation was performed with GC-TCD (7820A, Agilent Technologies), as previously described [40]. The total and soluble COD concentration ($gCOD_t\ L^{-1}$, $gCOD_s\ L^{-1}$) and VFA amount (in COD-basis, $gCOD_{VFA}\ L^{-1}$) of fermentation effluents were obtained daily, assuming a COD value for each VFA equal to 1.07, 1.51, 1.88, 1.88, 2.04, and 2.04 $gCOD_{VFA}/g_{VFA}$ for A, P, IB, B, IV, and V, respectively. COD was quantified using a Quick-COD analyser (LAR Process Analyser AG), following the ASTM D6238-98 method based on thermal oxidation at 1200 °C, following sample preparation methods detailed in [29]. H_s (M/atm) was determined by dividing the molar concentration of VFAs in the solution by the partial pressure (in v/v≈atm) obtained from analyte concentration in the headspace through the conversion factor of Sander *et al.* [30]. The temperature dependence of H_s was assumed to follow the van't Hoff equation.

2.2 Kinetics of VFA stripping on model solution

VFA stripping kinetics were evaluated through the experimental systems shown in Figure 2. Two 500 mL gas-tight round-bottom flasks, carefully sealed with glassware silicon and inspected for leaks before each test, were connected through a piping loop equipped with a 12V membrane pump. The flasks were mixed with a magnetic stirrer at 180 rpm and filled with 200 ml of broth (or mock broth composed of 10 g/L of A, P and B with a total VFAs concentration of 30 g/L and resulting pH=2.4) and 80 ml of 10% w/w $CaCO_3$ aqueous slurry, which was used as an alkaline trap. The pumping rate was adjusted by applying a variable voltage through a stabilised benchtop power supply. All the systems (excluding the pump engine) were kept at a set temperature through immersion in the water bath.

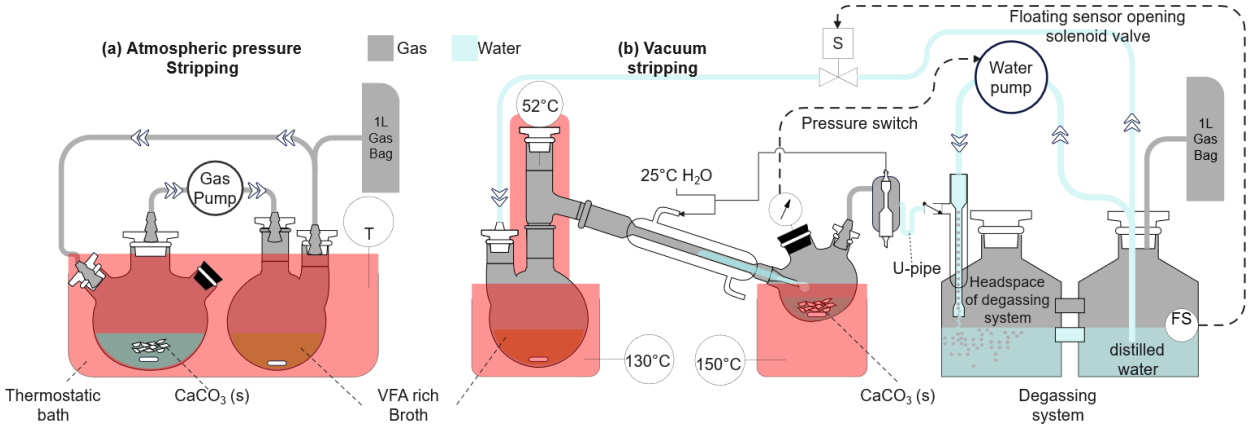


Figure 2: Scheme of apparatus for VFA recovery through stripping at atmospheric (a) and reduced pressure (b).

First attempts to decrease the operating pressure with the same device used for atmospheric stripping (Figure 2, a) led to leaks, a short pump lifespan, and unreliable operation. Most small-scale pumps are not compatible with hot condensing VFA vapours, and the volume of industrial pumps is too large for use in a small reactor (leading to leaks into the pump itself). To circumvent those limitations, a new pump-free system with standard glassware was designed and tested (Figure 2, b). The vacuum-based system was functionally identical to the first, but the low-pressure water vapour recirculation was obtained thermally through a different approach. This fermentation/stripping system consists of a modified distillation apparatus with both evaporation (fermentation broth or simulated broth) and condensation ($CaCO_3$ trap) flasks immersed in a hot silicone oil bath kept at 130°C and 150°C, respectively. Such a configuration heats the liquid inside the flask to the exact boiling temperature (52°C at 0.15 bar). The flow of steam (≈0.5-1 mL min^{-1}) evaporated from the broth (total volume 200 mL) is continuously condensed in the condensation tray and drops onto $CaCO_3$ slurry, which boils as well and, being at almost constant volume, evaporates again the water, delivering it to another downdraft cooler. The latter cooler is connected, with a U-shaped pipe, to the vacuum generation system, named VGS. The VGS operates discontinuously to keep the system at the target absolute pressure, concurrently removing the liquid accumulated in the U-shaped pipe and permanent gas generated by the system.

Condensate accumulates in a U-shaped pipe between each VGS activation, typically occurring every 5-10 min, allowing for the quantitation of the condensate. The rate of condensate accumulation was used to calculate steam flow through gas laws.

The VGS consisted of a venturi ejector fed with 60 L/min water (2-3 bar pressure delivered by a dual diaphragm pump), which was looped into a 5 L degassing system filled with deionised water. According to this structure, the gas produced by the fermentation was collected from a degasser headspace into a laminated gasbag (Supel™-Inert, Supelco). At the same time, the condensate increases the water level in the degassing system. To close the steam stripping loop, the degassing system was equipped with a floating sensor (detecting the level increase), and the bottom of the degassing system was connected to the fermentation broth through a solenoid valve actuated by the floating sensor. Since the volume of the $CaCO_3$ slurry remains almost constant throughout the experiment, this loop maintains the volume of the boiling broth constant (200 mL). In practice, the steam flow is provided by the temperature difference between the device's hot and cold portions. It is important to note that this approach, which proved highly reliable at the lab scale, can be used only to investigate lab-scale extractive fermentation. It is not intended to be a model for a real-scale fermenter.

2.3 Vacuum-enhanced Extractive Fermentation (EF)

After the VFAs collection system was tested with a model solution, it was modified as described in Figure 3 and used for EF. The extraction system used for EF is similar to that of Figure 2 (b), but without the $CaCO_3$ trap, thus delivering the VFA vapours directly to the 5 L degasser liquid. In addition, a thermally insulated 50 cm Vigreoux was placed between the broth and the condensation tray to deal with extensive foaming observed during initial EF. To simulate EF, the 0.2 L extraction system was flanked by a 0.4 L conventional Stirred Tank Reactor (STR), which was used to semi-continuously provide broth for extraction and receive extracted broth from the VFA extraction system. This configuration enabled the simulation of each step of EF and practically delivers an extraction performance identical to

the previous one. Still, as the small-scale and long-term experiment requires, it guarantees a more precise broth volume control. Chickpea flour and sunflower oil were selected based on their elemental composition and solubility properties to resemble food waste while being manageable and consistent. In a typical EF, 0.6 L of suspension was prepared by blending 28 g of chickpea flour and 1 g of sunflower oil into 0.6 L of anaerobic digestate. The latter was obtained from a pilot thermophilic Continuous-flow Stirred-Tank Reactor (CSTR) operated with a 30-day Hydraulic Residence Time (HRT) and a 30-day sludge residence time (SRT) for approximately one year, using an input (prepared with tap water) consisting of 200 g/L Chickpea flowers and 5 g/L sunflower oil. Inoculum was incubated for one week, exhausting residual biogas potential, delivering a slurry with minimal (<1 gCOD/L) soluble organics, 50 gCOD/L of suspended solids and 1.5 g/L of total ammonia nitrogen.

At the beginning of every experiment, the concentration of fermentable COD was equal to 53 gCOD/L, and total soluble alkalinity was 8±0.5 g/L $CaCO_3$ equivalent.

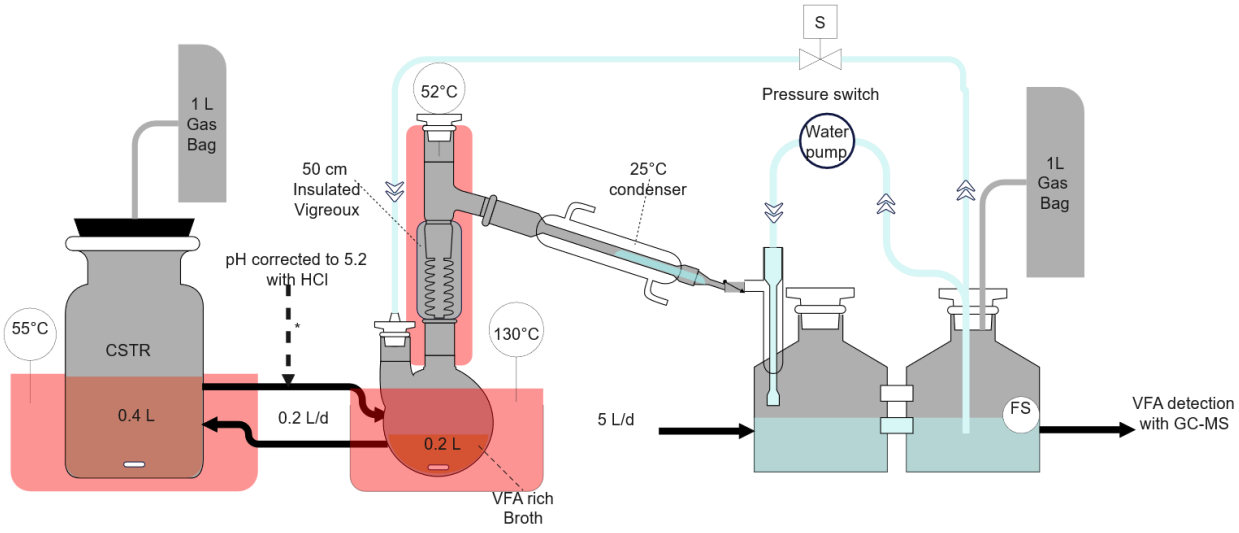


Figure 3: Scheme of the vacuum-enhanced extractive fermentation experiments. *performed only in the third EF experiment. STR: Stirred Tank Reactor.

185 0.4 L and 0.2 L of this suspension were transferred to the STR and extraction system, respectively. In
186 routine daily operations, about 0.2 L of the STR content was removed and transferred to the extraction
187 system, whose content was back-moved into the STR. To assess the extracted VFAs and minimise the
188 VFAs being returned to the broth, the degasser liquid was analysed and entirely replaced every daily
189 operation.

190 Three EFs were performed using slightly different initial conditions or adjusting pH before extraction.
191 The first EF was performed by mixing 28 g of chickpea flour and 1 g of sunflower oil into the inoculum as
192 received. The second EF was performed by mixing 28 g of chickpea flour and 1 g of sunflower oil into 0.3
193 L of inoculum and 0.3 L of distilled water. The third EF was performed as the first EF, but the pH of the
194 broth was routinely corrected to 5.2 (with HCl addition) before being transferred into the extraction
195 system.
196
197 *2.4 Analysis of microbial community during EF*

198 Samples for the analysis of the microbial population were obtained by centrifuging an aliquot of the
199 fermentation broth at 4000 × g. The wet solids obtained were freeze-dried at -65 °C and 1.0 mbar
200 vacuum conditions and stored at -4°C before the nucleic acid extraction and amplification. Nucleic acids
201 were extracted through a Qiagen protocol and Quacube HT instrument 3. A first amplification step of
202 the targets was done with universal primers Takahashi 2014 in 25 cycles. After an enzymatic purification,
203 samples were subjected to a second amplification step for the attachment of adapters and index
204 (Nextera XT) for the sequencing and identification, normalisation and multiplexing. Finally, sequencing
205 was performed on the Illumina MiSeq platform with a 2x300 bp format. Data from all samples were
206 subjected to Adapter trimming (Cutadapt) and read denoising (DADA2). Then feature filtering (0.01%)

and singleton discarding were made on data from each sample separately. Taxonomy was assessed using trained sequences (OTUs at 99%) from the Silva database version 138.

3. Results and discussion

3.1 Determination of VFAs gas/liquid partition in digestate

Preliminary evaluation of VFAs stripping within fermentation requires a complete picture of gas/liquid partition coefficients for each VFA under conditions comparable to those of fermentation, varying the temperature and pH. Practical acidogenesis temperature ranges between 35°C and 65°C, the latter considered as the threshold for the most ultra-thermophilic acidogenic microorganisms [31]. pH values can range between 4.5 (the minimum pH for an active fermentation) and 6.5, while more alkaline values do not allow any practical extraction of undissociated VFAs. In addition, MMC fermentation broths include suspended solids and VFAs mixture with an overall concentration above 10 g/L, falling out of the infinite dilution assumption of intrinsic Henry law solubility (H_s). Such peculiar features can influence the headspace content of VFAs, especially those with high K_{ow} (i.e., butyric and valeric acid), and demand for targeted determination of gas/liquid partition and related experimental H_s. Therefore, headspace analysis was performed with solutions of single VFA and VFAs mix, meanwhile varying temperatures, pH, and liquid matrix (digestate and water). Figure 4 plots the Henry solubility (H_s, M/atm) calculated from headspace analysis of two solutions at pH=2.35 prepared with 3.5 g/L of each VFA (as single solutes, Figure 4a) or with a mixture of 21 g/L of the 6 VFAs (3.5 g/L of each VFA within the same solution, Figure 4b).

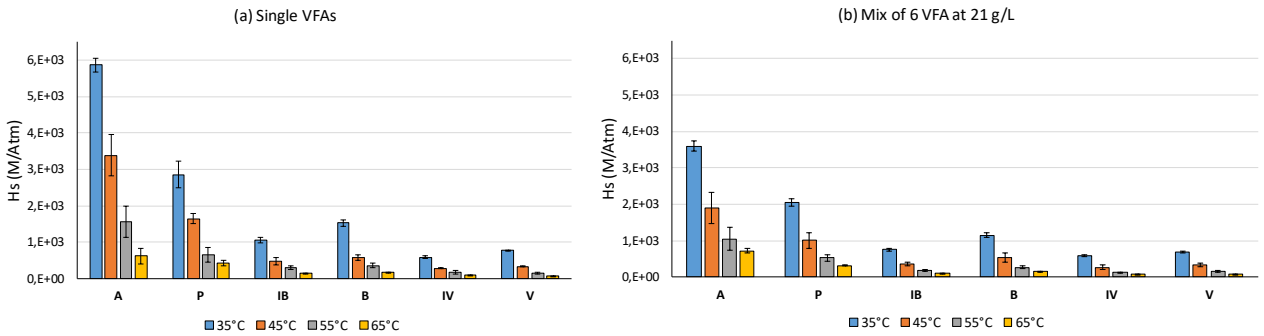


Figure 4: Plot of Henry solubility constants (H_s, M/Atm) obtained from (a) a 3.5 g/L solution of each VFA and (b) 6-VFAs mix (21 g/L total concentration).

For the single VFA solution, the H_s measured at 35°C were 5860±136, 2851±366, 1058±76, 1532±89, 588±31, and 773±6 M/atm respectively, for A, P, IB, B, IV, and V, highlighting a decrease for longer and more branched VFAs. As suggested by the Henry solubility definition, H_s is (linearly) proportional to solubility and inversely proportional to vapour pressure [32]. With the tested VFAs, a longer carbon chain results in a decrease in solubility that is sharper than the increase in vapour pressure, implying lower H_s for larger VFA [32] . With the same number of carbons, the branching of the chain also decreases H_s, suggesting that branching increases VFAs volatility more than boosting solubility. Increasing the temperature drastically decreases the H_s values, with a three-fold decrease for acetic acid and a five-fold decrease for valeric acid at 65°C compared to the H_s values obtained at 45°C. The absolute values and the observed temperature trend of H_s align with the literature [30,33]. H_s values obtained at 35°C with the 21 g/L mixed VFAs solution are lower than those obtained for the diluted solution of isolated compounds. This “mixture effect” is marked with shorter/water-soluble VFAs (e.g. A shows a 40% decrease of H_s in the mixture) but is less relevant with longer/hydrophobic VFAs, with almost no mixture effect for IV and V. Such an effect reflects non-ideal conditions due to the co-presence of different VFAs at significant concentrations. This effect was previously observed by Khiter and co-workers [34] in the context of membrane separation of VFAs. Looking at the definition of Henry's law, the H_s values of short VFAs are more influenced by water solubility than by boiling point. The

formation of carboxylic acid cyclic dimers could decrease the solubility of a VFAs as the total concentration of carboxylic acids increases in the % range [[35]. This phenomenon can be enhanced when hydrophobic VFAs "cap" the polar portion of shorter (more hydrophilic) VFAs, preventing the interaction with water and thus decreasing the affinity with the liquid. According to the results presented here, these phenomena can increase the volatilisation of VFAs within acidogenesis conditions and should be considered for stripping processes.

The H_s data obtained for B and V in the 6 VFAs mixture at 65°C approach the values that characterise substrates which could be recovered by stripping (< 100 M/atm), suggesting that stripping can be exploited for VFAs recovery from fermentation broth under thermophilic or hyperthermophilic conditions; the Henry constants at 55°C are 1.210±382, 570±95, 188±30, 286±53, 127±20, and 166±31 M/Atm, respectively for A, P, IB, B, IV, and V. In mass terms, this corresponds to K_{aw} partition coefficient (L_{aq}/m^3_{gas}) equal to 3±1%, 7±1%,20±2%,13±2%,30±3%, and 22±2% for A, P, IB, B, IV, and V.

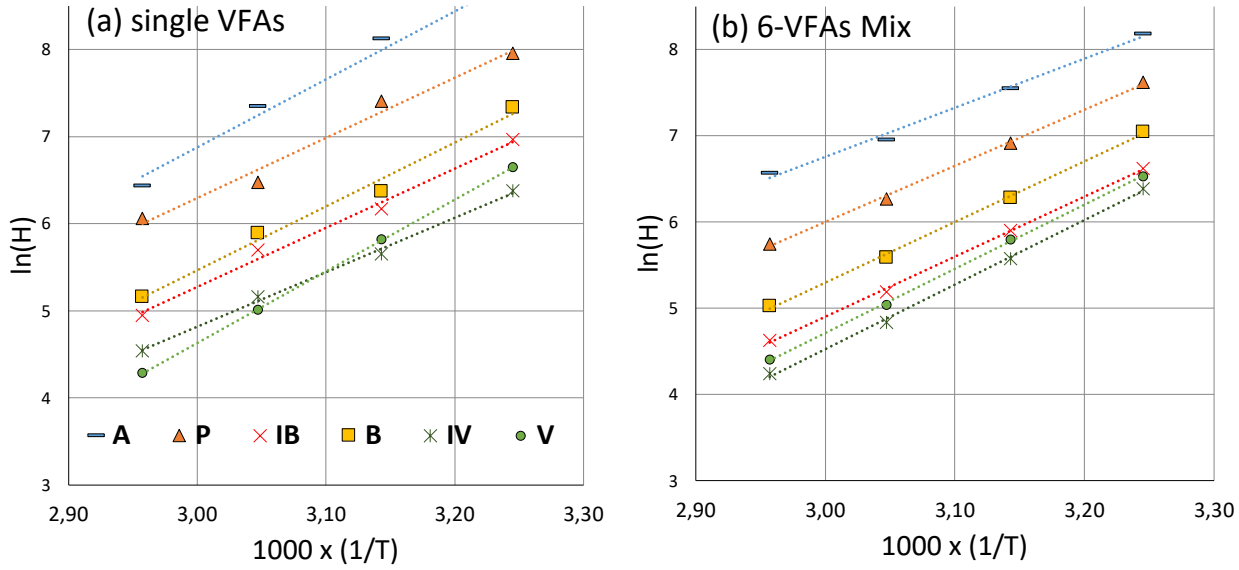


Figure 5: Logarithm vs reciprocal van't Hoff plot describing temperature effect on H_s, as experimentally observed for single VFAs (a) and 6-VFAs mix (b).

H_s at reduced pressure was evaluated at 35°C and 0.15 bar through the procedure reported by [28] applied to mixed VFAs. The value obtained was comparable (110±8%) to that obtained at 1 bar. This agreed with Henry and Raoult laws, which assume no molecular interaction in the gas phase.

Table 1: Van't Hoff plot coefficient for H_s of single VFA and VFAs mixture obtained from Figure 5.

	Single compound			**Mix of 6 VFAs**		
VFA	R^2	$H_{s,25C}$ (M/atm)	$dLnH_s/d(1/T)$	R^2	$H_{s,25C}$ (M/atm)	$dLnH_s/d(1/T)$
A	0,981	13707	7794	0,993	6679	5694
P	0,982	6034	6889	0,999	4144	6526
IB	0,992	2216	6788	0,999	1605	6972
B	0,987	3394	7312	0,998	2463	7028
IV	0,996	1161	6259	0,998	1345	7485
V	0,999	1894	8237	0,999	1545	7437

The effect of the temperature and co-solutes on H_s can be evaluated by plotting the natural logarithm of Hs versus the reciprocal of the temperature in Kelvin (Figures 5(a) and 5(b)). These graphs provide the coefficients of the van't Hoff equation, namely the H_s at 25°C ($H_{s,25}$, M/atm) and the temperature dependency thereof ($DlnH_s/D(1/T)$). A good linear regression was obtained for both single and a mixture of VFAs (Table 1).

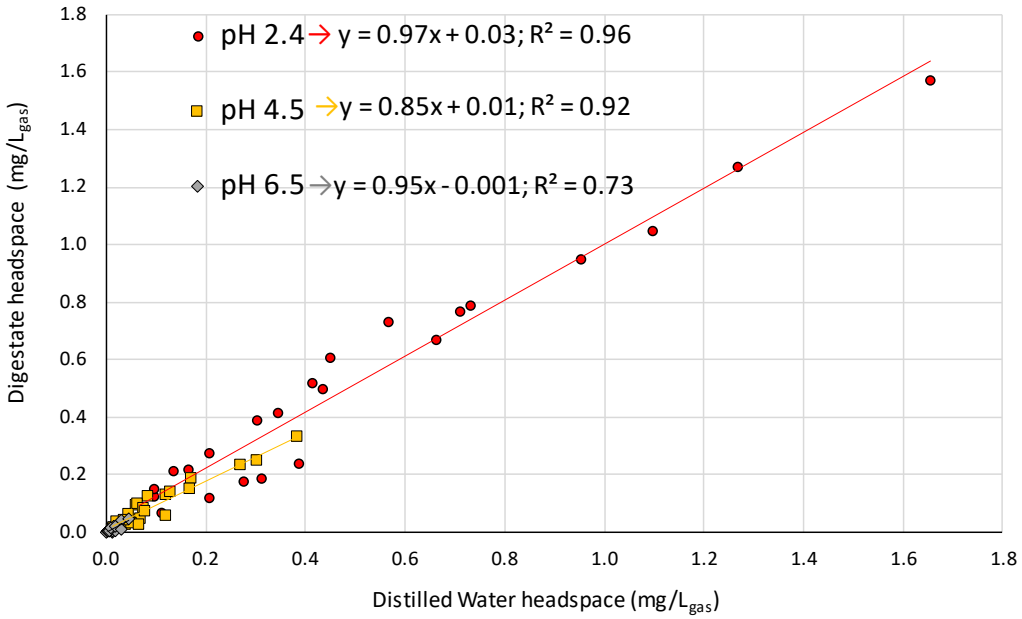


Figure 6: Scattering plot of headspace concentration in water and digestate with the same amount of VFAs and conditions.

To assess the partitioning between the liquid and gas phases at real fermentation conditions, different model solutions and real digestate with less than 0.1 g/L VFAs and containing 5% suspended solids were spiked with 3.5 g/L of each VFA and buffered to pH levels of 4.5, 5.5, and 6.5.

These liquids were brought to equilibrium with the headspace, in which VFAs were then analysed. This directly measured the amount of VFAs in the gas phase under conditions resembling acidogenesis. The effect of the real matrix in the gas/liquid partition of VFAs is detailed in Figure 6, showing the correlation between the headspace content (mg/L$_{gas}$) of all VFAs when in equilibrium with aqueous buffered water and digestate at pH 4.5, 5.5, and 6.5. The VFAs' headspace content of digestate is the same as that observed for aqueous solutions, with differences within the experimental variability. This suggests that the adsorption of less polar VFAs on organic matter is counterbalanced by the salting-out effect and other matrix effects from digestate constituents. Considering a real acidogenic broth, namely 3.5 g/L for each VFA, pH equal to 5.5 and thermophilic conditions, the expected equilibrium concentration of VFAs ranges

between 0.1 g/m^3 for acetic acid and 1 g/m^3 for valeric acid. Assuming a pressure drop equal to 1 kPa (e.g. bubbling the broth for a depth of 10 cm) and efficiency of gas pump equal to 80%, the energy consumption for pumping 1 m^3 is equal to (1 kPa · 1 m^3/0.8) 1.25 kJ/m^3 (0.35 Wh_{el}/m^3). Dividing this value by the concentration of VFA (g/m^3) in the gas, it is possible to determine the energy requirement for gas pumping. According to the equilibrium partitions, energy consumption ranges between 12.4 and 1.2 kJ/g of VFA. The comparison of these values with the energy requirement of other extraction methods [17,18] suggests that stripping is impractical for short VFAs. In contrast, if a concentration comparable to the partition equilibrium can be reached, stripping can be used to extract long VFAs. Actual feasibility depends on how far the stripping gas concentration is from the partition. The subsequent section evaluates the kinetics of the VFAs stripping to evaluate this aspect.

3.2 Kinetics of VFAs stripping with different pressure conditions

To assess the kinetics of VFAs volatilisation and trapping, 2 different experimental setups, detailed in Figure 2, were used. The first atmospheric-pressure system (Figure 2a) consisted of a conventional broth/trap system with gas recirculation and mechanical mixing of the liquid of both broth and alkaline traps. Preliminary tests were performed at mesophilic, thermophilic and hyperthermophilic temperatures. Only the application of very high temperature (70°C, 5 degrees above maximum practical temperature [31]) provided an appreciable extraction rate, Figure 7 (a). Then, to evaluate whether the vacuum offers a significant advantage over a conventional atmospheric system, low absolute pressure was tested under thermophilic conditions (52±3°C) using the system shown in Figure 2b. Figure 7 shows the extraction yield ($gVFA_{trapped}$/$gVFA_{broth,t0}$) obtained with stripping of model solutions (10 g/L of A, P, and B at pH=2.4) performed at 1 bar and the stripping made at 0.15 bar with vacuum-induced boiling.

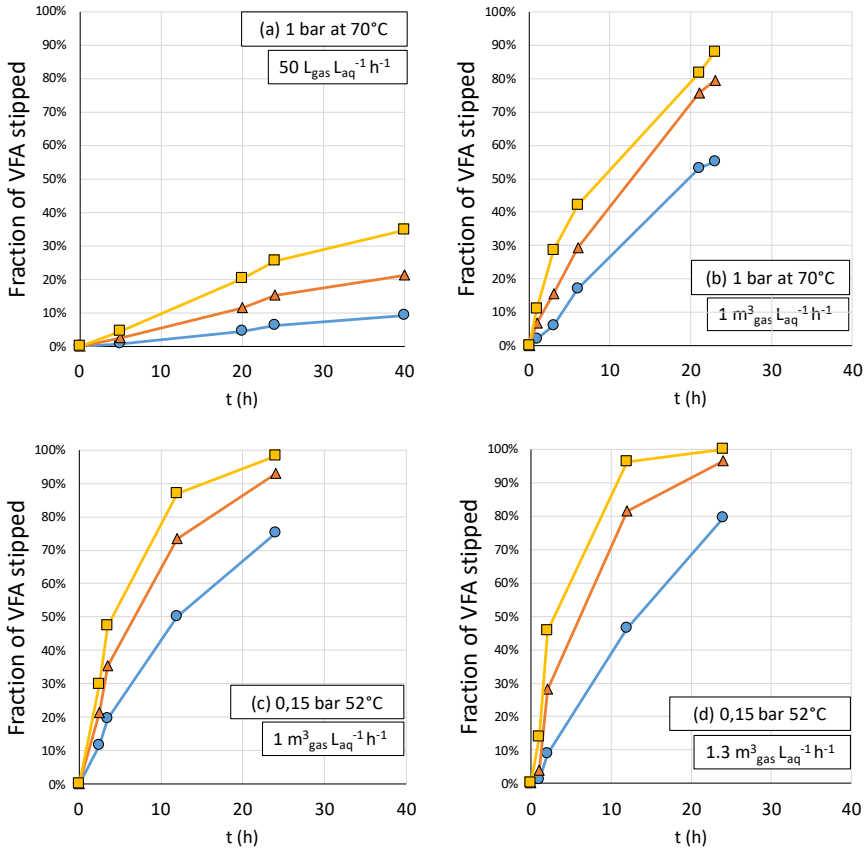


Figure 7. Time trend of extraction of A(blue -), P (red Δ) and B (orange □) from 10 g/L solution of A, P

Table 2. Kinetic parameters obtained for different VFA stripping configurations. T: temperature of the broth (°C); VSGF: volume-specific gas flow, namely gas flow (L h^{-1}) divided by stripping reactor volume (L); P: absolute pressure in bar; k: first-order decay constant (%h^{-1}); g_{COD} m^{-3}: COD concentration within stripping gas; ER: estimated energy requirement for VFA stripping (kJ/g_{COD}).

T	VSGF	P	Med.	pH	k(A)	k(P)	k(B)	g_{COD} m^{-3}
70	50	1.0	H_2O	2.4	0.2±0.01	0.6±0.02	1.1±0.04	6.5
70	1000	1.0	H_2O	2.4	3.5±0.09	6.8±0.1	8.8±0.3	3.0
52	1000	0.15	H_2O	2.4	5.8±0.06	11±0.1	17±0.2	5.3
52	1000	0.15	H_2O	2.4	6.3±0.3	14±0.2	27±0.3	4.3
52	1300	0.15	H_2O*	4.5	0.4±0.2	3.2±0.9	10±2.3	1.9
52	1300	0.15	digestate	4.8	0.4±0.03	1.8±0.3	5.1±1.3	1.0

316

317 and B in distilled water, as $g_{VFA}/g_{VFAbroth}$ obtained with different stripping gas rates and temperatures.
318 The observed trends fit with first-order kinetics with a good mass closure during the experiment,
319 confirming the efficiency of $CaCO_3$ trapping in the low-pressure system. As expected from H_s, the
320 extraction rate was B>P>A, with a higher extraction rate when using a larger flow rate and, noticeably,
321 lower absolute pressure. Table 2 quantitatively shows the kinetic parameters obtained from the Figure 7
322 trends and an analogous test made by spiking the buffered solution and digestate (initial pH equal to 4.5
323 and 4.8, respectively) [17,18]. The baseline extraction rate obtainable at 70°C, 1 bar and moderate gas
324 flow, was 0.001, 0.006, and 0.011 h^{-1}, respectively, for A, P, and B. In these conditions, the overall
325 concentration of VFAs was close to that expected from the equilibrium, suggesting that the limiting step
326 was the gas flow between the VFA-rich source and the $CaCO_3$ trap. Therefore, the volume-specific gas
327 flow (VSGF, namely the gas flow divided by stripped liquid volume) was increased from 50 L_{gas} h^{-1} L^{-1}_{broth}
328 to 1000 L_{gas} h-1 L^{-1}_{broth}. This brought the concentration of VFAs in the gas to about half of the
329 equilibrium, resulting in faster extraction kinetics. With these conditions, the extraction rates were
330 0.035, 0.068, and 0.088 h^{-1}. Considering the tested free VFA concentration of 10 g/L, A, P and B have a
331 VP equal to 8.4, 17, and 2 g L^{-1} d^{-1}. These VP values fall in the range of previously proposed
332 methodologies [15,16,18].

333 However, few environmental organisms can effectively convert complex substrates into VFAs at
334 temperatures higher than 65°C, and hyperthermophilic fermentation is challenging from several points of
335 view [31]. To achieve comparable results at a lower temperature, the same test was made with a low-
336 pressure system, working with a 0.15 bar absolute pressure and a 52 °C boiling system. Noticeably, this
337 configuration allowed a doubled extraction rate and a gas phase concentration very close to the saturation
338 level. Further increase of gas flow rate to 1300 L_{gas} h^{-1} L^{-1}_{broth} allowed an impressive removal rate equal to
339 0.063, 0.14, and 0.27 h^{-1} for A, P and B, with a complete stripping in a few hours. Finally, the same

experimental conditions were tested for extracting VFAs from a model solution buffered at pH 4.5 and VFA-spiked digestate (pH 4.8). Although the decrease of the extraction rate was more marked than that expected from simple pH increase, the resulting VP with these mock solutions were 58 and 30 gCOD L^{-1} d^{-1} for the model solution and VFA-spiked digestate; these values would correspond to a total volumetric productivity (VP) of 58 and 30 gCOD L^{-1} d^{-1} for water solution with pH=4.5 and digestate with pH=4.8.

3.3 Vacuum-Enhanced Stripping Fermentations

To explore the potential of EF using stripping, a real fermentation was made by blending simulated food waste with anaerobic digestate. This mixture was fermented for 28 days at thermophilic temperature (52-58°C) with the 400+200 mL reactor detailed in Figure 3 and Section 2.3.

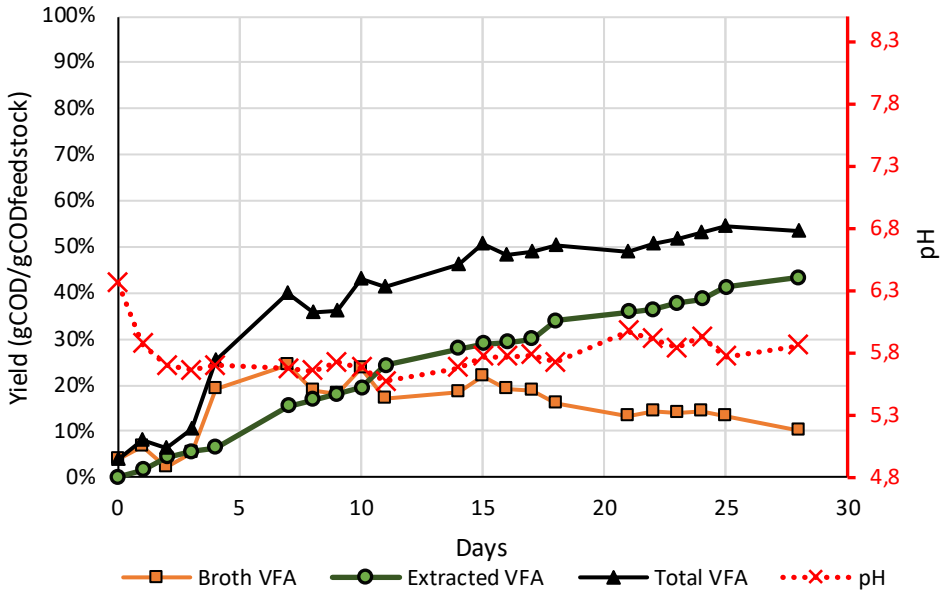
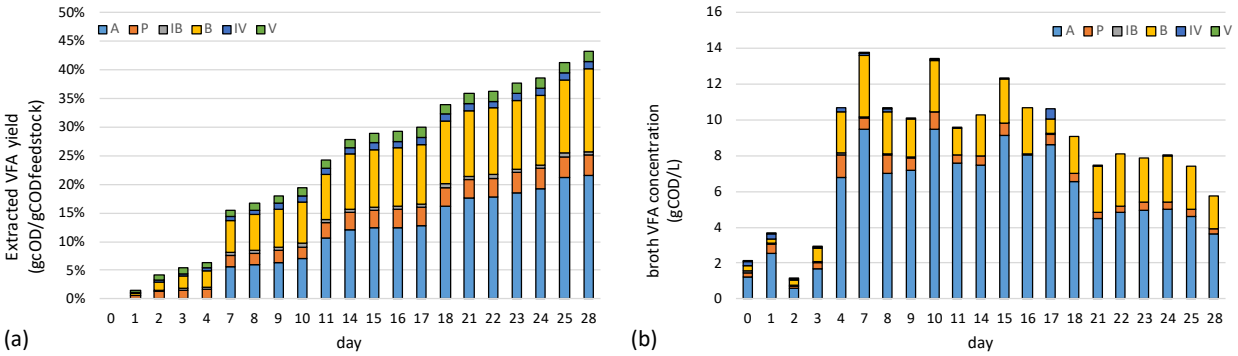


Figure 8. Time trend observed during the first EF experiment, showing Extracted VFAs, concentration of VFAs in the broth and pH of the broth.



353

354 **Figure 9.** Detailed composition of extracted VFAs (a, as gCOD/gCOD fed) and concentration of each VFA

355 in fermentation broth (b, data shown as gCOD/L of each VFA) observed during each day of the first EF.

356 The first test was performed by mixing 600 mL of inoculum, obtained from a thermophilic methanogenic

357 anaerobic digester, with 28 g of chickpea flour and 1 g of sunflower oil (Figure 8).

358 In less than 1 day, the system became acidogenic (pH<5.8) with few gCOD/L of VFAs, without any

359 significant methane production and just minor (few mL/d) H_2 production during the first days. The content

360 of VFAs in the fermentation broth (Figure 9,b) subsequently increased to 10.6 g COD/L in 4 days,

361 corresponding to a 20% conversion, as is typically observed in experiments without extractive VFAs

362 fermentation. At this point, the concentration of free VFAs (especially B) became sufficient, and the

363 stripped VFAs amount became relevant, namely 3% of the feedstock COD per day, corresponding to a VP

364 of approximately 1.6 gCOD L^{-1} d^{-1}. From this point, the VFAs concentration in the broth became stable

365 around 5-10 $gCOD_{VFA}$ L^{-1}, and the reactor delivered a stable amount of VFAs until exhaustion of the

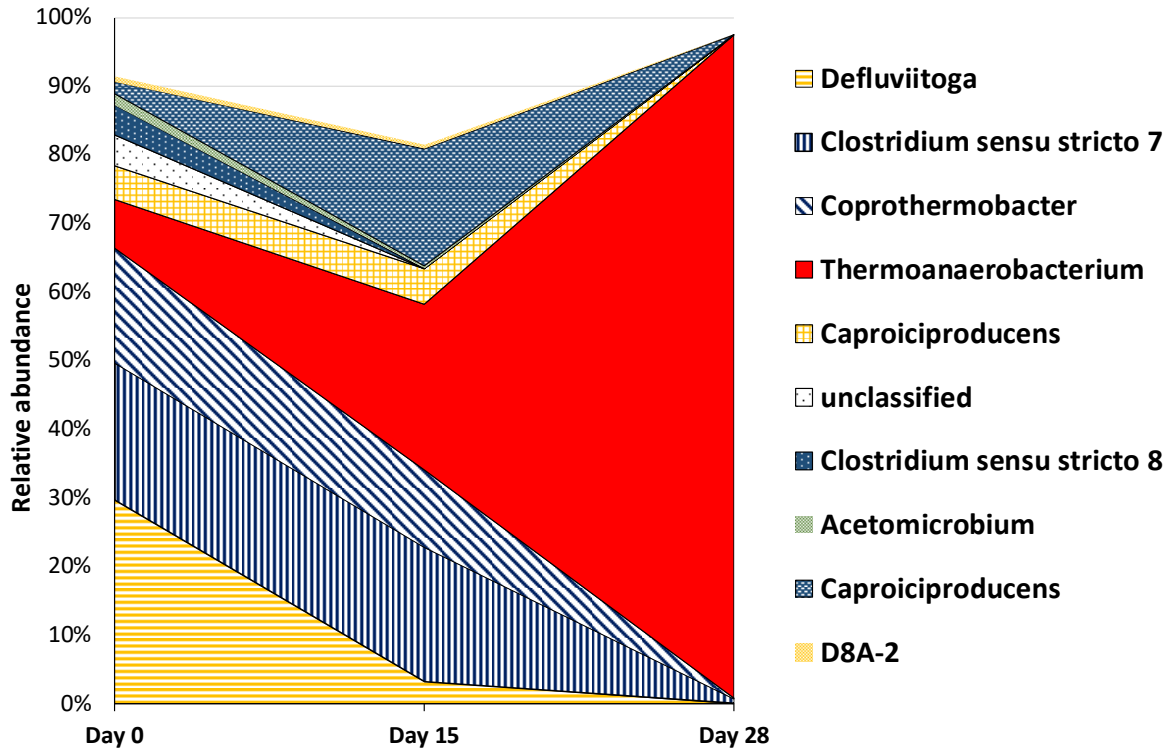
366 fermentation, which was detected as a gradual pH increase to values close to 6. To understand the

367 biological phenomena underlying this peculiar fermentation, the microbial population change while

368 moving to extractive fermentation was monitored through ribosomal RNA sequencing of samples taken

369 at day 0, 15 and 28 (Figure 10).

370



371

372 **Figure 10.** Absolute abundances of the top 10 genera of microbial population observed during the EF

373 experiment.

374 The microbial population at day 0 was characterised by a diversified population dominated by *Defluvitoga*,

375 *Clostridium* and *Coprothermobacter* genera. During the first 15 days of EF, *Thermoanaerobacterium*,

376 *Clostridium sensu stricto 7, Coprothermobacter,* and *Caproiciproducens* became predominant (24, 20, 11,

377 and 5% abundances, respectively), while the abundance of *Defluviitoga* dropped to 3%. On day 28, the

378 entire microbial population was dominated by Thermoanaerobacterium (97% of abundance), which is in

379 line with what was observed by Zhang et al.[36]*Archaea*, mostly *Methanobacterium,* were present in

380 minor amounts at time 0 and completely disappeared before day 15, as suggested by the negligible

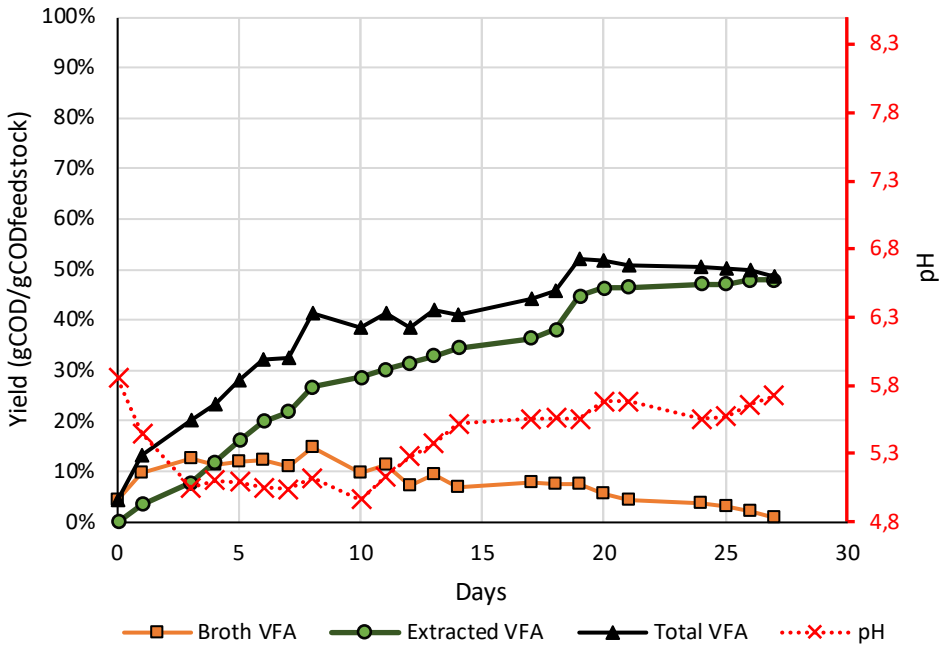
381 methane production during the experiment.

382

An overall yield of VFAs ($gCOD_{VFA}/gCOD_{feedstock}$) equal to 54% was obtained at the end of the fermentation, with 10% of the VFAs still in the broth (5.3 gCOD L^{-1}) and 44% of VFAs recovered as stripped VFAs. The relative composition of stripped VFAs was relatively constant during the study and similar to the average composition of VFAs in the fermentation broth during the experiment, with just a minor enrichment of longer VFAs, namely 0.5, 0.08, 0.01, 0.33, 0.03, and 0.04 $gCOD/gCOD_{extr}$ for A, P, IB, B, IV, and V. The relative composition of extracted VFAs fits with the broth VFAs composition, considering the H_s of each VFA.

Based on the VFAs produced and the COD analysis of the outputs, it was possible to make the COD balance of the whole fermentation experiment. The total input was 50 gCOD, made of 33.8 gCOD fermentable feedstock and 16 gCOD as inoculum (mostly suspended solids). The overall output fits reasonably with the input, namely 18±1 gCOD as VFAs (extracted and still in the broth), 17±3 gCOD as suspended solids, 12±2 gCOD as other soluble organics. To assess the purity of the stripped VFAs, an identical parallel fermentation was run with the device depicted in Figure 2 (stripping and reaction with $CaCO_3$ to form VFAs salts). At the end of the experiment, the final $CaCO_3$ slurry was filtered, and the aqueous filtrate was dried, yielding white crystals which consist of clean calcium-carboxylate crystals (>98% purity, checked through ATR-FTIR and VFAs analysis by GC-MS). The results suggest that equilibrium is reached when the VFAs extraction rate equates to VFAs biological production.

As pH is the most crucial parameter influencing the stripping rate (as seen in section 3.2), we explored the possibility of increasing the extraction rate by decreasing the alkalinity using less inoculum. Therefore, this second experiment was performed with 300 mL of fresh inoculum, 300 mL of distilled water, 28 g of chickpea flowers, and 1 g of sunflower oil (Figure 11).

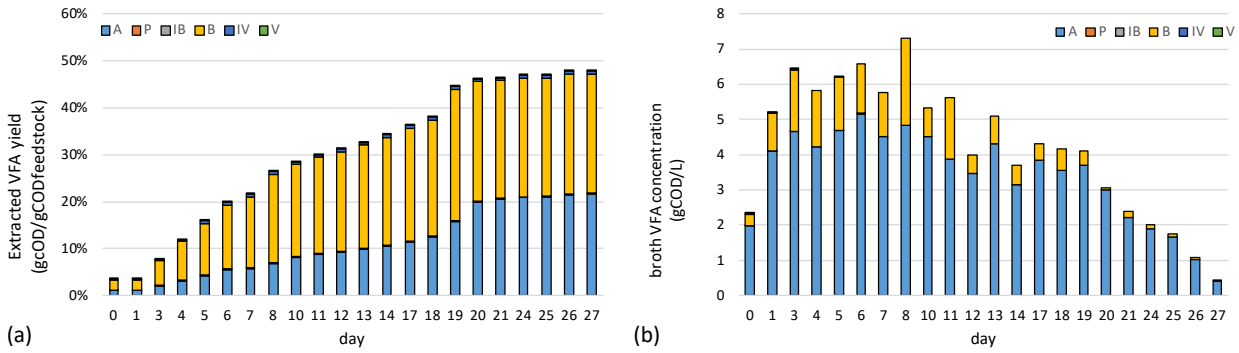


404

405 **Figure 11.** Time trend observed during the second EF experiment (performed with a lower

406 inoculum/substrate ratio) showing Extracted VFAs, concentration of VFAs in the broth and pH of the

407 broth.



408

409 **Figure 12.** Detailed composition of extracted VFAs (a, as gCOD/gCOD fed) and concentration of each VFA

410 in fermentation broth (b, data shown as gCOD/L of each VFA) observed during each day of the second

411 EF.

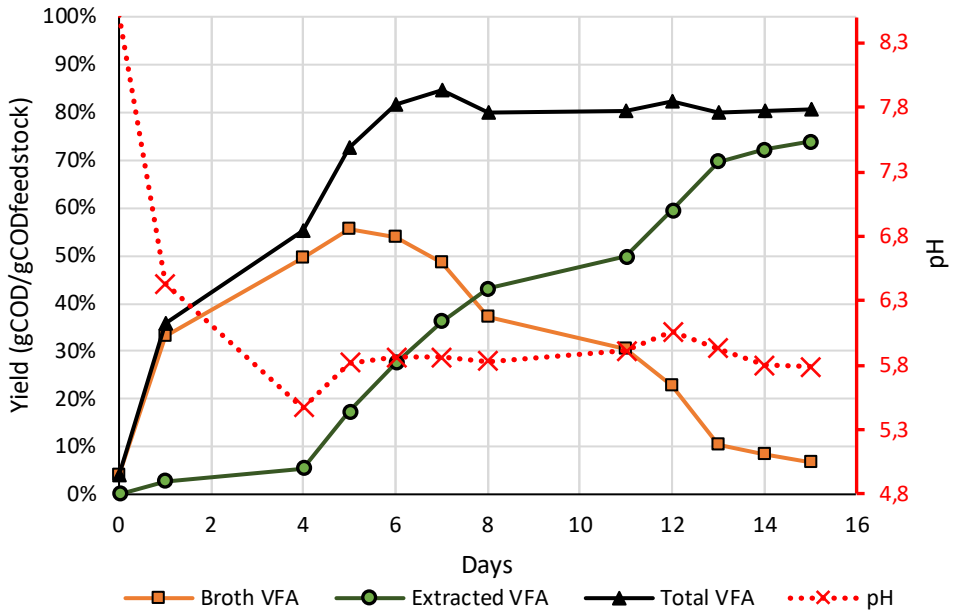
412 Fermentation started with an even sharper increase of VFAs in the fermentation broth in the first 3 days,

413 but thanks to lower alkalinity, the pH immediately dropped to pH 5. As a result, VP increased to 2 gCOD

414 L^{-1} d^{-1} with a steady state followed by exhaustion, similarly to what was observed in the first experiment.

However, a more pronounced pH change and an apparent stop of acidogenesis at day 20 were observed, with trends similar to what is expected in VFAs extraction without concurrent VFAs production from fermentation. At the end of the fermentation, an overall VFAs yield equal to 49% was obtained, with almost (48%) total stripping of the VFAs and a fermentation broth with just 0.5 gCOD L^{-1} of VFAs. The relative composition of VFAs obtained was constant during the study, with a final extracted mixture more enriched in B (0.53 $gCOD/gCOD_{extr}$) than in the previous experiment. This was due to a 50% higher B concentration in the broth, common for MMC fermentation when pH is maintained between 5 and 6 [37] due to microbial ecology and the higher thermodynamic stability of longer VFAs at low pH [38]. Although working at low pH drastically increases the extraction rate, the second experiment suggests that it concurrently increases the inhibition from free VFAs. Even if this increase significantly improves the yield of extracted VFAs, the overall amount of VFAs obtained in 28 days was lower than that obtained with fermentation at pH 5.8. Looking at the COD balance, a total input of 50 gCOD (33.8 gCOD fermentable feedstock and 10.6 gCOD from inoculum) gave 17±1 gCOD of VFAs, 13±5 gCOD of suspended solids, and 14±2 gCOD of other soluble organics, clearly suggesting an incomplete conversion of the substrate.

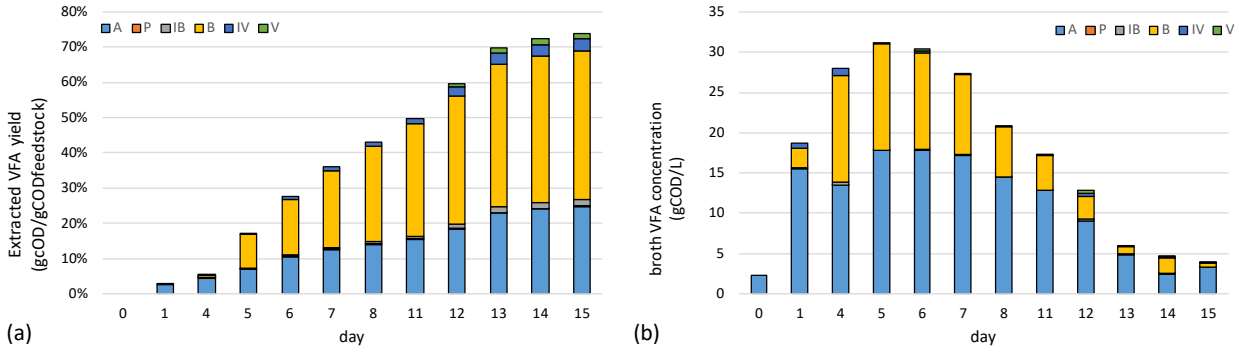
To combine the high rate of fermentation observed during the first experiment (pH between 5.6-6.0) and the high rate of extraction observed in the second experiment (pH between 4.9-5.6), a total alkalinity content and substrate/inoculum equal to that of the first experiment were used in combination with a pH control to 5.2 with HCl before stripping (Figure 13).



435

436 **Figure 13.** Time trend observed during the third EF experiment (performed controlling pH just before

437 stripping) showing Extracted VFAs, VFAs in the broth and pH of the broth.



438

439 **Figure 14.** Detailed composition of extracted VFAs (a, as gCOD/gCOD fed) and concentration of each VFA

440 in fermentation broth (b, data shown as gCOD/L of each VFA) observed during each day of the third EF.

441 The pH of the STR (Figure 13) was maintained between 5.4 and 6, avoiding excessive broth acidification

442 while ensuring a high VFAs extraction rate due to the lower pH during stripping. The difference in the

443 resulting VFAs trends was impressive, with an initial 4-day leap of VFAs concentration in the fermentation

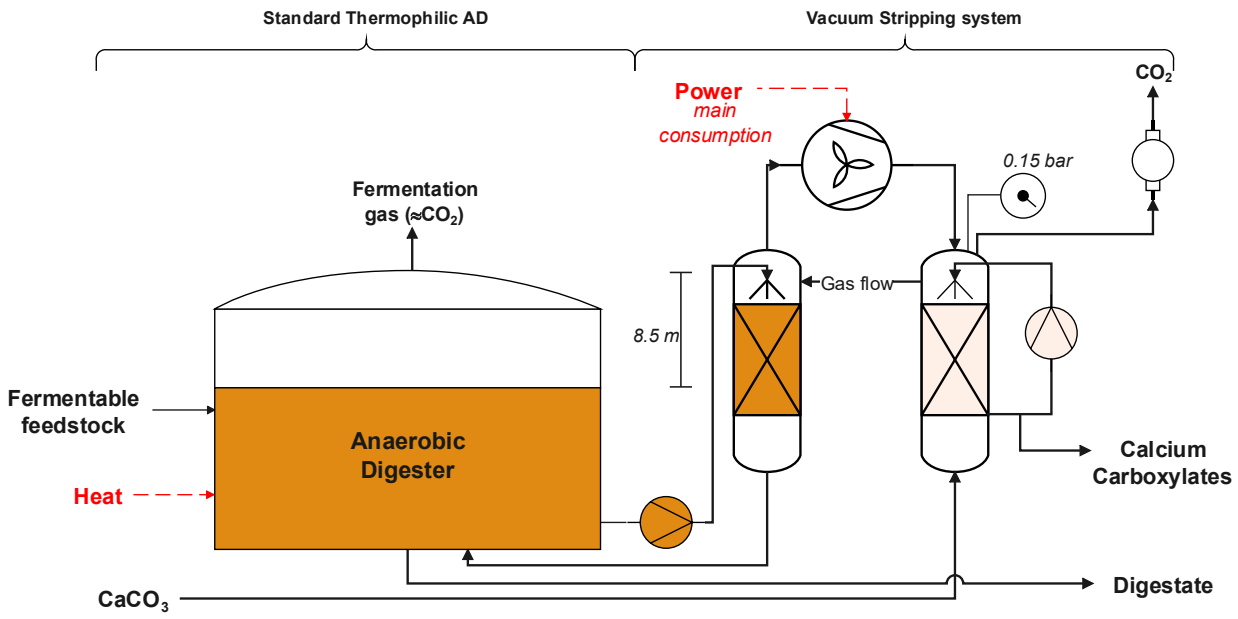
444 broth to 28 gCOD L^{-1} and an average extraction rate of 3.3 gCOD L^{-1} d^{-1} between days 5 and 15. Although

445 the trends were qualitatively identical to previous experiments, the timing of extractive fermentation and

process exhaustion sped up twice, giving an almost complete conversion of the feedstock in 15 days, as testified by soluble COD decrease below 6 gCOD L^{-1}. Decoupling of fermentation and stripping pH drastically enhances extractive fermentation's overall performance, allowing an outstanding VFAs yield equal to 81%, with 74% of feedstock COD obtained as clean stripped VFAs. Similarly to the previous experiment, the extracted VFAs mixture was selectively enriched in B (0.57 $gCOD/gCOD_{extr}$) and A (0.33 $gCOD/gCOD_{extr}$) but with a more complex relative composition, which probably arises from more complete conversion of the feedstock. Experimental evidence suggests that controlling the pH during extraction and fermentation is critical for the quick and quantitative conversion of the substrate into VFAs. It is worth noticing that the decoupling of stripping pH and fermentation pH was easily achieved through the addition of a relatively low amount of HCl (1.6 g for 27 g of VFAs obtained) and, in principle, could be obtained c pH swing before stripping [39].

3.4 Technical evaluation of EF and scale-up considerations

The stripping-based method presented here is a relatively simple extraction concept that does not require a particular solvent or specialised equipment, but rather a suitable reactor shape and a fan which delivers the required flow of stripping gas. For this reason, it is straightforward to define the functional units of EF and obtain a reliable estimate of the energy requirement (ER) without the need for complex Life Cycle Analysis (LCA). Figure 15 shows a proof-of-principle process scheme that can be applied at an industrial scale. Preliminary analysis on this highlights the main critical issue of stripping-based EF, namely the ER for moving a large volume of gas between the stripping and trapping sections of the vacuum system. As previously (section 3.1) described, ER depends on the concentration of VFAs in the stripping gas ($gCOD/m^3$) and energy consumption for pumping 1 m^3 gas, which is, by definition, the pressure drop ($Pa=N/m^2=J/m^3$) overcome by the fan.



469

470 **Figure 15.** Main functional units, input and output of the vacuum-enhanced system for EF.

471 Dividing the VP observed during EF by the VSGF used during the experiments (calculated as in Table 2), it
472 is possible to calculate the actual VFAs concentration in stripping gas (gCOD/m^3) and obtain the energy
473 requirement (ER, kJ/$gCOD_{VFA}$), which was calculated assuming a pressure drop of 1000 Pa, 80% fan
474 efficiency. Using gross VP values (gCOD/L divided by days of experiments), the ER ranges between 5.3
475 and 15 kJ/gCOD (0.7-4.1 kWh/kg COD). Using the maximum observed VP, which means assuming a
476 continuous and optimised system, provides an ER ranging from 2.4 to 5.8 kJ/gCOD (0.7-4.1 kWh/kg
477 COD). Such values, which can be decreased through the proper design of the apparatus (e.g. decreasing
478 pressure drop), can be considered economically and energetically acceptable for the obtainment of
479 more valuable VFAs (e.g. butyric acid). Generally, EF through vacuum-enhanced stripping requires less
480 energy than what is usually assumed for other EF processes [17,18].

481

4. Conclusions

Given the acid dissociation constants ($pk_a \approx 4.75$) and actual Henry constants, stripping can be potentially used for the extraction of the six fermentation-derived VFAs (acetic, propionic, isobutyric, butyric, isovaleric, and valeric acid) from slightly acidified fermentation broth. Experimental evaluation of VFA partitions in digestate revealed that the H_s of VFAs within mixed VFAs solution (e.g. 20-60 g/L overall concentration) with or without suspended solids, exert a higher partial pressure than expected., with absolute values that can easily surpass g/m^3 at high temperature (>50°C) as pH drops to 5. Evaluation of kinetics of stripping-based extraction of VFAs was evaluated with a model system, revealing that in conditions similar to that found in fermentation it is possible to achieve volumetric productivities higher than that obtained for methane production (e.g. >10 $gCOD_{VFA}\ L^{-1}\ d^{-1}$ [9]), especially when absolute pressure is close to the water vapour pressure at fermentation temperature. A simple vacuum-based setup for extractive fermentation was designed and tested, proving a low cost replicable experimental tool. To assess the actual performance of the process, the setup was employed to ferment a food waste analogue consisting of chickpea flour and vegetable oil. The experiments consistently produced clean VFAs salts, and outperformed non-extractive fermentation when pH was corrected before extraction, namely 74% conversion within 15 days.

This approach, which can be easily replicated with different fermentation conditions, offers promising opportunities for efficient, solventless VFA extraction from biowastes using a microbial mixed culture system. Testing of hyperthermophilic conditions (raising VFAs gas concentration) and bioaugmented thermophilic/acidophiles inocula (delivering higher volumetric productivity at low pH), as well as coupling extractive fermentation with innovative pH control methods (like electrochemical pH swing) are just some of the possible aspects that could be evaluated in the future.

CRediT authorship contribution statement

Cristian Torri: conceptualisation, methodology, data analysis, Formal analysis, Investigation, Writing – original draft, visualisation, project administration, funding acquisition; Federica Zimbardi: formal analysis, data analysis, Investigation; Andrea Facchin: conceptualisation, methodology, software, writing – review & editing, visualisation.

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