

1 Fermentative hydrogen production from cheese
2 whey with *in-line*, concentration gradient-driven
3 butyric acid extraction

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24 **Abstract**

25 Hydrogen (H₂) generation from cheese whey, with simultaneous production and
26 extraction of volatile fatty acids (VFAs), was studied in UASB reactors at two
27 temperatures (20 and 35°C) and pH values (5.0 and 4.5). The extraction module,
28 installed through a recirculation loop, was a silicone tube coil submerged in water,
29 which allows concentration-driven extraction of undissociated VFAs. Operating
30 conditions were selected as a compromise for the recovery of both H₂ and VFAs. Batch
31 experiments showed a higher yield (0.9 mol H₂ mol⁻¹ glucose_{eq.}) at 35°C and pH 5.0,
32 regardless of the presence of the extraction module, whereas lower yields were obtained
33 at pH 4.5 and 20°C (0.5 and 0.3 mol H₂ mol⁻¹ glucose_{eq.}, respectively). VFAs crossed
34 the silicone membrane, with a strong preference for butyric over propionic and acetic
35 acid due to its higher hydrophobicity. Sugars, lactic acid and nutrients were retained,
36 resulting in an extracted solution of up to 2.5 g L⁻¹ butyric acid with more than 90%
37 purity. Continuous experiment confirmed those results, with production rates up to 2.0
38 L H₂ L⁻¹ d⁻¹ and butyric acid extraction both *in-line* (from the UASB recirculation) and
39 *off-line* (from the UASB effluent). *In-line* VFA extraction can reduce the operating
40 costs of fermentation, facilitating downstream processing for the recovery of marketable
41 VFAs without affecting the H₂ production.

42

43 **Keywords:** Biohydrogen; Butyric acid; Dairy wastewater; Pertraction; Selective
44 extraction; Waste biorefinery

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47

48 **1. Introduction**

49 The increasing societal need for energy and materials, along with population growth,
50 fossil fuel depletion and growing interest in environmental issues, are driving a global
51 shift towards a sustainable and circular economy. In 2018, an updated bioeconomy
52 strategy has been adopted by the European Union, along with the Paris Agreement
53 commitments, to achieve the sustainable growth and environmental protection goals
54 included in the 2030 agenda [1]. In this view, biodegradable waste streams are proposed
55 as renewable substrates for energy and chemical production, partially replacing fossil
56 fuels [2,3]. This context encourages production systems to implement the biorefinery
57 concept [4], where waste is considered as an opportunity to diversify the product
58 spectrum while reducing the costs of biomass supply and waste treatment, thereby
59 meeting the increasingly stringent legislation on emissions.

60

61 The dairy industry processes 170 billion L of milk per year in Europe [5], generating an
62 average of 2.5 L wastewater per L of milk processed and 9-10 L cheese whey (CW) per
63 kg of cheese produced. When CW is discharged without proper treatment, it can have
64 serious adverse effects on the environment, *i.e.* rising of eutrophication in water bodies
65 or decreased crop yields and oxygen availability in agricultural land [6]. CW
66 management mainly involves whey protein recovery, animal feeding, or treatment in
67 dedicated wastewater treatment plants, depending on the size of the dairy industry and
68 the production context [7]. However, the high concentration of readily degradable
69 compounds (50-100 g_{COD} L⁻¹, 90% of which in the form of lactose) makes CW an
70 outstanding substrate for biological production of energy and chemical commodities [8],
71 not fully exploited so far. Physicochemical and biological processes can be synergically

72 implemented, according to the waste biorefinery concept, to convert CW to valuable
73 products such as methane [9], hydrogen [10], volatile fatty acids (VFAs) [11], alcohols
74 [12], lactic acid [13], electric energy [14], or bioplastics [15].

75

76 Among the suitable processes, dark fermentation is considered the core of a waste
77 biorefinery scheme, as it enables biological simplification and conversion of organic
78 substrates to a carbon-neutral energy carrier (H_2) and building blocks (VFAs) suitable
79 for downstream applications [3,16]. Since sugars are the preferred substrate for
80 fermentative microorganisms, CW is a substrate of particular interest for dark
81 fermentation. CW fermentation results in H_2 yields typically spanning between 1 and 4
82 mol mol^{-1} lactose (or 0.5 and 2 mol mol^{-1} glucose_{eq.}) depending on the operating
83 conditions such as pH, temperature and organic loading rate [10,17–19].

84

85 Besides H_2 , up to 20-30 g L^{-1} VFAs, mainly acetic, propionic, and butyric acid are
86 produced through CW fermentation, at different mass proportions depending on the
87 operating parameters, pH in particular [10,11,20]. Typically, the operating conditions
88 that foster H_2 production in CW fermentation also favour butyric acid production
89 among the soluble organic fermentation products [10,21]. Butyric acid finds numerous
90 applications in the chemical, pharmaceutical, perfume and animal feed sectors [22],
91 with a market size of about 125 M€ ([https://www.marketsandmarkets.com/Market-](https://www.marketsandmarkets.com/Market-Reports/butyric-acid-market-76962011.html)
92 [Reports/butyric-acid-market-76962011.html](https://www.marketsandmarkets.com/Market-Reports/butyric-acid-market-76962011.html)) which is expected to further increase by
93 15.1% year⁻¹, as a response to its approval as food flavouring agent by the U.S. Food
94 and Drug Administration (FDA) [23]. This already favourable context could further
95 benefit, in the next decade, by the development of the bioplastic sector, as butyric acid

96 is a precursor for polyhydroxyalkanoates (PHA) production [24]. Thus, the
97 development of a process for the combined production of H₂ and butyric acid
98 substantially contributes to a modern and environmentally sustainable CW
99 management.

100

101 Several technologies are available for VFAs extraction, including physical
102 (nanofiltration, liquid-liquid extraction, vapour permeation, membrane contactors, gas
103 stripping and distillation), chemical (adsorption and solvent extraction) and
104 electrochemical (electrodialysis) methods [23,25]. However, the development of a low-
105 cost system to selectively extract the target compound from a VFAs mixture is still a
106 challenge. **Outram and Zhang** [26] recently showed that concentration-gradient-driven
107 liquid-liquid extraction (pertraction) through a non-porous silicone membrane, using
108 distilled water as the draw solution, can be applied to recover VFAs. Furthermore, it
109 was shown that longer-chain VFAs migrate faster than shorter-chain VFAs through the
110 silicone membrane due to their higher hydrophobicity [26]. This represents a
111 remarkable feature, as it would enable the selective extraction of butyric acid over other
112 typical CW fermentation products (i.e. acetic, propionic and lactic acid).

113

114 The aim of the present study was to study the performance of a novel reactor concept
115 for simultaneous H₂ and butyric acid recovery from CW, where an *in-line* silicone
116 membrane extraction module is implemented into a fermentative UASB reactor through
117 a recirculation loop. **The operating conditions were chosen as a compromise between H₂
118 production (optimal pH between 5.5 and 6.0) and VFA extraction (requiring pH below
119 the pK_a of VFA).** First, inoculum and up-flow velocity were optimised for H₂ and

120 butyric acid production. Then, the effects of pH (5.0 vs. 4.5) and temperature (35 vs. 20
121 °C) on H₂ production and butyric acid recovery were evaluated in the UASB operated
122 either under batch or continuous mode. Finally, the extraction efficiencies achieved
123 were compared to those obtained by operating an *off-line* butyric acid extraction system
124 fed with the fermentative UASB effluent.

125

126 **2. Materials and methods**

127 ***2.1 Source of inoculum and pretreatment***

128 The inoculum used in this study was either activated or digested sludge from the
129 wastewater treatment plant of a dairy industry (Dairygold, Mitchelstown, Ireland). The
130 activated and digested sludge had a total solids concentration of 42.7 ± 0.8 and $66.0 \pm$
131 3.0 g L^{-1} , and a volatile solids concentration of 24.8 ± 0.4 and $49.8 \pm 2.6 \text{ g L}^{-1}$,
132 respectively. Heat pretreatment was done by heating thin tubes containing 5 mL of
133 sludge in a dry bath (Fisher Scientific) at 90°C for 15 minutes.

134

135 ***2.2 Synthetic medium and cheese whey composition***

136 The synthetic medium used for inoculum screening was the DSMZ (Deutsche
137 Sammlung von Mikroorganismen und Zellkulturen) medium nr. 141 with the following
138 modifications: lactose (10 g L^{-1}) was used instead of glucose as the substrate, and yeast
139 extract, tryptone, resazurine and Na₂S were not added. CW from cow milk processing
140 was collected from **the dairy industry (Dairygold)**, stored at -20°C after transportation to
141 the lab, and defrosted to 4°C 24 hours prior to utilization to prevent acidification. The
142 CW composition was as specified in Table 1.

143

144 **Table 1.** Cheese whey characterization

| Parameter | Unit | Values |
|---|---------------------|--|
| Total Solids (TS) | g L ⁻¹ | 69.98 ± 1.94 |
| Volatile Solids (VS) | g L ⁻¹ | 64.04 ± 1.76 |
| Total suspended solids (TSS) | g L ⁻¹ | 1.18 ± 0.11 |
| Volatile suspended solids (VSS) | g L ⁻¹ | 1.17 ± 0.05 |
| pH | - | 6.42 |
| Conductivity | mS cm ⁻¹ | 5.24 |
| COD | g L ⁻¹ | 66.96 ± 4.80 |
| TOC _{sol} | g L ⁻¹ | 20.82 ± 1.08 |
| Total dissolved saccharides | g L ⁻¹ | 41.70 ± 0.91 |
| Acetic acid | mg L ⁻¹ | 262 ± 5 |
| Propionic acid | mg L ⁻¹ | 83 ± 2 |
| Lactic acid | mg L ⁻¹ | 926 |
| Total P | mg L ⁻¹ | 308 ± 22 |
| Anions (Cl ⁻ , NO ₂ ⁻ , NO ₃ ⁻ , PO ₄ ³⁻ , SO ₄ ²⁻) | mg L ⁻¹ | 436 ± 23, <10, <10, 188 ± 3, 23 ± 1 |
| Cations (Ca ²⁺ , K ⁺ , Na ⁺ , NH ₄ ⁺) | mg L ⁻¹ | 266 ± 56, 1702 ± 177, 441 ± 76, 83 ± 8 |
| Soluble proteins | g L ⁻¹ | 2.30 ± 0.01 |

145

146 **2.3 Inoculum screening**

147 Four inocula, *i.e.* activated or digested sludge with or without heat-shock pretreatment,
 148 were compared for H₂ production from lactose in a preliminary batch experiment. The
 149 experiment was conducted in triplicate 120 mL serum bottles with 48 mL of synthetic
 150 medium and 2 mL of each inoculum. The initial pH was adjusted to 7.0 using 1 M
 151 NaOH. Abiotic (without inoculum) and no-substrate (without lactose) controls were
 152 also prepared. The bottles were sparged with N₂ for 5 min prior to incubation at 35°C
 153 for about 17 days with 150 rpm shaking in an orbital shaker incubator
 154 (ThermoScientific MaxQ 8000).

155

156 **2.4 Effect of up-flow velocity on hydrogen production from CW**

157 The effect of the up-flow velocity on H₂ production was studied in 1 L recirculated
 158 UASB reactors operated in batch mode, and maintained at 35°C using a water bath with

159 recirculation (Grant Tc120, UK). A controller (Cole-Parmer 300, USA) connected to a
160 pH probe (VWR, USA) and a peristaltic pump (Verdeflex, The Netherlands) **was** used
161 to keep the pH above 5.0 in the UASB reactor by addition of 5M NaOH from a bottle
162 under N₂ atmosphere. After sparging with N₂, the reactors were fed with 700 mL CW
163 using a peristaltic pump (Masterflex L/S, Cole-Parmer, USA). Heat-treated digested
164 sludge (4%) was added as inoculum from a sampling port. The CW was recirculated
165 from the top to the bottom of the bioreactor, using a peristaltic pump (Masterflex), to
166 achieve an up-flow velocity of 0.1, 0.5, 1.0 or 2.0 m h⁻¹. The gas produced was collected
167 in a gas bag, and the batch experiments were stopped when no H₂ production was
168 observed anymore for at least 3 consecutive days (after 8-11 days fermentation).

169

170 ***2.5 Batch experiments with in-line VFAs extraction***

171 UASB reactors, with the same configuration as the previous experiment, were used for
172 evaluating the effect of *in-line* VFAs extraction on CW fermentation at different
173 temperature (20 and 35°C) and pH (4.5 and 5.0) using heat-treated digested sludge as
174 inoculum and an up-flow velocity of 1.0 m h⁻¹. The VFAs extraction module included a
175 silicone tube coil (2 and 4 mm internal and external diameter, VWR, The Netherlands)
176 with a total length of 4.2-4.4 m, submerged into 700 mL distilled water (draw solution)
177 in a conical 1 L flask. The flask was sealed at the top with a rubber stopper and
178 connected to the gas line outlet (Fig. 1) to recover the gas **diffusing** through the silicone
179 membrane. The extraction module was installed to the UASB reactor through a
180 recirculation loop. One UASB reactor was operated without *in-line* **VFA** extraction as
181 control. Since the working volume increased due to the addition of the extraction unit,

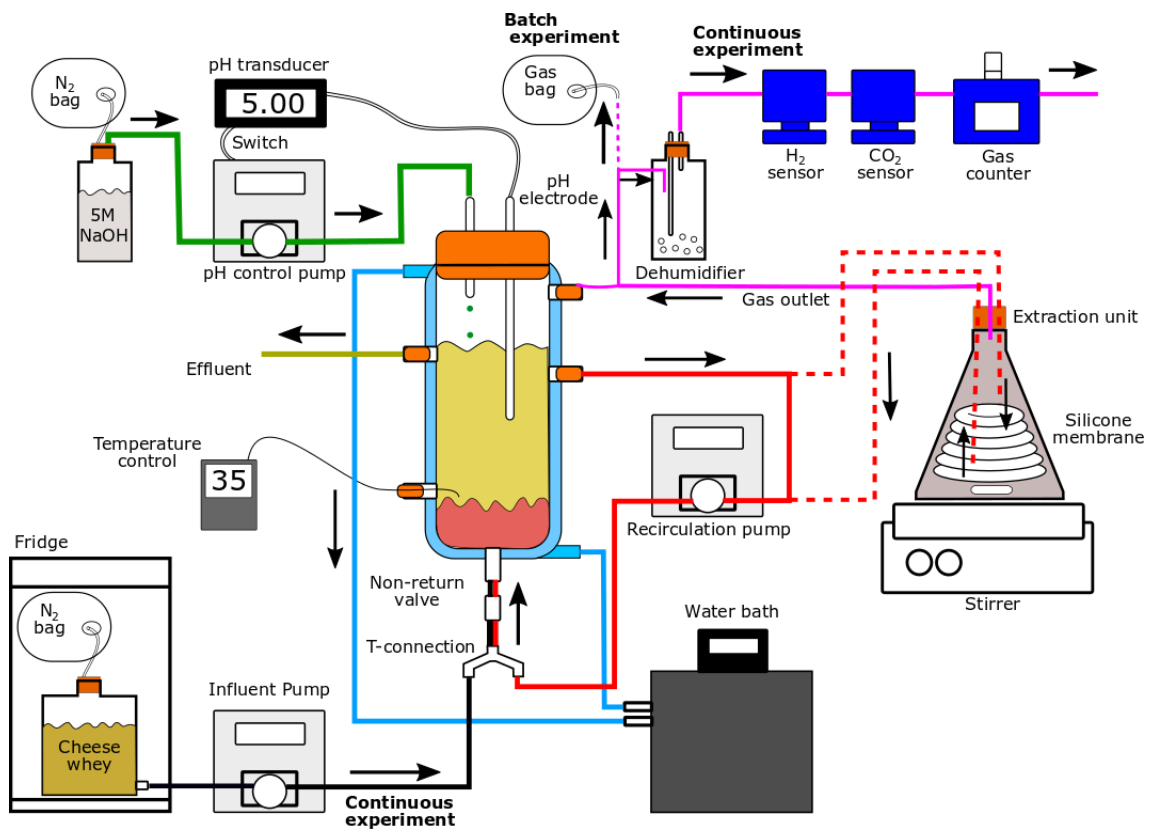
182 the UASB reactors were fed with 830-850 mL of CW as compared to the 700 mL of the
183 preliminary experiment on the up-flow velocity (section 2.4).

184

185 **2.6 Continuous experiment with in-line VFA extraction**

186 For the continuous experiments, an influent supply tank, kept at 4-6°C inside a fridge,
187 was connected to two UASB reactors through a pump (Masterflex), and an on-line gas
188 monitoring system composed by a V-count gas counter and H₂ and CO₂ sensors
189 (BlueSens, Germany) was installed (Fig. 1). Two UASB reactors (namely, UASB-A
190 and UASB-B) were run in parallel, according to the experimental stages reported in
191 Table 2. After a 5-day start-up in batch mode, CW was fed continuously at 24 hours
192 hydraulic retention time (HRT) to compare the performance of the UASB reactors in the
193 presence and absence of the extraction module, and then to study the response of the
194 integrated system to pH changes.

195



196 **Figure 1.** UASB reactor configuration adopted for the experiments in batch and
 197 continuous operation mode. The coloured lines represent the influent (black, only for
 198 the continuous experiment), recirculation (red), effluent (yellow), pH control (green),
 199 water jacket (blue) and gas (magenta) lines.

200

201 **Table 2.** Overview of the UASB reactor operation with the experiments in continuous
 202 mode. All experiments were performed at 35°C.

| Reactor | Days | Operation mode | pH control | Extraction unit |
|---------|-------|--|------------|-----------------|
| UASB-A | 0-5 | Batch (Start-up) | 5.0 | No |
| | 6-42 | Continuous | 5.0 | No |
| | 43-64 | Continuous (restarted with fresh inoculum) | 4.5 | Yes |
| | 65-84 | Continuous | 5.0 | Yes |
| UASB-B | 0-5 | Batch (Start-up) | 5.0 | No |
| | 6-48 | Continuous | 5.0 | Yes |
| | 49-74 | Continuous | 4.5 | Yes |
| | 75-84 | Continuous | 5.0 | Yes |

203

204 **2.7 Off-line extraction experiment**

205 Fermentate from both UASB reactors, when operated in continuous mode at pH 5.0 and
 206 4.5, respectively, was collected on day 71 and used for *off-line* extraction tests in batch.

207 The fermentate was acidified to pH 3 by HCl addition prior to starting the experiment.

208 A flask containing CW fermentate (500 mL) was connected through a pump to the
 209 extraction module containing either 500 mL distilled water or 0.5 M NaOH as the draw
 210 solution through a recirculation loop. The recirculation flow was 21 mL min⁻¹, the same
 211 applied to the UASB reactor to obtain an up-flow velocity of 1.0 m h⁻¹.

212

213 **2.8 Monitoring and analytical methods**

214 Gas produced during the inoculum screening tests was quantified using a syringe
215 method [27]. Gas produced during the UASB batch tests, including the gas diffusing
216 through the silicone membrane (Fig. 1), was collected in 5 L gas bags and measured
217 using the water displacement method. For all the batch experiments, gas samples (5 mL)
218 were collected either from the headspace of the serum bottles or from the gas bags and
219 stored in 5.9 mL gas collection vials (Exetainer[®], Labco, UK) at ambient temperature
220 for analysis. Gas composition (H₂, CH₄ and CO₂) was analysed using a gas
221 chromatograph (GC) (Agilent 7890A, USA) equipped with a thermal conductivity
222 detector (TCD) and a 80/100 Hayesep Q column. Argon was the carrier gas with a flow
223 of 24 mL min⁻¹, and oven, injector and detector were kept at 90, 90 and 200°C,
224 respectively. For the continuous experiment, both gas lines were connected to the on-
225 line monitoring sensors and gas counter (BlueSens, Germany).

226

227 Liquid samples were collected from the serum bottles (2 mL), from a sampling port in
228 the recirculation tube of the UASB reactors (4 mL), as well as from a sampling tube
229 submerged in the draw solution (2 mL), and stored at -20°C in plastic tubes for analysis.

230 Sugars, carboxylic acid and alcohol concentrations in liquid samples were analysed
231 using a liquid chromatograph (LC) (1260 Infinity II, Agilent, USA) equipped with a
232 refractive index detector (RID) and a Hi-Plex H column (300×7.7 mm) held at 60°C.

233 The mobile phase was H₂SO₄ (5 mM) at a flow rate of 0.7 mL min⁻¹. Total dissolved
234 saccharides were measured using a phenol-sulphuric colorimetric method [28] with a
235 spectrophotometer (Shimadzu UV-1900, Japan) at 485 nm.

236

237 Total solids (TS), total suspended solids (TSS), volatile solids (VS), volatile suspended
238 solids (VSS) and chemical oxygen demand (COD) were measured according to the
239 APHA procedures [29]. Total organic carbon (TOC) was analysed using a TOC
240 analyser (TOC-L CSN Analyser, Shimadzu, Japan). Conductivity and pH were
241 measured with a conductivity meter (Mettler Toledo, USA) and with a pH controller
242 (Cole Parmer 300, UK) connected to a pH probe (SlimTrode, Hamilton, Switzerland),
243 respectively. Cations and anions were measured via ionising coupled plasma - optical
244 emission spectroscopy (ICP-OES 5110, Agilent, USA) and ion chromatography (IC
245 AS-DV, Thermo Scientific, USA), respectively. Total phosphorus, ammonium and
246 soluble proteins were measured using a Nutrient analyser (Gallery Plus, Thermo
247 Scientific, USA).

248

249 ***2.9 Calculations***

250 The modified Gompertz model was applied as reported in [Asunis et al.](#) [10]. Carbon
251 balances were made based on the carbon content of liquid and gas products detected. A
252 carbon content of 46% was assumed for proteins [30]. The organic loading rate was
253 calculated based on COD. The acidification degree was calculated according to
254 [Bengtsson et al.](#) [31]. Fluxes and mass transfer coefficients (K_{OV}) were calculated
255 according to [Outram and Zhang](#) [26].

256

257 **3. Results and discussion**

258 ***3.1 Inoculum screening and optimal up-flow velocity***

259 When incubated in batch with the lactose-containing synthetic medium, heat-treated
260 digested sludge gave a significantly **higher H_2 production rate ($0.66 \text{ L L}^{-1} \text{ d}^{-1}$)** and yield

261 (0.92 ± 0.38 mol mol⁻¹ glucose_{eq.}), as well as a higher butyric acid yield (0.27 ± 0.12
262 mol mol⁻¹ glucose_{eq.}) than the other inocula tested, *i.e.* non-treated digested sludge, and
263 both treated and non-treated activated sludge (Table S1). Thus, heat-treated digested
264 sludge was selected as the inoculum for all follow-up experiments.

265

266 A remarkable effect on the H₂ production from **cheese whey** was observed for the
267 different up-flow velocities tested (0.1, 0.5, 1.0 and 2.0 m h⁻¹) in recirculated UASB
268 reactors operated in batch mode **when controlling the pH at 5.0**. Up-flow velocities of
269 1.0 and 2.0 m h⁻¹ resulted in a H₂ yield of about 1.0-1.1 mol mol⁻¹ glucose_{eq.}, 40 and
270 60% higher than the yields obtained at 0.5 and 0.1 m h⁻¹, respectively (Fig. S1, Table
271 S2), as a result of the higher mixing and gas stripping from the fermentation broth.
272 **Methane was not detected at any condition tested, due to the quick acidification of the**
273 **medium, with pH dropping from 6.4 to 5.0 within one operation day**. Based on these
274 results, an up-flow velocity of 1.0 m h⁻¹ was selected for further experiments.

275

276 **3.2 Batch cheese whey fermentation in UASB reactors and VFA extraction**

277 **3.2.1 Effect of in-line VFAs extraction, pH and temperature on H₂ production**

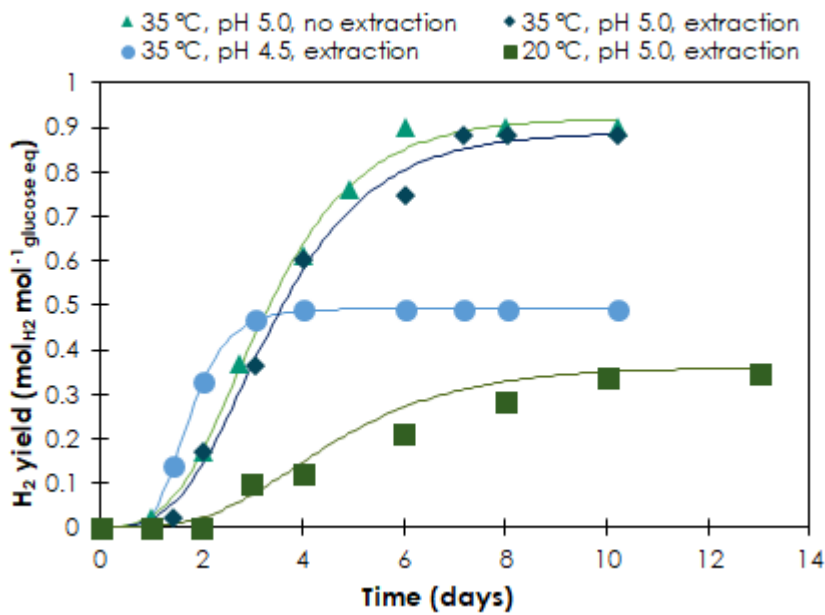
278 Similar yields of about 0.9 mol H₂ mol⁻¹ glucose_{eq.} and maximum production rates of
279 about 0.26 mol H₂ mol⁻¹ glucose_{eq.} d⁻¹ (Fig. 2; Table 3) were observed in the UASB
280 reactors operated in batch at 35°C and pH 5.0 with and without the *in-line* VFA
281 separation module. Therefore, the VFAs extraction module had a minimum impact on
282 CW fermentation in UASB reactors. The results were also similar to those obtained in
283 the preliminary test at up-flow velocities of 1.0 and 2.0 m h⁻¹ (Fig. S1), confirming the
284 replicability of the fermentation process. This is further confirmed by the fact that the

285 obtained H₂ yield was comparable to the results achieved in previous studies on CW
286 fermentation [10,32].

287

288 Decreasing the operating temperature to 20°C, or pH to 4.5, resulted in a lower H₂ yield
289 of 0.36 and 0.49 mol H₂ mol⁻¹ glucose_{eq.}, respectively. The fermentation kinetics of CW,
290 and in particular lactose hydrolysis, are indeed slower at low temperature [33]. At pH
291 4.5, despite the relatively fast kinetics (0.35 mol H₂ mol⁻¹ glucose_{eq.} d⁻¹) and short lag-
292 phase (1.0 d) (Table 3), H₂ production was likely inhibited by the accumulation of
293 butyric acid in its undissociated form (7.6 g L⁻¹, 68% of the total), which can penetrate
294 the bacterial cells suppressing growth and metabolic activity [34]. H₂ yields obtained
295 from CW-based substrates using mixed cultures at pH below 5.0 are typically low,
296 although a H₂ yield of 1.83 mol H₂ mol⁻¹ glucose_{eq.} was reported from a diluted CW
297 powder solution (4.9 g lactose L⁻¹) in a fluidized bed reactor operated at pH 4.0-4.5
298 under thermophilic conditions (55°C) [19].

299



300

301 **Figure 2.** Evolution of H₂ yields over time for the UASB fermentation tests performed
 302 at 20 and 35°C, pH 5.0 and 4.5, with or without *in-line* VFA extraction. Scatter plots
 303 represent the experimental data, and continuous lines represent the Gompertz model
 304 fitting.

305
 306 **Table 3.** Gompertz model parameters as calculated for the fermentation tests performed
 307 at different operating conditions.

| Parameter | Measure unit | 35°C, pH 5.0, no extraction | 35°C, pH 5.0, extraction | 35°C, pH 4.5, extraction | 20°C, pH 5.0, extraction |
|-------------------------------------|---|--------------------------------|-----------------------------|-----------------------------|-----------------------------|
| H ₂ yield _{max} | mol H ₂ mol ⁻¹ glucose _{eq.} | 0.921 | 0.888 | 0.491 | 0.360 |
| R _{max} | mol H ₂ mol ⁻¹ glucose _{eq.} d ⁻¹ | 0.259 | 0.238 | 0.354 | 0.075 |
| λ | d | 1.379 | 1.474 | 1.049 | 2.041 |
| t _{95-H2} | d | 7.214 | 6.908 | 3.106 | 9.100 |
| R ² | - | 0.996 | 0.994 | 1.000 | 0.981 |

308

309 3.2.2 Effect of *in-line* VFA extraction, pH and temperature on fermentation pathways

310 In all conditions tested, fermentation evolved according to three subsequent degradation
 311 stages. Lactose was first hydrolysed to glucose at different rates depending on the
 312 operating conditions, and then converted to lactic acid via homolactic fermentation.
 313 Galactose, the other monomeric sugar **formed during** lactose hydrolysis, was always
 314 below detection, suggesting its rapid conversion to glucose 6-phosphate, since there is
 315 no catabolic pathway to metabolize it [35]. Lactic acid was then converted to H₂, CO₂
 316 and VFAs, with a prevalence of butyric acid which was produced up to 15 and 20 g L⁻¹
 317 regardless of temperature and pH. **The highest butyrate production rate of 5.7 g L⁻¹ d⁻¹**
 318 **was obtained at pH 5 in the UASB without extraction module, after about two days of**
 319 **lag-phase (Fig. 3).** The full conversion of lactic acid to VFAs was achieved at pH 5.0,
 320 within 6-8 days at 35°C and around 10 days at 20°C, whilst the same fate was not
 321 observed at pH 4.5, likely due to inhibition of the fermentative microorganisms [34].

322

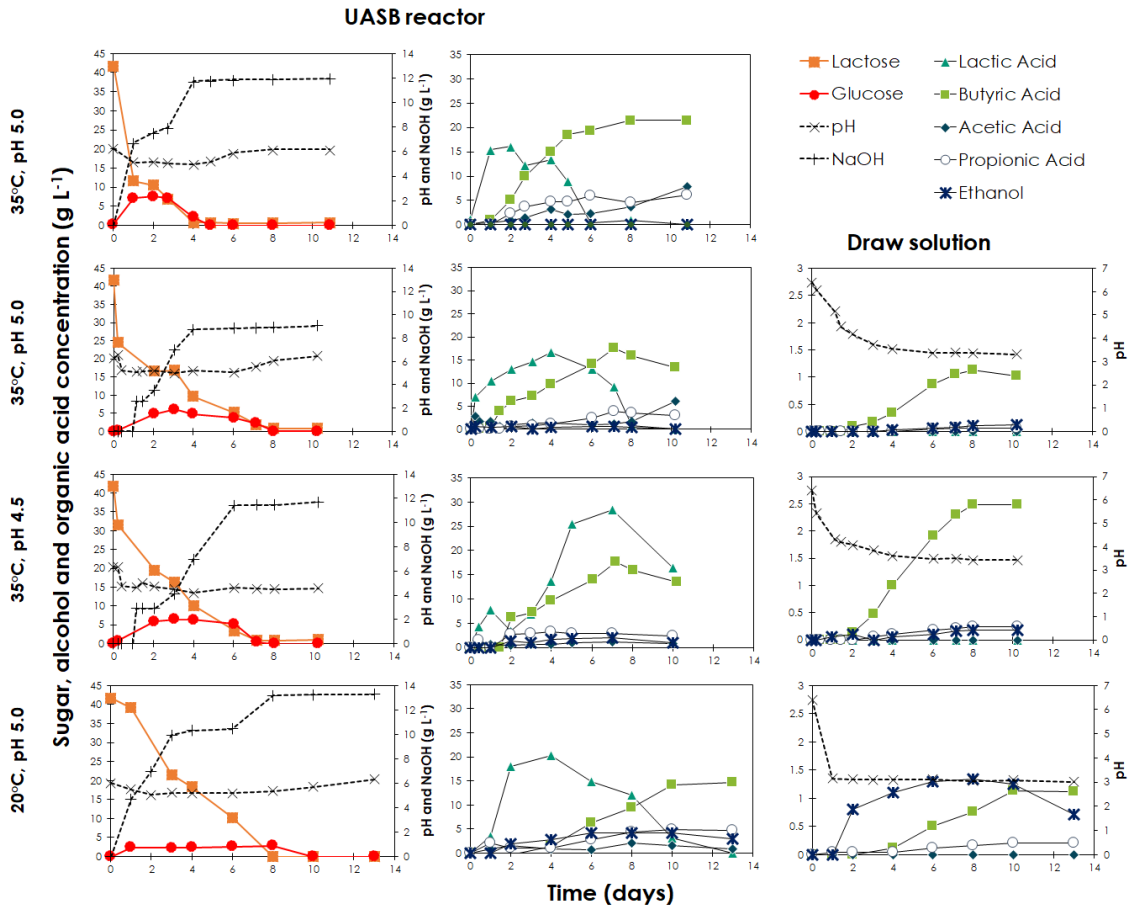
323 In both UASB reactor tests at 35°C and pH 5.0, the acetic acid concentration increased
324 after 6-8 operation days (Fig. 3), suggesting the onset of homoacetogenic pathways with
325 related negative effects on the H₂ yields [36,37]. Although the optimum growth pH of
326 propionate producing microorganisms is typically around 7 [38], propionic acid was
327 detected in all tests at pH 5.0, with a final concentration of 4-5 g L⁻¹, and even at pH 4.5
328 with a final concentration of 2.3 g L⁻¹. Significant ethanol production, up to 5 g L⁻¹, was
329 obtained only at 20°C, suggesting a shift from homolactic to heterolactic fermentation.
330 In this case, the overlapping pathways may have been caused by the slower sugar
331 consumption rates due to the lower temperature [39].

332

333 Under all conditions investigated, the pH dropped from the initial value of 6.3 to either
334 5.0 or 4.5 within 1-2 days (at 35°C) or 3 days (at 20°C), and a further decrease was
335 avoided only by automatic NaOH dosing. In the UASB reactors operated at pH 5.0,
336 once the sugars were fully consumed, the pH raised again likely due to protein
337 hydrolysis [40], and the consequent ammonium release. Indeed, about 430 and 460 mg
338 L⁻¹ ammonium was found upon CW fermentation at 20 and 35°C, respectively, against
339 the 83 mg L⁻¹ detected in the CW prior to fermentation (Table 1). The pH increase was
340 more evident in the UASB reactor with the extraction module, due to the VFAs
341 crossover through the silicone membrane, resulting in a final pH of 6.5 as compared to a
342 pH of 6.2 observed in the UASB reactor without extraction module (Fig. 3). As an
343 interesting consequence, a 25% lower NaOH dosage (9.0 g L⁻¹ CW) was required in the
344 UASB reactor provided with the extraction module than in the UASB reactor where

345 VFAs were not extracted (12.0 g L⁻¹ CW) in order to maintain pH values above 5.0,
 346 which, in turn, significantly reduces the operating costs in full-scale application.

347



348

349 **Figure 3.** Sugar, alcohol and VFAs concentration profiles, pH and NaOH dosage for the
 350 UASB fermentation tests at different temperature (20 or 35°C), pH (4.5 or 5.0) with or
 351 without the silicone membrane extraction module. The column “Draw solution” refers
 352 to the VFAs and alcohol extracted from the UASB reactors through the silicone
 353 membrane, and the resulting pH profiles.

354

355 *3.2.3 Effect of pH and temperature on VFAs and alcohol extraction through silicone*
 356 *membrane*

357 At 35°C, in the UASB reactor equipped with in-line extraction, irrespectively of the
358 operating pH values (5.0 or 4.5), butyric acid was the main metabolite extracted,
359 accounting for more than 90% of the carbon content (Table 4). Indeed, butyric acid
360 migrates faster than shorter chain acids through the silicone membrane matrix due to its
361 higher hydrophobicity [26]. Sugars, lactic acid, and nutrient sources such as proteins, P,
362 anions (Cl⁻, NO₂⁻, NO₃⁻, PO₄³⁻, and SO₄²⁻) and cations (Ca²⁺, K⁺, Na⁺, and NH₄⁺) were,
363 however, retained in the UASB reactor **mixed liquor**. This confirms that the extraction
364 module prevents the migration of substrates and nutrients, which could inhibit the
365 fermentation process, besides reducing costs for pH control, and allows recovery of
366 butyric acid with more than 90% purity (on carbon content basis), simplifying
367 downstream processing. However, when the extraction module was installed into the
368 UASB, 16.6–19.9% of the inlet carbon was not detected as fermentation product,
369 against only 3.1% unaccounted carbon in the control UASB reactor (Table 4),
370 suggesting VFA adsorption on the silicone membrane. **This hypothesis is supported by**
371 **the higher butyric acid concentration obtained at pH 5 and 35°C without, rather than**
372 **with, the extraction module (Table 4), despite the similar butyric acid yield expected**
373 **based on the similar H₂ yield obtained in the two UASBs (Fig. 2).** This would be,
374 nevertheless, a minor issue in continuous operation, since the membrane will be quickly
375 saturated with VFAs, after which a further loss will not occur.

376

377 In the test at 20°C, it is worth underlining that also ethanol was produced and extracted
378 through the silicone membrane, opening up further fermentation-related applications.
379 However, from day 8 **onwards**, ethanol concentrations in the draw solution decreased

380 (Fig. 3), likely due to **its** volatilization, a feature to be considered when ethanol is the
381 target of the separation process, which was not the case in this study.

382

383 In the UASB reactor operated at 35°C and pH 5.0 with in-line VFAs extraction, a total
384 of 14 g L⁻¹ butyric acid was produced, 1 g L⁻¹ of which was recovered in the draw
385 solution upon extraction (Fig. 3). The butyric acid flux through the silicone membrane
386 reached a maximum of 0.41 g m⁻² h⁻¹ on day 6 (Fig. 4). At pH 5.0, only 45% of the
387 butyric acid (pK_a=4.82), *i.e.* about 6.3 g L⁻¹, was in the undissociated form, which is a
388 requisite for crossing the silicone membrane [26]. Furthermore, from day 6, the
389 concentration of undissociated acid further decreased to <10% (*i.e.*, < 1.4 g L⁻¹) due to
390 the pH raise above 6.0, whereas the pH of the draw solution dropped to 3.5 (Fig. 3). The
391 decreasing concentration gradient between fermentate and draw solution caused a
392 decrease of the butyric acid flux, which became even negative on day 10 (Fig. 4),
393 suggesting that a small amount of butyric acid was **diffusing** back towards the
394 fermentation compartment. This issue can be mitigated in continuous operation, since
395 continuous carbohydrate fermentation would prevent a pH raise, and butyric acid
396 migration would thus continue as long as a concentration gradient is kept between the
397 fermentate and the draw solution.

398

399 To maintain the concentration gradient between the fermentation broth and draw
400 solution as high as possible, VFAs can be periodically or continuously extracted from
401 the draw solution, e.g. using electrodialysis technology [41]. Electrodialysis has
402 previously been applied to extract VFAs directly from a fermentation broth [41,42], but
403 its application is limited by the fact that all the anions are unselectively extracted, and

404 by biofouling. Both issues are avoided, or at least mitigated, if a silicone membrane
405 separation module is installed prior to the electro dialysis unit. Jones et al. (2017)
406 reported also a 3.75 higher H₂ production in a bioreactor operated with *in-line* VFAs
407 extraction *via* electro dialysis, with respect to a control reactor without extraction unit.
408 Such a beneficial effect was, nevertheless, not evident in this study (Fig. 2), likely due
409 to the substantially higher VFA concentrations (20-35 g L⁻¹ total VFAs) in the
410 fermentation broth (Fig. 3) compared to those (3-4 g L⁻¹ total VFAs) reported in Jones et
411 al. [42]. Indeed, although lower VFA concentrations were measured in the presence,
412 than in the absence, of the extraction unit (Fig. 3), the mitigation effect of the *in-line*
413 VFA extraction was not enough to impact the H₂ yield.

414

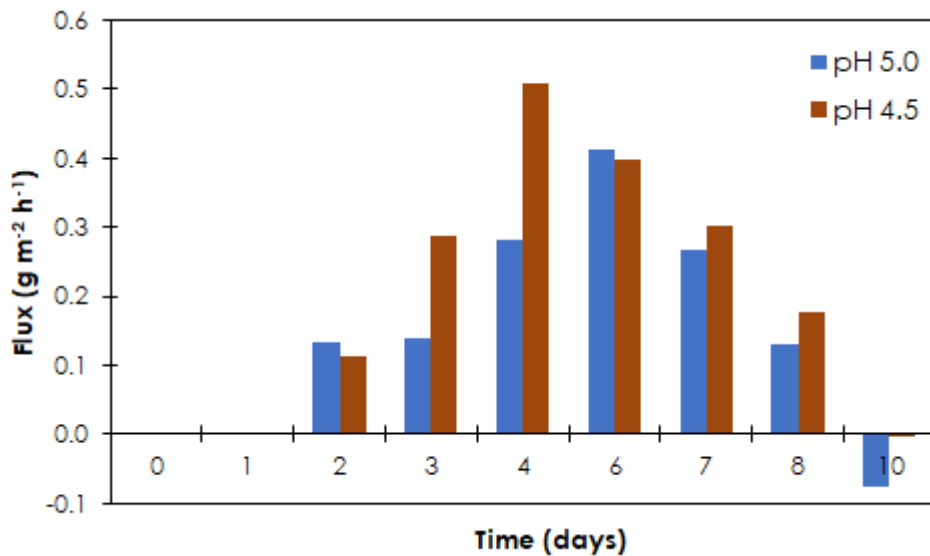
415 Fermentation at pH 4.5, which led to about 70% of the produced butyric acid in
416 undissociated form, resulted in a 240% higher butyric acid extraction (2.5 g L⁻¹, 40% of
417 the theoretical maximum value) through the silicone membrane than at pH 5.0 (Fig. 3).
418 The butyric acid flux reached a peak of 0.51 g m⁻² h⁻¹ on day 4, higher than the
419 maximum flux of 0.41 g m⁻² h⁻¹ obtained at pH 5.0 (Fig. 4). Ultimately, the butyric acid
420 extraction process can be facilitated by lowering the pH in the fermentation reactor,
421 although this would be detrimental to the H₂ production (Fig. 2). An acidification-
422 neutralization step could also be included into the extraction loop, but this would result
423 in higher operation costs.

424 **Table 4.** Carbon balances (in g L⁻¹) of the fermentation tests performed at different temperature (20 or 35°C) and pH (4.5 or 5.0) using
 425 UASB reactors with or without silicone membrane extraction unit.

| Compound (g C L ⁻¹) | Cheese whey | 35°C, pH 5.0, no extraction | 35°C, pH 5.0, extraction | | 35°C, pH 4.5, extraction | | 20°C pH 5.0, extraction | |
|------------------------------------|--------------|--------------------------------|--------------------------|---------------|--------------------------|---------------|-------------------------|---------------|
| | | Fermentate | Fermentate | Draw solution | Fermentate | Draw solution | Fermentate | Draw solution |
| Lactose | 17.54 | - | - | - | - | - | - | - |
| Lactic acid | 0.37 | - | - | - | 6.54 | - | - | - |
| Acetic acid | 0.10 | 1.46 | 2.44 | - | 0.34 | - | 0.41 | - |
| Propionic acid | 0.04 | 2.18 | 1.47 | 0.07 | 1.12 | 0.12 | 1.94 | 0.10 |
| Butyric acid | - | 11.69 | 7.32 | 1.03 | 3.61 | 1.36 | 8.03 | 0.61 |
| Ethanol | - | - | 0.27 | 0.13 | 0.55 | 0.10 | 1.55 | 0.21 |
| CO ₂ | - | 3.11 | 1.68 | 1.22 | 0.76 | 0.74 | 0.81 | 0.99 |
| Proteins ^a | 1.06 | 0.07 | 0.30 | - | 0.07 | - | 0.46 | - |
| Total | 19.11 | 18.51 | 15.93 | | 15.31 | | 15.11 | |
| Balance | 100% | 96.9% | 83.4% | | 80.1% | | 79.1% | |

426 ^a Calculated assuming that 46% of the protein weight is carbon [30]

427



428

429 **Figure 4.** Evolution of the butyric acid flux through the silicone membrane over time
430 during the UASB batch experiments performed at 35°C and at different pH.

431

432 **3.3 Continuous cheese whey fermentation in UASB reactors and VFA extraction**

433 **3.3.1 Effect of in-line VFA extraction on H₂ production**

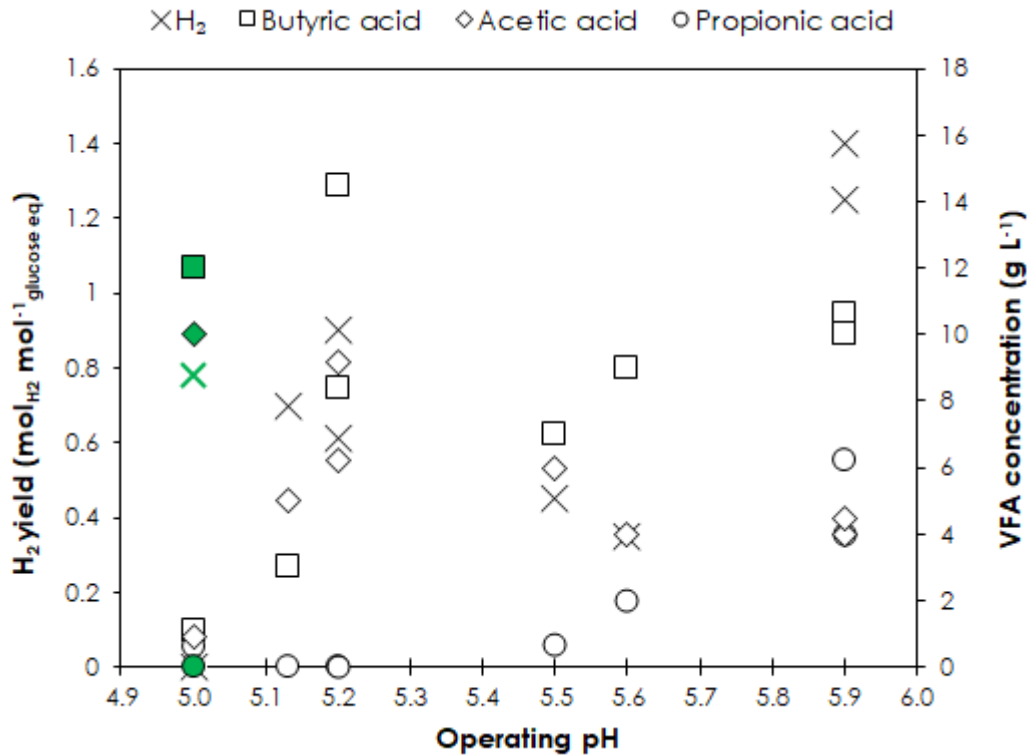
434 After a 5-day start-up in batch, both UASB-A (without extraction module) and UASB-B
435 (with extraction module) were operated in continuous mode, with a HRT of 24 hours,
436 reaching the same maximum HPR of 1.9-2.0 L L⁻¹ d⁻¹ within 20 and 37 days operation,
437 respectively (Fig. 5). This confirms that the use of the *in-line* VFA extraction module
438 implemented in this study had a minimum impact on the achievable H₂ production. The
439 presence of the long silicone spiral (4.2-4.4 m in this study) in the recirculation line
440 may, however, impact the contact time between the substrates and microorganisms,
441 particularly during continuous operation, resulting in a slower onset of the H₂
442 production (Fig. 5). The gas produced by CW fermentation was mainly composed of H₂
443 and CO₂, with a H₂ concentration of 35-37% in UASB-A and 27-28% in UASB-B. The

444 observed difference in gas composition was attributed to the lower solubility of CO₂ (<
445 10⁻⁴ g kg⁻¹) as compared to H₂ (1.6 × 10⁻³ g kg⁻¹) in distilled water (the draw solution) at
446 the low pH (<4) caused by the extracted VFAs. In line with a waste biorefinery
447 approach, the produced CO₂ can be converted to value-added products through algae- or
448 cyanobacteria-based processes [43,44], or microbial electrosynthesis [45].

449

450 Providing a UASB reactor with an extraction module also enabled a more stable
451 process, particularly on days 39-48, as underlined by the HPR values which spanned
452 between 0.8-1.1 L L⁻¹ d⁻¹ in UASB-B, as compared to UASB-A (0.6 and 1.6 L L⁻¹ d⁻¹).
453 The performance of both UASB reactors (average HPR of 1.0-1.3 L L⁻¹ d⁻¹, with peaks
454 of about 2.0 L L⁻¹ d⁻¹, and a highest yield of 0.7-0.8 mol H₂ mol⁻¹ glucose_{eq.}) was
455 remarkable, since it fairly compares with the highest HPR obtained through continuous
456 dark fermentation of CW. Castelló et al. [46] operated a UASB reactor at 30°C and
457 average pH 5, reporting a low HPR of only 0.12 L L⁻¹ d⁻¹ due to the onset of
458 methanogenesis, an issue which did not occur at any stage in the present study.
459 However, methane production cannot be fully excluded over extended operation
460 periods, due to the acclimation of the consortium and the possible presence of
461 favourable micro-environments [37]. A slightly higher average HPR of 1.6 L L⁻¹ d⁻¹ was
462 obtained by Blanco et al. [21], who used a novel structured-bed reactor configuration,
463 operated at pH 5, 25°C and an OLR of 24 g COD L⁻¹ d⁻¹, though fed with synthetic
464 cheese whey. Higher HPRs can be potentially obtained at a pH close to 6 (Fig. 5), but
465 this will result in a much lower VFA recovery through the silicone membrane due to
466 acid dissociation. For instance, high HPRs (28 and 25 L L⁻¹ d⁻¹) have been obtained by
467 CW powder fermentation at pH 5.9, when applying an OLR of 150-160 g_{COD} L⁻¹ d⁻¹

468 [20,47], more than three times higher than the OLR applied in this study. However, at
 469 pH 5.9, only a small fraction (8%) of the butyric acid is undissociated, making *in-line*
 470 extraction through the silicone membrane ineffective.

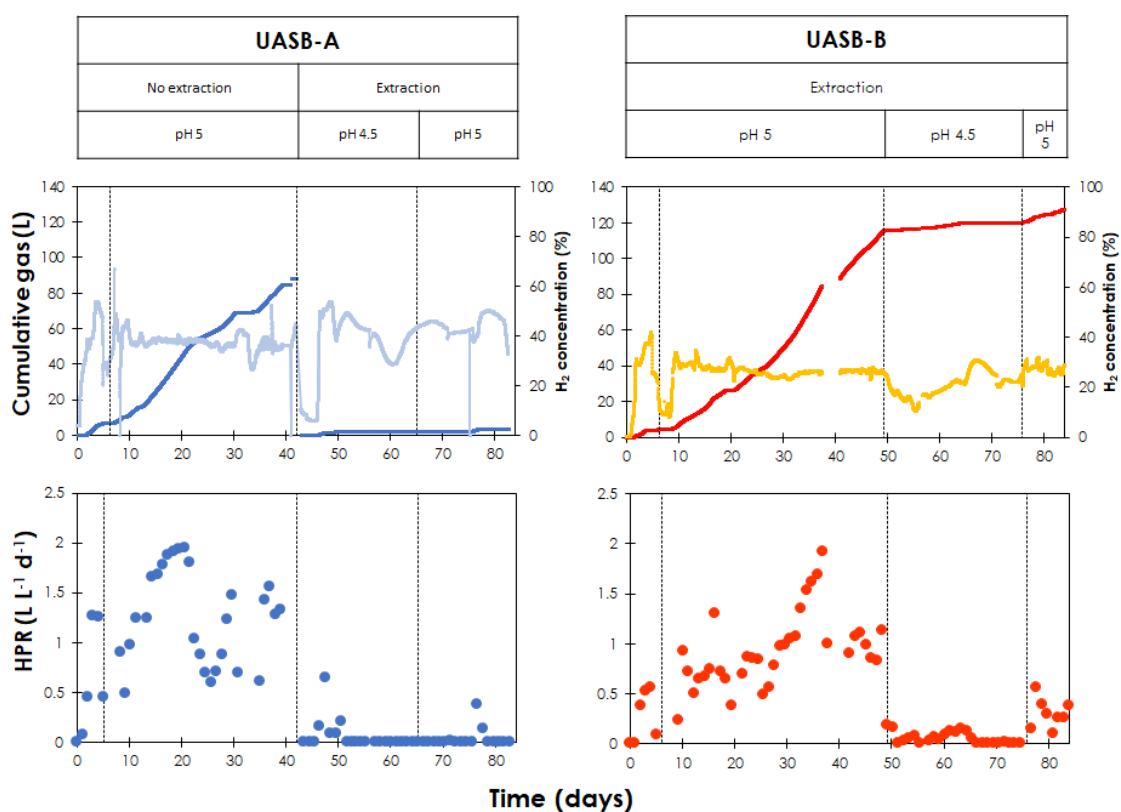


471
 472 **Figure 5.** Highest H₂ yield and VFA concentrations obtained by fermentation of cheese
 473 whey or cheese whey powder under mesophilic conditions at different controlled pH, as
 474 reported in references [20,21,32,46–50]. The green values refer to the present study.

475
 476 **3.3.2 Effect of pH on H₂ production**

477 Since a low pH is preferable for VFAs extraction through the silicone membrane (Fig.
 478 4), two different strategies were attempted to adapt the microorganisms to ferment CW
 479 at low pH. On day 42, operation of UASB-A was stopped, and the reactor was restarted
 480 at pH 4.5 with fresh inoculum, whereas the pH of UASB-B was decreased from 5.0 to
 481 4.5 on day 49. Both UASB reactors were operated with in-line VFAs extraction during

482 this stage (Table 2). In both reactors, the low pH caused a HPR below $0.2 \text{ L L}^{-1} \text{ d}^{-1}$,
 483 substantially lower than those obtained at pH 5.0 (Fig. 6). Furthermore, in UASB-A, a
 484 consistent H_2 production was not achieved even after raising the pH to 5.0 (on day 65),
 485 whereas the H_2 production was resumed in UASB-B, though with an average HPR of
 486 only $0.4 \text{ L L}^{-1} \text{ d}^{-1}$ during days 75-84 (Fig. 6). This suggests that the microbial
 487 community enriched at pH 5.0 was resilient, and able to resume the H_2 production after
 488 a pH shock, but was unable to fully restore its productivity in the short term.
 489



490

491 **Figure 6.** Cumulative gas production (primary axis, blue or red), H_2 concentration
 492 (secondary axis, light blue or orange) and daily average H_2 production rate (HPR) of the
 493 two UASB reactors throughout the experiment. The experimental stages, separated by
 494 the vertical dotted lines, refer to Table 2. In UASB-A, gas production stopped on days

495 31-34 due to influent pump failure. For UASB-B, data are missing on days 37-39 due to
496 a sensor failure.

497

498 3.3.3 Cheese whey fermentation pathways under different operation conditions

499 During continuous operation, a partial conversion of lactose to lactic acid occurred
500 already in the supply tank, despite it was regularly cleaned, re-supplied with fresh CW
501 and maintained at 4-6°C. This led to an influent lactose concentration of 10-30 g L⁻¹ or
502 even lower (Fig. 7), against 41.7 g L⁻¹ in the fresh CW (Table 1). Partial acidification of
503 the CW (average acidification degree of 20%) resulted in an average pH of 4.5 ± 0.3,
504 and an OLR ranging between 40 and 80 g_{COD} L⁻¹ d⁻¹, or even lower (Fig. 7). Ethanol
505 was produced from day 28 in the supply tank, and its concentration reached 10-15 g L⁻¹
506 on day 65, suggesting the onset of heterolactic fermentation. Although the variability of
507 the influent characteristics affected the execution of the experimental tests, it is
508 unavoidable and also occurs in full-scale applications. This issue can be mitigated by
509 minimising preliminary storage prior to fermentation and keeping the distance between
510 the dairy factories where CW is produced and the treatment plant as short as possible.

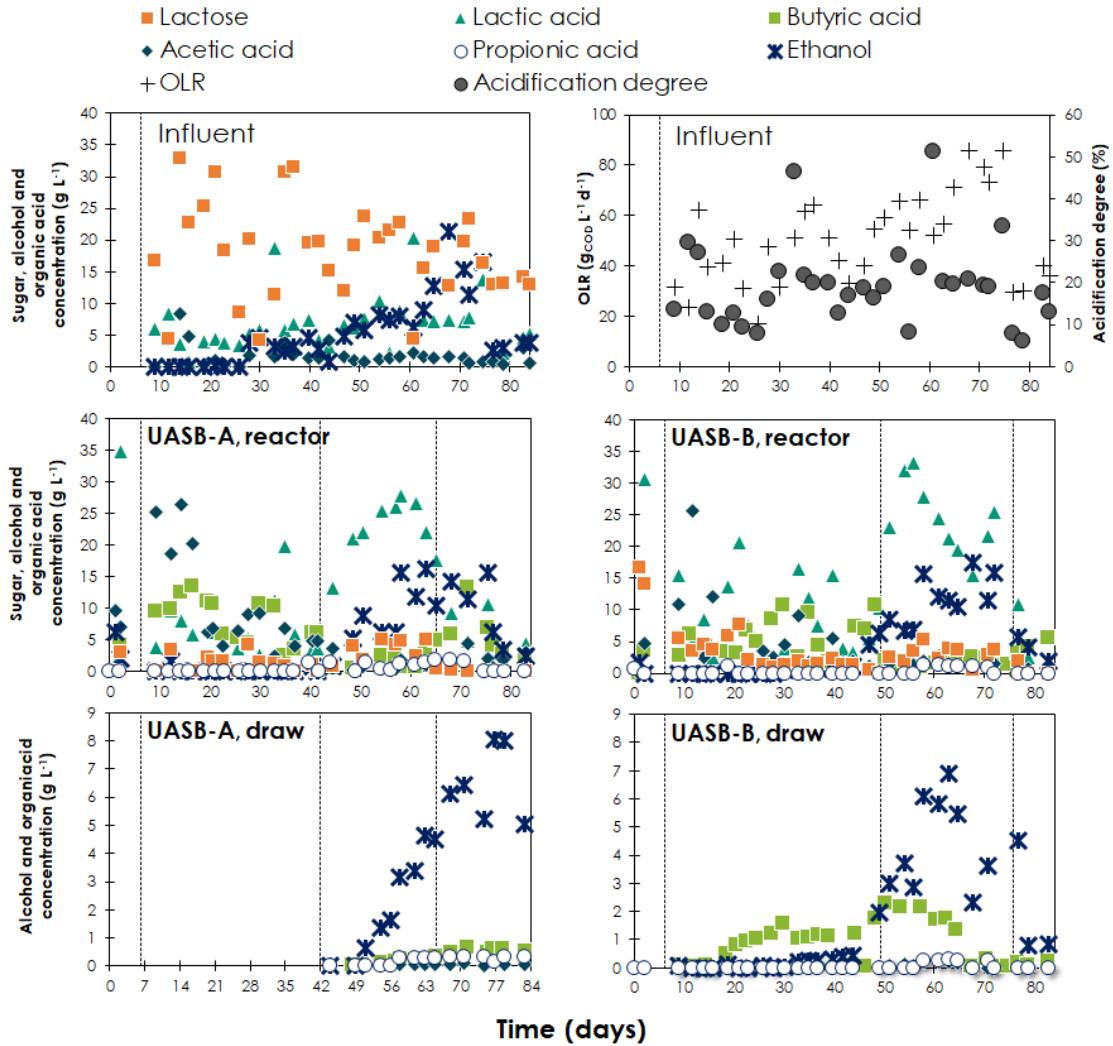
511

512 During the first ten days of continuous operation at pH 5.0 (day 6-16), acetic acid was
513 the main metabolite produced in both UASB reactors, reaching concentrations up to 25
514 g L⁻¹ (Fig. 7), which subsequently decreased to <10 g L⁻¹. After this initial stage,
515 butyric acid was the main VFA produced at pH 5.0 in both UASB reactors, with
516 fluctuating concentrations (between 5 and 12 g L⁻¹) due to the unstable influent lactose
517 concentration (Fig. 7). The achieved butyric acid concentration of 12 g L⁻¹ is among the
518 highest reported in literature (Fig. 5). The trend of butyric acid concentrations reflects

519 that of the H₂ production (Fig. 6), suggesting that H₂ was mainly produced via lactic
520 acid conversion to butyric acid, as previously reported [10,20]. In both UASB reactors,
521 when operated under continuous mode at pH 5.0, over 80% of the influent sugars were
522 consumed, whereas lactic acid conversion was incomplete resulting in an average
523 residual concentration of 5.9 and 8.7 g L⁻¹ in UASB-A and UASB-B, respectively (Fig.
524 7). Longer HRTs may allow a full sugar and lactic acid conversion to VFAs, but this
525 will cause an increase of the required reactor volume, resulting in higher costs, probably
526 not balanced by the advantages that can be obtained.

527

528 A drastic decrease in butyric acid production, along with H₂ production, was observed
529 when both UASB reactors were operated at pH 4.5, although the sugar consumption
530 remained over 80% (Fig. 7). Most sugars were indeed converted to lactic acid, which
531 accumulated up to 25-30 g L⁻¹, but further conversion of lactic acid to VFAs was
532 inhibited by the low pH, resulting in VFAs concentrations below 2 g L⁻¹. Interestingly,
533 at pH 4.5, the ethanol concentration increased up to 15 g L⁻¹ in both UASB reactors,
534 besides being produced already in the supply tank (Fig. 7). When the pH was increased
535 back from 4.5 to 5.0, the lactic acid concentration immediately decreased to < 10 g L⁻¹
536 in both UASB reactors, and the butyric acid concentration increased back to about 5 g
537 L⁻¹.



539

540 **Figure 7.** Sugar, alcohol, and carboxylic acid concentration in the influent, the two
 541 UASB reactor effluents and the respective draw solutions during the continuous
 542 experiment. The organic loading rate (OLR) and acidification degree of the influent are
 543 also reported. The experimental stages, separated by the vertical dotted lines, refer to
 544 Table 2.

545

546 *3.3.4 Continuous VFA extraction through silicone membrane*

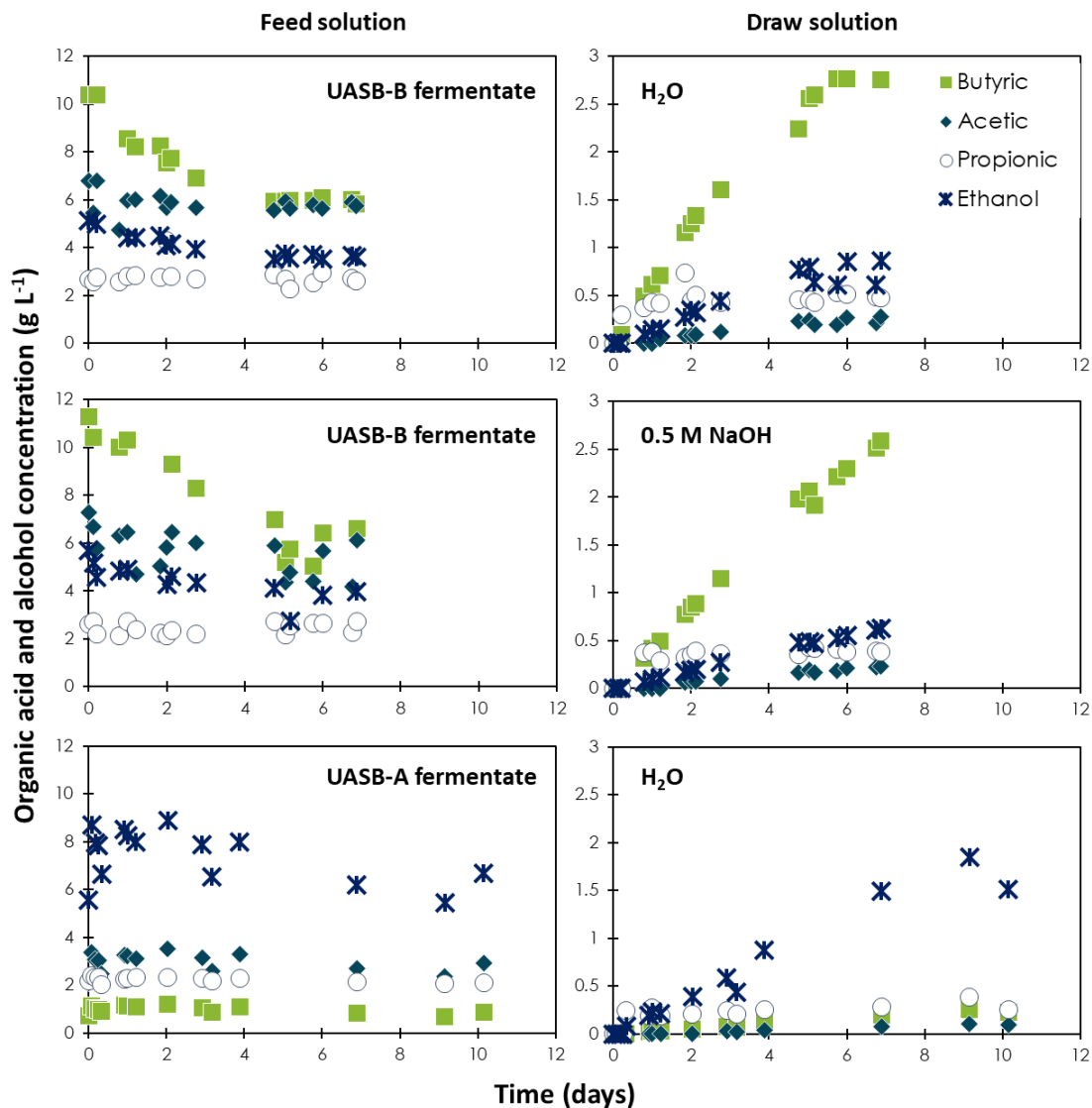
547 In UASB-B, butyric acid was extracted at pH 5.0 up to a concentration of 1.0-1.5 g L⁻¹
548 within 7 days, confirming the results obtained under batch conditions. The extraction of
549 butyric acid benefited from the decrease of fermentation pH down to 4.5, which enabled
550 to reach concentrations up to 2.2 g L⁻¹ in the draw solution on days 54-58. However,
551 starting from day 59, the butyric acid extraction was affected by the low production in
552 UASB-B, which caused an inversion of the concentration gradient (Fig. 7). When the
553 UASB reactors were operated at pH 4.5, ethanol was produced in both UASB reactors
554 and extracted up to a concentration of 7 g L⁻¹ (Fig. 7), suggesting that non-porous
555 silicone membranes can be used for alcohol extraction as well. Unlike carboxylic acids,
556 alcohols do not dissociate in water, and the extraction is therefore not affected by the
557 pH.

558

559 *3.4 Off-line VFAs and alcohol extraction from cheese whey fermentate*

560 The effluents of UASB-A and UASB-B were collected on day 71, while the reactors
561 were operated at pH 4.5 and 5.0, respectively, and tested for off-line VFAs and alcohol
562 extraction upon acidification (pH 3.0). The fermentate of UASB-B **mainly consisted** of
563 butyric acid (10.8 ± 0.4 g L⁻¹), **with** lower concentrations of ethanol (5.4 ± 0.3 g L⁻¹) and
564 acetic (7.0 ± 0.2 g L⁻¹), propionic (2.7 ± 0.0 g L⁻¹) and lactic (2.9 ± 0.1 g L⁻¹) acid,
565 whereas the fermentate of UASB-A contained mainly lactic acid (19.4 ± 1.1 g L⁻¹) and
566 ethanol (5.9 ± 0.4 g L⁻¹) with lower concentrations of acetic (2.2 ± 0.1 g L⁻¹), propionic
567 (2.2 ± 0.0 g L⁻¹) and butyric (0.8 ± 0.0 g L⁻¹) acid.

568



569

570 **Figure 8.** Off-line VFAs and alcohol extraction from the UASB reactor effluents,
 571 acidified to pH 3.0, via silicone membrane pertraction at 20°C. The UASB-A and
 572 UASB-B fermentate was collected while the reactors were operated at pH 4.5 and 5.0,
 573 respectively. Lactic acid data are omitted since it was not detected in the draw solutions.

574

575 Acidifying the fermentate to pH 3.0, and, in turn, increasing the share of undissociated
 576 VFAs, fostered their diffusion rate through the silicone membrane. Butyric acid was
 577 extracted with a maximum flux of $0.53 \text{ g m}^{-2} \text{ h}^{-1}$, exceeding the concentration of 2 g L^{-1}

578 in the draw solution within 5 days (Fig. 8). Indeed, at pH 3.0, over 99% of the butyric
579 acid is in undissociated form, as compared to the 40% undissociated at pH 5.0. On the
580 other hand, by decreasing the pH the diffusion of acetic and propionic acid is favoured
581 as well, leading to a lower purity of butyric acid in the draw solution (65.4% and 69.2%
582 on carbon content basis, using water and 0.5 M NaOH, respectively, as draw solution).
583 In contrast, an over 90% purity was observed in the experiments performed at pH 5.0
584 and 4.5 (Fig. 3).

585

586 Using NaOH as the draw solution resulted in a slower VFAs migration (maximum flux
587 of $0.26 \text{ g d}^{-1} \text{ h}^{-1}$), but a concentration gradient was maintained due to the dissociation of
588 the extracted VFAs, and the subsequent formation of sodium salts. In contrast, a plateau
589 was reached when pure water was used as the draw solution (Fig. 8). The overall mass
590 transfer coefficient (K_{OV}) of butyric acid from the UASB-B fermentate was 0.109 and
591 $0.101 \text{ } \mu\text{m s}^{-1}$ using, respectively, water and NaOH as the draw solution. Those values
592 are comparable to the K_{OV} of $0.157 \text{ } \mu\text{m s}^{-1}$ previously obtained for butyric acid
593 extraction from acidified fish fermentate using silicone membrane [26].

594

595 Despite the high lactic acid concentration (19.4 g L^{-1}) in the UASB-A fermentate, 85%
596 of which in undissociated form at pH 3, lactic acid did not migrate through the
597 membrane, resulting in concentrations below the detection limit in the draw solution.
598 This is due to its low volatility (Henry constant $9.6 \times 10^{-9} \text{ atm m}^{-3} \text{ mol}^{-1}$) and solubility in
599 water, whereas the more volatile ethanol (Henry constant $5.0 \times 10^{-6} \text{ atm m}^{-3} \text{ mol}^{-1}$)
600 migrated with a K_{OV} of $0.083\text{-}0.096 \text{ } \mu\text{m s}^{-1}$.

601

602 **3.5 Future research directions**

603 Despite the promising results obtained in this study, more research efforts are required
604 to advance the technology readiness level of the integrated process. Further studies
605 should focus on membrane characteristics (material, length, and thickness) and process
606 operating parameters (pH, temperature, and recirculation flow). A second process to be
607 downstream implemented, e.g. electrodialysis-based technologies [42], is required to
608 concentrate the VFAs extracted and, at the same time, to avoid their accumulation in the
609 draw solution, keeping a sufficient concentration gradient between the fermentation
610 broth and the draw solution to allow VFA migration. Enhancing in-line VFA extraction
611 further reduces their toxic effect on the microorganisms, and thus positively affects H₂
612 yields [42], resulting in further economic benefits.

613

614 **4. Conclusions**

615 This study proposes a novel approach for cheese whey valorisation that can also be
616 applied to other waste streams, where dark fermentation is combined to a relatively low-
617 cost extraction process to obtain H₂ and high-purity butyric acid. This study showed
618 that:

619

- 620 • HPR up to 2.0 L L⁻¹ d⁻¹ can be obtained by fermenting cheese whey at pH 5.0;
- 621 • Non-porous silicone membranes favours extraction of longer chain fatty acids
622 over short chain acids, which is a unique advantage for processes generating
623 VFA mixtures;
- 624 • Up to 3 g L⁻¹ of high purity (>90% on carbon content basis) butyric acid can be
625 in-line extracted without affecting the steady-state H₂ production, decreasing the

626 NaOH requirement and saving the energy which is otherwise required for
627 extraction;

628 • Ethanol can be extracted using silicone membranes, whereas sugars and
629 nutrients are retained;

630 • Low pH values increase VFAs extraction rate in silicone membrane pertraction,
631 but drastically decrease the HPR and can negatively affect the selectivity of the
632 extraction process.

633

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642

643 **Conflict of interest**

644 The authors declare no conflict of interest.

645

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