Elsevier Editorial System(tm) for
International Journal of Hydrogen Energy
Manuscript Draft

Manuscript Number:

Title: Fermentative hydrogen production from cheese whey with in-line, concentration gradient-driven butyric acid extraction

Article Type: Full Length Article

Section/Category: Bio Hydrogen / Bio Gasification

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

Corresponding Author: Dr. Fabiano Asunis, Ph.D.

Corresponding Author's Institution: University of Cagliari

First Author: Paolo Dessì, Ph.D.

Order of Authors: Paolo Dessì, Ph.D.; Fabiano Asunis, Ph.D.; Harish Ravishankar, Ph.D.; Francesco G Cocco, M.Sc.; Giorgia De Gioannis, Ph.D.; Aldo Muntoni, Ph.D.; Piet Lens, Ph.D.

Abstract: Hydrogen generation from cheese whey, with simultaneous production and extraction of volatile fatty acids (VFAs), was studied in UASB reactors. The extraction module was a silicone tube coil submerged in distilled water, which allows concentration-driven VFA extraction . An Higher H2 yield of 0.9 mol H2 mol-1 glucoseeq was obtained in batch assays at 35°C and pH 5.0, regardless of the presence of the extraction module, than at pH 4.5 and 20°C. VFAs crossed the silicone only in the undissociated form, with preference for butyricover propionic and acetic acid. Sugars, lactic acid and nutrients were retained, resulting in an extracted solution of 2.5 g L-1 butyric acid with more than 90% purity. Results of continuous operation confirmed those obtained in batch, with H2 production rates up to 2.0 L H2 L-1 d-1 and effective butyric acid extraction. In-line VFA extraction facilitates downstream processing for VFA recovery, without affecting hydrogen production.

Università degli Studi di Cagliari

DIPARTIMENTO DI INGEGNERIA CIVILE, AMBIENTALE E ARCHITETTURA

DICAAR

To

International Journal of Hydrogen Energy

Editorial office

Dear Editor,

On behalf of co-authors, please find enclosed our manuscript entitled "FERMENTATIVE HYDROGEN PRODUCTION FROM CHEESE WHEY WITH IN-LINE, CONCENTRATION GRADIENT-DRIVEN BUTYRIC ACID EXTRACTION".

The present work proposes a novel approach for resource recovery from cheese whey (CW), where dark fermentation (DF) is coupled to a relatively low-cost extraction process, through a recirculation loop, in order to obtain bio-H_2 and valuable butyric acid from this important agroindustrial biowaste. We investigated such an integrated system, end the effect of key operating parameters such as temperature and pH, in both batch and continuous UASB experiments.

DF of biowastes has been studied extensively during recent decades since it is closely related to the transition towards an innovative approach for biowaste valorisation, the so-called waste biorefinery concept. DF can convert the high amount of CW lactose into bio-H_2 and a mixture of valuable Volatile Fatty Acids (VFAs), as a function of operating parameters adopted. The pool of VFAs can be used in the chemical industry, as building block chemicals or as a feedstock for further biological valorisation, e.g. PHA production or methanization. Nevertheless, the exploitation of such a VFAs requires a cost-effective separation and recovery method, which is among the major bottlenecks that hinder the diffusion of those biobased chemicals as a competitive alternative to the non-renewable counterpart.

The use of silicone membranes has been recently proposed as a cost-effective environmental alternative to other membrane extraction possibilities being the silicone a wide-spread commercial material and because of the use of water as extractant instead of organic solvents.

This work reports that the VFAs produced by DF could be effectively and selectively extracted from the fermentation broth by using silicone membranes without affecting the overall bio-H_2 yields. In particular, the silicone membranes favour the selective extraction of the most hydrophobic CW fermentation product (i.e. butyric acid), retaining in the fermentation broth the other products (i.e. acetic and propionic acid). Sugars and nutrients are also retained by the silicone membrane, resulting in the extracted of butyric acid with >90% purity (on a carbon basis). We consider this a unique advantage for combined bio-H_2 production and VFA recovery from mixtures, compared to competing technologies.

DICAAR, via Marengo, 2- 09123 CAGLIARI

Tel. 070.675. 5520 - email: fabiano.asunis@unica.it

http://dipartimenti.unica.it/ingegneriacivileambientaleearchitettura/

Also, these findings are expected to open up the path for further research aimed at implementing *in-line* VFA extraction in hydrogen-based biorefinery schemes and highly interesting for *International Journal of Hydrogen Energy* readers.

The manuscript's Category is "Bio Hydrogen / Bio Gasification". Being the paper related to a topic of valid and relevant scientific and technical interest, it is appealing to both a scientific and a technical audience.

All the authors mutually agree that the manuscript should be submitted to *International Journal of Hydrogen Energy*. The manuscript is the original work of the authors and it has not been published elsewhere, nor it is currently under consideration for publication elsewhere.

Thank you for your consideration of this manuscript.

With kind regards,

Cagliari, March 20st 2020

Fabiano Asunis

Corresponding author

SUGGESTED REVIEWERS

Peter Bakonyi, PhD

Research Institute on Bioengineering, Membrane Technology and Energetics, University of Pannonia, Veszprém (Hungary)

bakonyip@almos.uni-pannon.hu

Prof. Bakonyi is an expert in gaseous biofuel (in particular hydrogen) fermentation and upgrading by membrane separation technology

Venkata Mohan, PhD

Principal Scientist, Indian Institute of Chemical Technology (India)

vmohan_s@yahoo.com

Dr. Venkata Mohan is an expert in biotechnology and bioenergy.

Jukka Rintala, PhD

Professor, Faculty of Engineering and Natural Sciences, Tampere University (Finland)

jukka.rintala@tuni.fi

Prof. Rintala is an expert on biological process and head of the research group on Bio- and Circular Economy at Tampere University (TAU)

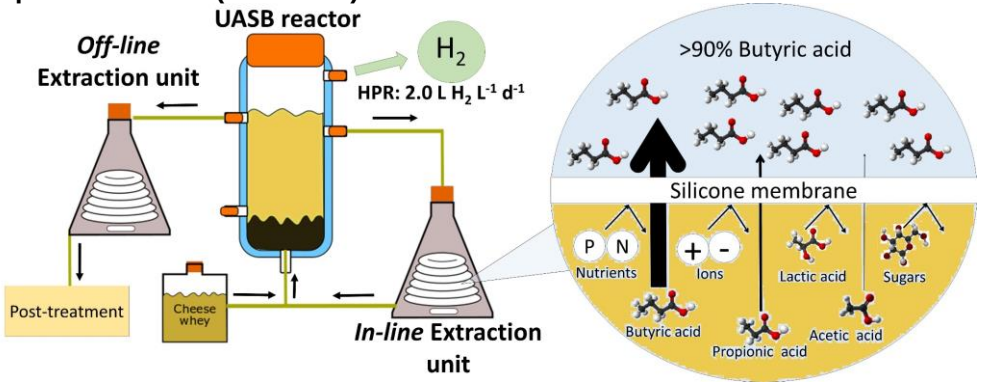
Mohammad Taherzadeh, PhD

Department of Resource Recovery and Building Technology, University of Borås (Sweden)

mohammad.taherzadeh@hb.se

Prof. Taherzadeh is an expert in resource recovery from biowaste and his research is focused on the conversion of waste materials to several products such as ethanol, biogas, and hydrogen.

Graphical Abstract (for review)



Highlights

- Cheese whey fermentation was studied in UASB rectors with in-line VFA extraction
- Butyric acid was selectively extracted from the broth using a silicone membrane
- Sugars, lactic acid, and nutrients are retained by the silicone membrane
- HPR up to 2 L/L/d and butyric extraction up to 2.5 g/L butyric acid were achieved
- Low pH enhances VFA migration through the silicone membrane

*Manuscript
Click here to view linked References

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

Paolo Dessì [a,#], *Fabiano Asunis* [a,b,*,#], *Harish Ravishankar* [a], *Francesco Giuseppe Cocco* [a,b], *Giorgia De Gioannis* [b], *Aldo Muntoni* [b], *Piet N. L. Lens* [a]

[a] *National University of Ireland Galway, University Road, Galway, H91 TK33, Ireland*

[b] *Department of Civil and Environmental Engineering and Architecture, University of Cagliari, Via Marengo 2, 09123 Cagliari, Italy*

[#] *These authors contributed equally to the manuscript*

Manuscript submitted to: *International Journal of Hydrogen Energy*

*****Corresponding author: e-mail: fabiano.asunis@unica.it, mail: Department of Civil and Environmental Engineering and Architecture, University of Cagliari, Via Marengo 2, 09123 Cagliari, Italy

Abstract

Hydrogen generation from cheese whey, with simultaneous production and extraction of volatile fatty acids (VFAs), was studied in UASB reactors. The extraction module was a silicone tube coil submerged in distilled water, which allows concentration-driven VFA extraction. Higher H_2 yield of 0.9 mol H_2 mol^{-1} $glucose_{eq}$ was obtained in batch assays at 35°C and pH 5.0, regardless of the presence of the extraction module, than at pH 4.5 and 20°C. VFAs crossed the silicone only in the undissociated form, with a preference for butyric over propionic and acetic acid. Sugars, lactic acid and nutrients were retained, resulting in an extracted solution of 2.5 g L^{-1} butyric acid with more than 90% purity. Results of continuous operation confirmed those obtained in batch, with H_2 production rates up to 2.0 L H_2 L^{-1} d^{-1} and effective butyric acid extraction. In-line VFA extraction facilitates downstream processing for VFA recovery, without affecting hydrogen production.

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

1. Introduction

The increasing societal need for energy and materials, along with population growth, fossil fuel depletion and growing interest in environmental issues, are driving a global shift towards a sustainable and circular economy. In 2018, an updated bioeconomy strategy has been adopted by the European Union, along with the Paris Agreement commitments, to achieve the sustainable growth and environmental protection goals included in the 2030 agenda [1]. In this view, biodegradable waste streams are proposed

as renewable substrates for energy and chemical production, partially replacing fossil fuels [2,3]. This context encourages production systems to implement the waste biorefinery concept [4], where waste is considered as an opportunity to diversify the product spectrum while reducing the costs of biomass supply and waste treatment, thereby meeting the increasingly stringent legislation on emissions.

The dairy industry processes 170 billion liters of milk per year in Europe [5], generating an average of 2.5 L wastewater per L of milk processed and 9-10 L cheese whey (CW) per kg of cheese produced. When CW is discharged without proper treatment, it can have serious adverse effects on the environment, i.e. rising of eutrophication in water bodies or decreased crop yields and oxygen availability in agricultural land [6]. CW management mainly involves whey protein recovery, animal feeding, or treatment in dedicated wastewater treatment plants, depending on the size of the dairy industry and the production context [7]. However, the high concentration of readily degradable compounds (50-100 g_{COD} L^{-1}, 90% of which in the form of lactose) makes CW an outstanding substrate for biological production of energy and chemical commodities [8], not fully exploited so far. Physicochemical and biological processes can be synergically implemented, according to the waste biorefinery concept, to convert CW to valuable products such as methane [9], hydrogen [10], volatile fatty acids (VFAs) [11], alcohols [12], lactic acid [13], electric energy [14], or bioplastics [15].

Among the suitable processes, dark fermentation is considered the core of a waste biorefinery scheme, as it enables biological simplification and conversion of organic substrates to a carbon-neutral energy carrier (H_2) and building blocks (VFAs) suitable

for downstream applications [3,16]. Since sugars are the preferred substrate for fermentative microorganisms, CW is a substrate of particular interest for dark fermentation. CW fermentation results in H_2 yields typically spanning between 1 and 4 mol mol^{-1} lactose (or 0.5 and 2 mol mol^{-1} $glucose_{eq.}$) depending on the operating conditions such as pH, temperature and organic loading rate [10,17–19].

Besides H_2, up to 20-30 g L^{-1} VFAs, mainly acetic, propionic, and butyric acid are produced through CW fermentation, at different mass proportions depending on the operating parameters, pH in particular [10,11,20]. Interestingly, the operating conditions that foster H_2 production also favour butyric acid production among soluble organic fermentation products [10,21]. Butyric acid finds numerous applications in the chemical, pharmaceutical, perfume, and animal feed sectors [22], with a market size of about 125 M€ (https://www.marketsandmarkets.com/Market-Reports/butyric-acid-market-76962011.html) which is expected to further increase by 15.1% $year^{-1}$, as a response to its approval as a food flavouring agent by the U.S. Food and Drug Administration (FDA) [23]. This already favorable context could further benefit, in the next decade, by the development of the bioplastic sector, as butyric acid is a precursor for polyhydroxyalkanoates (PHA) production [24]. Thus, the development of a process for the combined production of H_2 and butyric acid substantially contributes to a modern and environmentally sustainable CW management.

Several technologies are available for VFAs extraction, including physical (nanofiltration, liquid-liquid extraction, vapour permeation, membrane contactors, gas stripping and distillation), chemical (adsorption and solvent extraction) and

electrochemical (electrodialysis) methods [23,25]. However, the development of a low-cost system to selectively extract the target compound from a VFAs mixture is still a challenge. Outram and Zhang (2018) recently showed that concentration-gradient-driven liquid-liquid extraction (pertraction) through a non-porous silicone membrane, using distilled water as the draw solution, can be applied to recover VFAs. Furthermore, it was shown that longer-chain VFAs migrate faster than shorter-chain VFAs through the silicone membrane due to their higher hydrophobicity [26]. This represents a remarkable feature, as it would enable the selective extraction of butyric acid over other typical CW fermentation products (i.e. acetic, propionic and lactic acid).

The present study aimed to study the performance of a novel reactor concept for simultaneous H_2 and butyric acid recovery from CW, where an *in-line* silicone membrane extraction module is implemented into a fermentative UASB reactor through a recirculation loop. First, inoculum and upflow velocity were optimised for H_2 and butyric acid production. Then, the effects of pH (5.0 *vs.* 4.5) and temperature (35 *vs.* 20 °C) on H_2 production and butyric acid recovery were evaluated in the UASB operated either under batch or continuous mode. Finally, the extraction efficiencies achieved were compared to those obtained by operating an *off-line* butyric acid extraction system fed with the fermentative UASB effluent.

2. Materials and methods

2.1 Source of inoculum and pretreatment

The inoculum used in this study was either activated or digested sludge from the wastewater treatment plant of a dairy industry (Dairygold, Mitchelstown, Ireland). The

activated and digested sludge had a total solids concentration of 42.7 ± 0.8 and 66.0 ± 3.0 g L^{-1}, and a volatile solids concentration of 24.8 ± 0.4 and 49.8 ± 2.6 g L^{-1}, respectively. Heat pretreatment was done by heating thin tubes containing 5 mL of sludge in a dry bath (Fisher Scientific) at 90°C for 15 minutes.

2.2 Synthetic medium and cheese whey composition

The synthetic medium used for inoculum screening was the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) medium nr. 141 with the following modifications: lactose (10 g L^{-1}) was used instead of glucose as the substrate, and yeast extract, tryptone, resazurine and Na_2S were not added. CW from cow milk processing was collected from the mentioned dairy industry, stored at -20°C after transportation to the lab, and defrosted to 4°C 24 hours before utilization to prevent acidification. The CW composition was as specified in Table 1.

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

2.3 Inoculum screening

Four inocula, i.e. activated or digested sludge with or without heat shock pretreatment were compared for H_2 production from lactose in a preliminary batch experiment. The experiment was conducted in triplicate 120 mL serum bottles with 48 mL of synthetic medium and 2 mL of each inoculum. The initial pH was adjusted to 7.0 using 1 M NaOH solution. Abiotic (without inoculum) and no-substrate (without lactose) controls were also prepared. The bottles were sparged with N_2 for 5 min prior to incubation at 35°C for about 17 days with 150 rpm shaking in an orbital shaker incubator (ThermoScientific MaxQ 8000).

2.4 Effect of upflow velocity on hydrogen production from CW

The effect of the upflow velocity on hydrogen production was studied in 1 L recirculated UASB reactors operated in batch mode. The UASB reactor was maintained at 35°C using a water bath with recirculation (Grant Tc120, UK). A controller (Cole-Parmer 300, USA) connected to a pH probe (VWR, USA) and a peristaltic pump (Verdeflex, The Netherlands) were used to keep the pH above 5.0 in the UASB reactor by addition of 5M NaOH from a bottle under N_2 atmosphere. After sparging with N_2, the reactors were fed with 700 mL CW using a peristaltic pump (Masterflex L/S, Cole-Parmer, USA). Heat-treated digested sludge (4%) was added as inoculum from a sampling port. The CW was recirculated from the top to the bottom of the bioreactor, using a peristaltic pump (Masterflex), to achieve an upflow velocity of 0.1, 0.5, 1.0 or 2.0 m h^{-1}. The gas produced was collected in a gas bag, and the batch experiments were

158 stopped when no H_2 production was observed anymore for at least 3 consecutive days
159 (after 8-11 days fermentation).
160
161 ***2.5 Batch experiments with in-line VFAs extraction***
162 UASB reactors, with the same configuration as the previous experiment, were used for
163 evaluating the effect of *in-line* VFAs extraction on CW fermentation at different
164 temperature (20 and 35°C) and pH (4.5 and 5.0) using heat-treated digested sludge as
165 inoculum and an upflow velocity of 1.0 m h^{-1}. The VFAs extraction module included a
166 silicone tube coil (2 and 4 mm internal and external diameter, VWR, The Netherlands)
167 with a total length of 4.2-4.4 m, submerged into 700 mL distilled water (draw solution)
168 in a conical 1 L flask. The flask was sealed at the top with a rubber stopper and
169 connected to the gas line outlet (Fig. 1) to recover the gas flowing through the silicone
170 membrane. The extraction module was installed to the UASB reactor through a
171 recirculation loop. One UASB reactor was operated without *in-line* extraction as
172 control. Since the working volume increased due to the addition of the extraction unit,
173 the UASB reactors were fed with 830-850 mL of CW as compared to the 700 mL of the
174 preliminary experiment on the up-flow velocity (section 2.4).
175
176 ***2.6 Continuous experiment with in-line VFA extraction***
177 For the continuous experiments, an influent supply tank, kept at 4-6°C inside a fridge,
178 was connected to two UASB reactors through a pump (Masterflex), and an on-line gas
179 monitoring system composed by a V-count gas counter and H_2 and CO_2 sensors
180 (BlueSens, Germany) was installed (Fig. 1). Two UASB reactors (namely, UASB-A
181 and UASB-B) were run in parallel, according to the experimental stages reported in

Table 2. After a 5-day start-up in batch mode, CW was fed continuously at 24 hours hydraulic retention time (HRT) to compare the performance of the UASB reactors in the presence and absence of the extraction module, and then to study the response of the integrated system to pH changes.

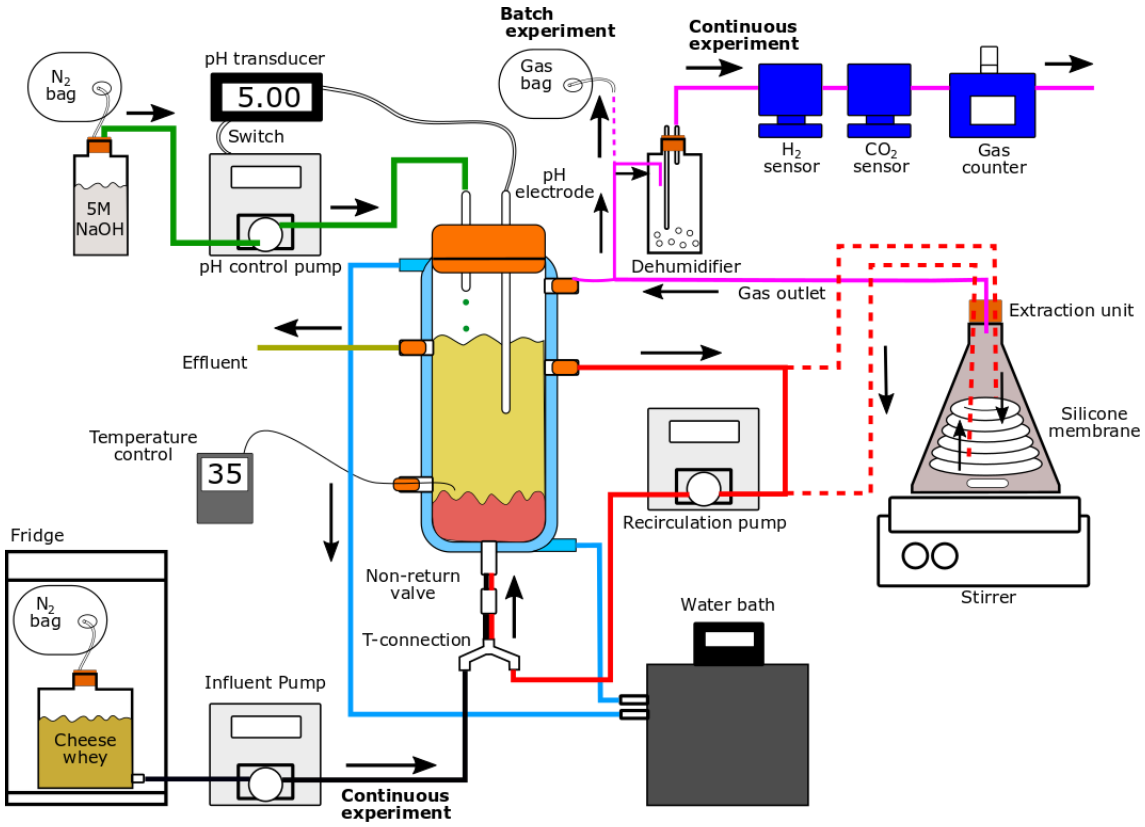


Figure 1. UASB reactor configuration adopted for the experiments in batch and continuous operation mode. The coloured lines represent the influent (black, only for the continuous experiment), recirculation (red), effluent (yellow), pH control (green), water jacket (blue) and gas (magenta) lines.

Table 2. Overview of the UASB reactor operation with the experiments in continuous 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

2.7 Off-line extraction experiment

Fermentate from both UASB reactors, when operated in continuous mode at pH 5.0 and 4.5, respectively, was collected on day 71 and used for *off-line* extraction tests in batch. The fermentate was acidified to pH 3 by HCl addition before starting the experiment. A flask containing CW fermentate (500 mL) was connected through a pump to the extraction module containing either 500 mL distilled water or 0.5 M NaOH as the draw solution through a recirculation loop. The recirculation flow was 21 mL min^{-1}, the same applied to the UASB reactor to obtain an upflow velocity of 1.0 m h^{-1}.

2.8 Monitoring and analytical methods

Gas produced during the inoculum screening tests was quantified using the syringe method [27]. Gas produced during the UASB batch tests, including the gas diffusing through the silicone membrane (Fig. 1), was collected in 5 L gas bags and measured using the water displacement method. For all the batch experiments, gas samples (5 mL) were collected either from the headspace of the serum bottles or from the gas bags and stored in 5.9 mL gas collection vials (Exetainer®, Labco, UK) at ambient temperature for analysis. Gas composition (H_2, CH_4 and CO_2) was analysed using a gas chromatograph (GC) system (Agilent 7890A, USA) equipped with a thermal

conductivity detector (TCD) and an 80/100 Hayesep Q column. Argon was the carrier gas with a flow of 24 mL/min, and oven, injector and detector were kept at 90, 90 and 200°C, respectively. For the continuous experiment, both gas lines were connected to the on-line monitoring sensors and gas counter (BlueSens, Germany).

Liquid samples were collected from the serum bottles (2 mL), from a sampling port in the recirculation tube of the UASB reactors (4 mL), and a sampling tube submerged in the draw solution (2 mL), and stored at -20°C in plastic tubes for analysis. Sugars, carboxylic acid and alcohol concentrations in liquid samples were analysed using a liquid chromatograph (LC) (1260 Infinity II, Agilent, USA) equipped with a refractive index detector (RID) and a Hi-Plex H column. The mobile phase was H_2SO_4 (5 mM) at a flow rate of 0.7 mL min^{-1}. Total dissolved saccharides were measured using a phenol-sulphuric colorimetric method [28] with a spectrophotometer (Shimadzu UV-1900, Japan) at 485 nm.

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

proteins were measured using a Nutrient analyser (Gallery Plus, Thermo Scientific, USA).

2.9 Calculations

The modified Gompertz model was applied as reported in Asunis et al. (2019). Carbon balances were made based on the carbon content of liquid and gas products detected. Carbon content of 46% was assumed for proteins [30]. The organic loading rate was calculated based on COD. The acidification degree was calculated according to Bengtsson et al. (2008). Fluxes and mass transfer coefficients (K_{OV}) were calculated according to Outram and Zhang (2018).

3. Results and discussion

3.1 Inoculum screening and optimal up-flow velocity

When incubated in batch with the lactose-containing synthetic medium, heat-treated digested sludge gave a significantly higher H_2 (0.92 ± 0.38 mol mol^{-1} $glucose_{eq.}$) and butyric acid (0.27 ± 0.12 mol mol^{-1} $glucose_{eq.}$) yields than the other inocula tested, i.e. non-treated digested sludge, and both treated and non-treated activated sludge (Table S1). Thus, heat-treated digested sludge was selected as the inoculum for all follow-up experiments.

A remarkable effect on the H_2 production was observed for the different upflow velocities tested (0.1, 0.5, 1.0 and 2.0 m h^{-1}) in recirculated UASB reactors operated in batch mode. Upflow velocities of 1.0 and 2.0 m h^{-1} resulted in an H_2 yield of about 1.0-1.1 mol mol^{-1} $glucose_{eq.}$, 40 and 60% higher than the yields obtained at 0.5 and 0.1 m h^{-}

^{1}, respectively (Fig. S1, Table S2), as a result of the higher mixing and gas stripping from the fermentation broth. Based on these results, an upflow velocity of 1.0 m h^{-1} was selected for further experiments.

3.2 Batch cheese whey fermentation in UASB reactors and VFA extraction

3.2.1 Effect of in-line VFAs extraction, pH and temperature on hydrogen production

Similar yields of about 0.9 mol H_2 $mol^{-1}glucose_{eq.}$ and maximum production rates of about 0.26 mol H_2 mol^{-1} $glucose_{eq.}$ d^{-1} (Fig. 2; Table 3) were observed in the UASB reactors operated in batch at 35°C and pH 5.0 with and without the *in-line* VFA separation module. Therefore, the VFAs extraction module had a minimum impact on CW fermentation in UASB reactors. The results were also similar to those obtained in the preliminary test at upflow velocities of 1.0 and 2.0 m h^{-1} (Fig. S1), confirming the replicability of the fermentation process. This is further confirmed by the fact that the obtained H_2 yield was comparable to the results achieved in previous studies on CW fermentation [10,32].

Decreasing the operating temperature to 20°C, or pH to 4.5, resulted in a lower H_2 yield of 0.36 and 0.49 mol H_2 mol^{-1} $glucose_{eq.}$, respectively. The fermentation kinetics of CW, and in particular lactose hydrolysis, are indeed slower at lower temperatures [33]. At pH 4.5, despite the relatively fast kinetics (0.35 mol H_2 mol^{-1} $glucose_{eq.}$ d^{-1}) and short lag-phase (1.0 d) (Table 3), H_2 production was likely inhibited by the accumulation of butyric acid in its undissociated form (7.6 g L^{-1}, 68% of the total), which can penetrate the bacterial cells suppressing growth and metabolic activity [34]. It is worth mentioning that H_2 yields obtained from CW-based substrates using mixed cultures at

pH below 5.0 are typically low, although an H_2 yield of 1.83 mol H_2 mol^{-1} $glucose_{eq.}$ was reported from a diluted CW powder solution (4.9 g lactose L^{-1}) in a fluidized bed reactor operated at pH 4.0-4.5 under thermophilic conditions (55°C) [19].

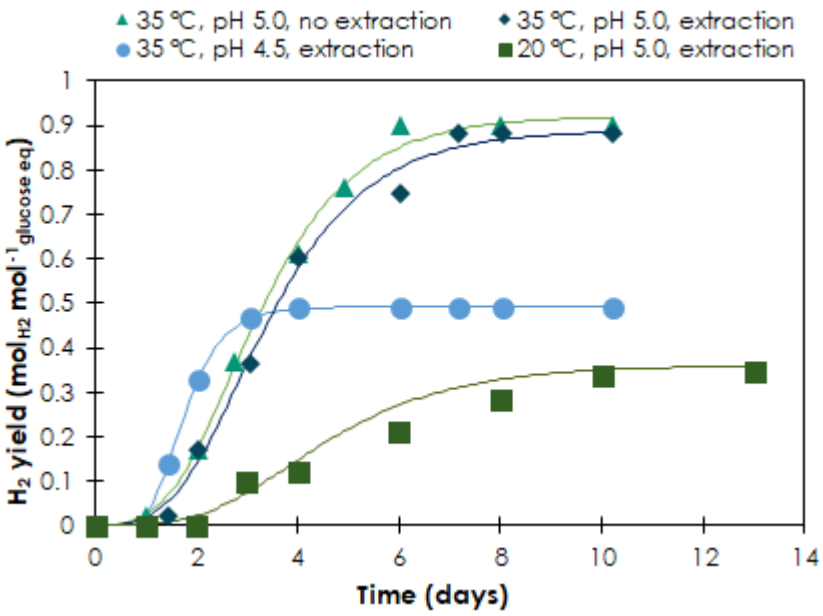


Figure 2. Evolution of H_2 yields overtime for the UASB fermentation tests performed at 20 and 35°C, pH 5.0 and 4.5, with or without *in-line* VFA extraction. Scatter plots represent the experimental data, and continuous lines represent the Gompertz model fitting.

Table 3. Gompertz model parameters as calculated for the fermentation tests performed under 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
$t_{95\text{-}H2}$	d	6.800	7.000	3.100	9.100
R^2	-	0.996	0.994	1.000	0.981

299 *3.2.2 Effect of in-line VFA extraction, pH and temperature on fermentation pathways*

300 In all conditions tested, fermentation evolved according to three subsequent degradation
301 stages. Lactose was first hydrolysed to glucose at different rates depending on the
302 operating conditions, and then converted to lactic acid via homolactic fermentation.
303 Galactose, the other monomeric sugar expected from lactose hydrolysis, was always
304 below detection, suggesting its rapid conversion to glucose 6-phosphate, since there is
305 no catabolic pathway to metabolize it [35]. Lactic acid was then converted to H_2, CO_2
306 and VFAs, with a prevalence of butyric acid which was produced up to 15 and 20 g L^{-1}
307 regardless of temperature and pH. The full conversion of lactic acid to VFAs was
308 achieved at pH 5.0, within 6-8 days at 35°C and around 10 days at 20°C, whilst the same
309 fate was not observed at pH 4.5, likely due to inhibition of the fermentative
310 microorganisms [34].
311
312 In both UASB reactor tests at 35°C and pH 5.0, the acetic acid concentration increased
313 after 6-8 operation days (Fig. 3), suggesting the onset of homoacetogenic pathways with
314 related negative effects on the hydrogen yields [36]. Propionic acid was produced in all
315 tests at pH 5.0, resulting in a final concentration of 4-5 g L^{-1}, whereas significant
316 ethanol production, up to 5 g L^{-1}, was obtained only at 20°C, suggesting a shift from
317 homolactic to heterolactic fermentation. In this case, the overlapping pathways may
318 have been caused by slower sugar consumption rates due to the lower temperature [37].
319
320 Under all the conditions investigated, the pH dropped from the initial value of 6.3 to
321 either 5.0 or 4.5 within 1-2 days (at 35°C) or 3 days (at 20°C), and a further decrease
322 was avoided only by automatic NaOH dosing. In the UASB reactors operated at pH 5.0,

once the sugars were fully consumed, the pH raised again likely due to protein hydrolysis [38], and the consequent ammonium release. Indeed, about 430 and 460 mg L^{-1} ammonium was found upon CW fermentation at 20 and 35°C, respectively, against the 83 mg L^{-1} detected in the CW prior to fermentation (Table 1). The pH increase was more clear in the UASB reactor with the extraction module, due to the VFAs crossover through the silicone membrane, resulting in a final pH of 6.5 as compared to a pH of 6.2 observed in the UASB reactor without extraction module (Fig. 3). As an interesting consequence, a 25% lower NaOH dosage (9.0 g L^{-1} CW) was required in the UASB reactor provided with the extraction module than in the UASB reactor where VFAs were not extracted (12.0 g L^{-1} CW) to maintain pH values above 5.0, which, in turn, significantly reduces the operating costs in full-scale application.

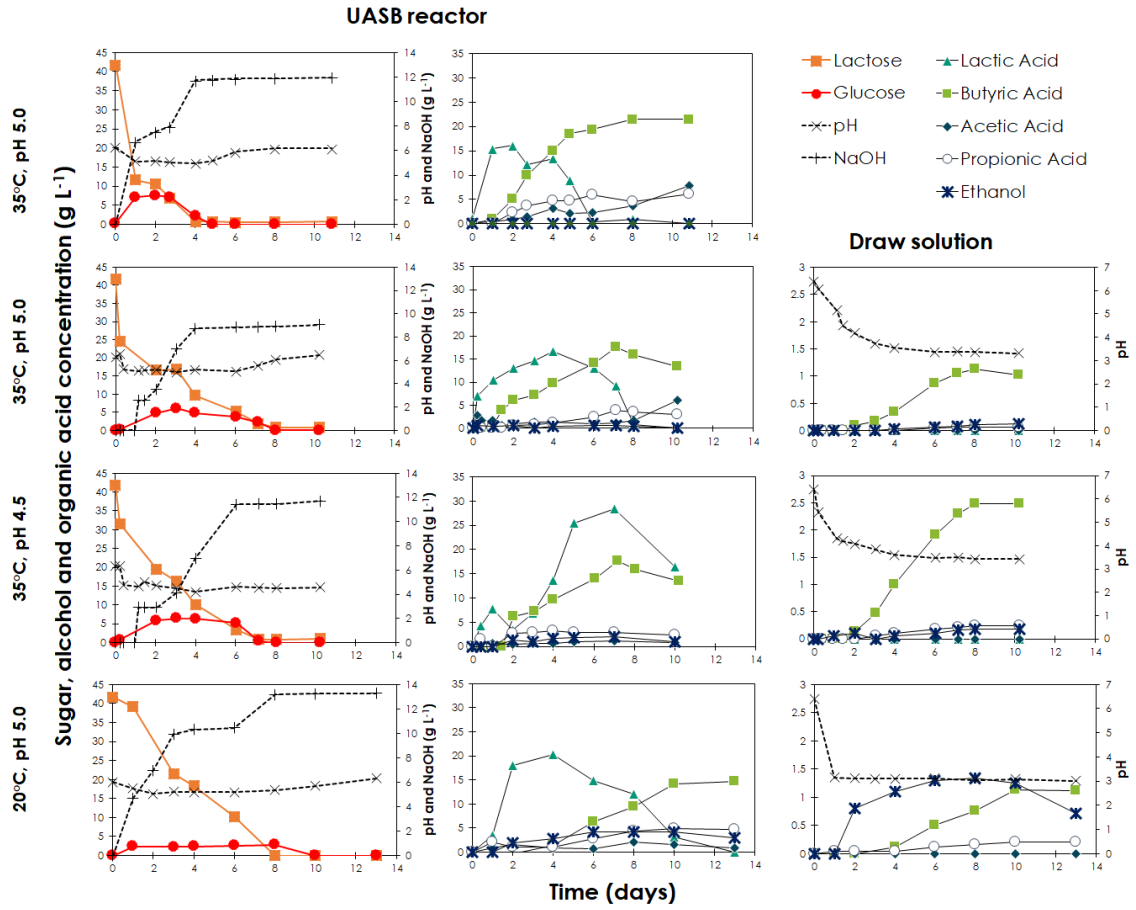


Figure 3. Sugar, alcohol and VFAs concentration profiles, pH and NaOH dosage for the UASB fermentation tests at different temperature (20 or 35°C), pH (4.5 or 5.0) with or without the silicone membrane extraction module. The column "Draw solution" refers to the VFAs and alcohol extracted from the UASB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

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

At 35°C, in the UASB reactor equipped with *in-line* extraction, irrespectively of the operating pH values (5.0 or 4.5), butyric acid was the main metabolite extracted,

346 accounting for more than 90% of the carbon content (Table 4). Indeed, butyric acid
347 migrates faster than shorter chain acids through the silicone membrane matrix due to its
348 higher hydrophobicity [26]. Sugars, lactic acid, and nutrient sources such as proteins, P,
349 anions (Cl^-, NO_2^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}) and cations (Ca^{2+}, K^+, Na^+, and NH_4^+) were,
350 however, retained in the UASB reactor. This confirms that the extraction module
351 prevents the migration of substrates and nutrients, which could inhibit the fermentation
352 process, besides reducing costs for pH control, and allows recovery of butyric acid with
353 more than 90% purity (on carbon content basis), simplifying downstream processing.
354 However, when the extraction module was installed into the UASB, 16.6–19.9% of the
355 inlet carbon was not detected as fermentation product, against only 3.1% unaccounted
356 carbon in the control UASB reactor (Table 4), suggesting VFA adsorption on the
357 silicone membrane. This would be, nevertheless, a minor issue in continuous operation,
358 since the membrane will be quickly saturated with VFAs, after which a further loss will
359 not occur.
360
361 In the test at 20°C, it is worth underlining that also ethanol was produced and extracted
362 through the silicone membrane, opening up further fermentation-related applications.
363 However, from day 8, ethanol concentrations in the draw solution decreased (Fig. 3),
364 likely due to volatilization, a feature to be considered when ethanol is the target of the
365 separation process, which was not the case in this study.
366
367 In the UASB reactor operated at 35°C and pH 5.0 with *in-line* VFAs extraction, a total
368 of 14 g L^{-1} butyric acid was produced, 1 g L^{-1} of which was recovered in the draw
369 solution upon extraction (Fig. 3). The butyric acid flux through the silicone membrane

reached a maximum of 0.41 g m^{-2} h^{-1} on day 6 (Fig. 4). At pH 5.0, only 45% of the butyric acid (pKa=4.82), i.e. about 6.3 g L^{-1}, was in the undissociated form, which is a requisite for crossing the silicone membrane [26]. Furthermore, from day 6, the concentration of undissociated acid further decreased to <10% (i.e., < 1.4 g L^{-1}) due to the pH rise above 6.0, whereas the pH of the draw solution dropped to 3.5 (Fig. 3). The decreasing concentration gradient between fermentate and draw solution caused a decrease of the butyric acid flux, which became even negative on day 10 (Fig. 4), suggesting that a small amount of butyric acid was flowing back towards the fermentation compartment. This issue can be mitigated in continuous operation, since continuous carbohydrate fermentation would prevent a pH raise, and butyric acid migration would thus continue as long as a concentration gradient is kept between the fermentate and the draw solution.

To maintain the concentration gradient between the fermentation broth and draw solution as high as possible, VFAs can be periodically or continuously extracted from the draw solution, e.g. using electrodialysis technology [39]. Electrodialysis has previously been applied to extract VFAs directly from a fermentation broth [39,40], but its application is limited by the fact that all the anions are unselectively extracted, and by biofouling. Both issues are avoided, or at least mitigated, if a silicone membrane separation module is installed prior to the electrodialysis unit. Jones et al. (2017) reported also a 3.75 higher H_2 production in a bioreactor operated with *in-line* VFAs extraction *via* electrodialysis, with respect to a control reactor without extraction unit. Such a beneficial effect was, nevertheless, not evident in this study (Fig. 2), likely due to the substantially higher VFAs concentrations (20-35 g L^{-1} total VFAs) in the

fermentation broth (Fig. 3) compared to those (3-4 g L^{-1} total VFAs) reported in Jones et al. (2017). Indeed, although lower VFA concentrations were measured in the presence, than in the absence, of the extraction unit (Fig. 3), the mitigation effect of the *in-line* VFA extraction was not enough to impact the H_2 yield.

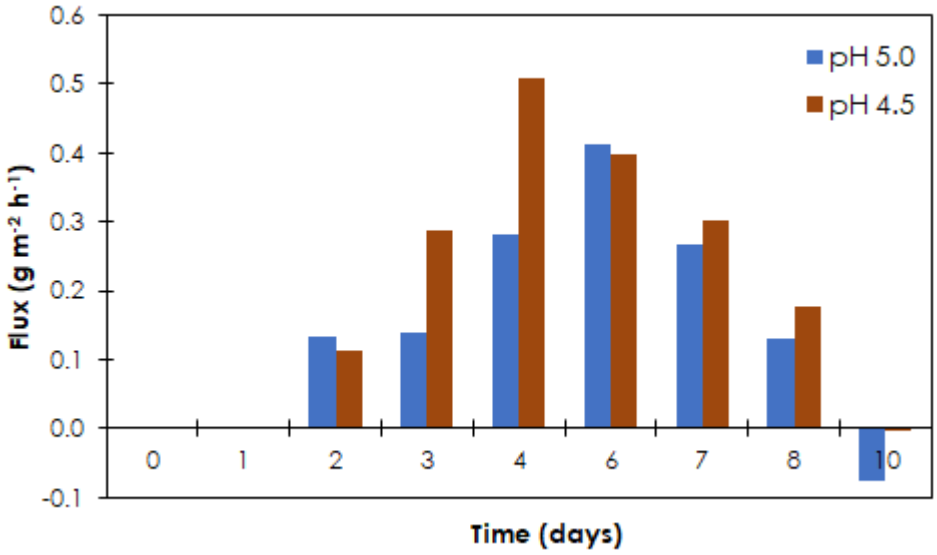
Fermentation at pH 4.5, which led to about 70% of the produced butyric acid in undissociated form, resulted in a 240% higher butyric acid extraction (2.5 g L^{-1}, 40% of the theoretical maximum value) through the silicone membrane than at pH 5.0 (Fig. 3). The butyric acid flux reached a peak of 0.51 g m^{-2} h^{-1} on day 8, higher than the maximum flow of 0.41 g m^{-2} h^{-1} obtained at pH 5.0 (Fig. 4). Ultimately, the butyric acid extraction process can be facilitated by lowering the pH in the fermentation reactor, although this would be detrimental to the H_2 production (Fig. 2). An acidification-neutralization step could also be included in the extraction loop, but this would result in higher operating costs.

Table 4. Carbon balances (in g L^{-1}) of the fermentation tests performed at different temperatures (20 or 35°C) and pH (4.5 or 5.0) using 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%**	

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

411



412

413 **Figure 4.** Evolution of the butyric acid flow through the silicone membrane during the

414 UASB batch experiments performed at 35°C and different pH.

415

416 ***3.3 Continuous cheese whey fermentation in UASB reactors and VFA extraction***

417 *3.3.1 Effect of in-line VFAs extraction on hydrogen production*

418 After a 5-day start-up in batch, both UASB-A (without extraction module) and UASB-B

419 (with extraction module) were operated in continuous mode, with an HRT of 24 hours,

420 reaching the same maximum HPR of 1.9-2.0 L L^{-1} d^{-1} within 20 and 37 days operation,

421 respectively (Fig. 5). This confirms that the use of the *in-line* VFAs extraction module

422 implemented in this study had a minimum impact on the achievable hydrogen

423 production. The presence of the long silicone spiral (4.2-4.4 m in this study) in the

424 recirculation line may, however, impact the contact time between the substrates and

425 microorganisms, particularly during continuous operation, resulting in a slower onset of

426 the H_2 production (Fig. 5). The gas produced by CW fermentation was mainly

427 composed of H_2 and CO_2, with H_2 concentration of 35-37% in UASB-A and 27-28% in

UASB-B. The observed difference in gas composition was attributed to the lower solubility of CO_2 ($<10^{-4}$ g kg^{-1}) as compared to hydrogen (1.6×10^{-3} g kg^{-1}) in distilled water (the draw solution) at the low pH (<4) caused by the extracted VFAs. In line with a waste biorefinery approach, the produced CO_2 can be converted to value-added products through algae- or cyanobacteria-based processes [41,42], or microbial electrosynthesis [43].

Providing a UASB reactor with an extraction module also enabled a more stable process, particularly on days 39-48, as underlined by the HPR values which spanned 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}). The performance of both UASB reactors (average HPR of 1.0-1.3 L L^{-1} d^{-1}, with peaks of about 2.0 L L^{-1} d^{-1}, and the highest yield of 1.5-1.6 mol H_2 mol^{-1} $glucose_{eq.}$) was remarkable since it fairly compares with the highest HPR obtained through continuous dark fermentation of CW. Castelló et al. (2009) operated a UASB reactor at 30°C and average pH 5, reporting a low HPR of only 0.12 L L^{-1} d^{-1} due to the onset of methanogenesis, an issue which did not occur at any stage in the present study. A slightly higher average HPR of 1.6 L L^{-1} d^{-1} was obtained by Blanco et al. (2019) who used a novel structured-bed reactor configuration, operated at 25°C and an OLR of 24 g COD L^{-1} d^{-1}, though fed with synthetic CW.

3.3.2 Effect of pH on hydrogen production

Since a low pH is preferable for VFAs extraction through the silicone membrane (Fig. 4), two different strategies were attempted to adapt the microorganisms to ferment CW at low pH. On day 42, the operation of UASB-A was stopped, and the reactor was

restarted at pH 4.5 with fresh inoculum, whereas the pH of UASB-B was decreased from 5.0 to 4.5 on day 49. Both UASB reactors were operated with *in-line* VFAs extraction during this stage (Table 2). In both UASB reactors, the low pH caused an HPR below 0.2 L L^{-1} d^{-1}, substantially lower than those obtained at pH 5.0 (Fig. 5). Furthermore, in UASB-A, a consistent H_2 production was not achieved even after raising the pH to 5.0 (on day 65), whereas the H_2 production was resumed in UASB-B, though with an average HPR of only 0.4 L L^{-1} d^{-1} during days 75-84 (Fig. 5). This suggests that the microbial community enriched at pH 5.0 was resilient, and able to resume the H_2 production after a pH shock, but was unable to fully restore its productivity in the short term.

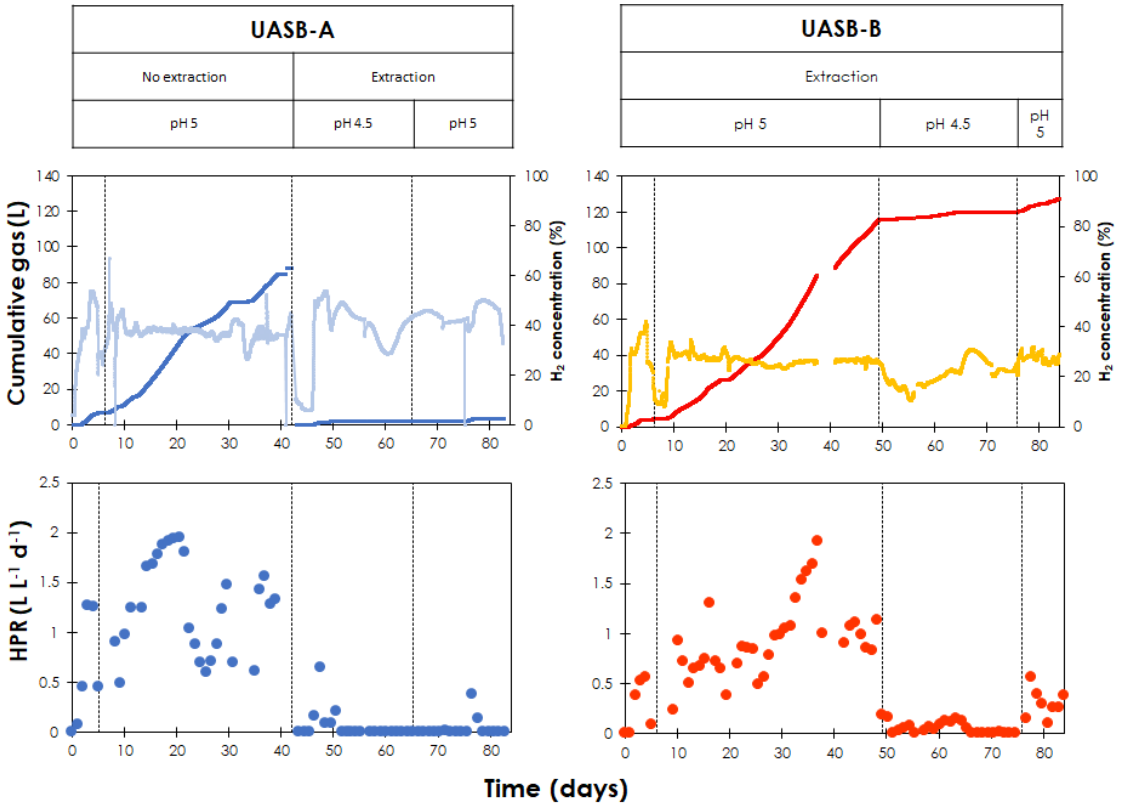


Figure 5. Cumulative gas production (primary axis, blue or red), hydrogen concentration (secondary axis, light blue or orange) and daily average hydrogen

production rate (HPR) of the two UASB reactors through the experiment. The experimental stages, separated by the vertical dotted lines, refer to Table 2. In UASB-A, gas production stopped on days 31-34 due to influent pump failure. UASB-B, data are missing on days 37-39 due to a sensor failure.

3.3.3 Cheese whey fermentation pathways under different operation conditions

During continuous operation, despite the CW supply tank was regularly cleaned, re-supplied with fresh CW and maintained at 4-6°C, a partial conversion of lactose to lactic acid occurred already in the supply tank, which led to the reduction of the lactose concentration from 41.7 g L^{-1} to 10-30 g L^{-1} or even lower (Fig. 6). Partial acidification of the CW (average acidification degree of 20%) resulted in an average pH of 4.5 ± 0.3, and an OLR ranging between 40 and 60 g_{COD} L^{-1} d^{-1}, or even lower (Fig. 6). Ethanol was produced from day 28 in the supply tank, and its concentration reached 10-15 g L^{-1} on day 65, suggesting the onset of heterolactic fermentation. Although the variability of the influent characteristics affected the execution of the experimental tests, it is unavoidable and also occurs in full-scale applications. This issue can be mitigated by minimising preliminary storage before fermentation and optimising the distance between the dairy factories where CW is produced and the treatment plant.

During the first ten days of continuous operation at pH 5.0 (day 6-16), acetic acid was the main metabolite produced in both UASB reactors, reaching concentrations up to 25 g L^{-1} (Fig. 6), which subsequently decreased to <10 g L^{-1}. After this initial stage, butyric acid was the main VFA produced at pH 5.0 in both UASB reactors, with fluctuating concentrations ranging between 5 and 12 g L^{-1}, due to the unstable influent

lactose concentration (Fig. 6). The trend of butyric acid concentrations reflects that of the H_2 production (Fig. 5), suggesting that H_2 was mainly produced via lactic acid conversion to butyric acid, as previously reported [10,20]. In both UASB reactors, when operated under continuous mode at pH 5.0, over 80% of the influent sugars were consumed, whereas lactic acid conversion was incomplete resulting in an average residual concentration of 5.9 and 8.7 g L^{-1} in UASB-A and UASB-B, respectively (Fig. 6). Longer HRTs may allow a full sugar and lactic acid conversion to VFAs, but this will cause an increase of the required reactor volume, resulting in higher costs, probably not balanced by the advantages that can be obtained.

A drastic decrease in butyric acid production, along with H_2 production, was observed when both UASB reactors were operated at pH 4.5, although the sugar consumption remained over 80%. Most sugars were indeed converted to lactic acid, which accumulated up to 25-30 g L^{-1} (Fig. 6), but further conversion of lactic acid to VFAs was inhibited by the low pH, resulting in VFAs concentrations below 2 g L^{-1}. Interestingly, at pH 4.5, the ethanol concentration increased up to 15 g L^{-1} in both UASB reactors, besides being produced already in the supply tank (Fig. 6). When the pH was increased back from 4.5 to 5.0, the lactic acid concentration immediately decreased to < 10 g L^{-1} in both UASB reactors, and the butyric acid concentration increased back to about 5 g L^{-1}.

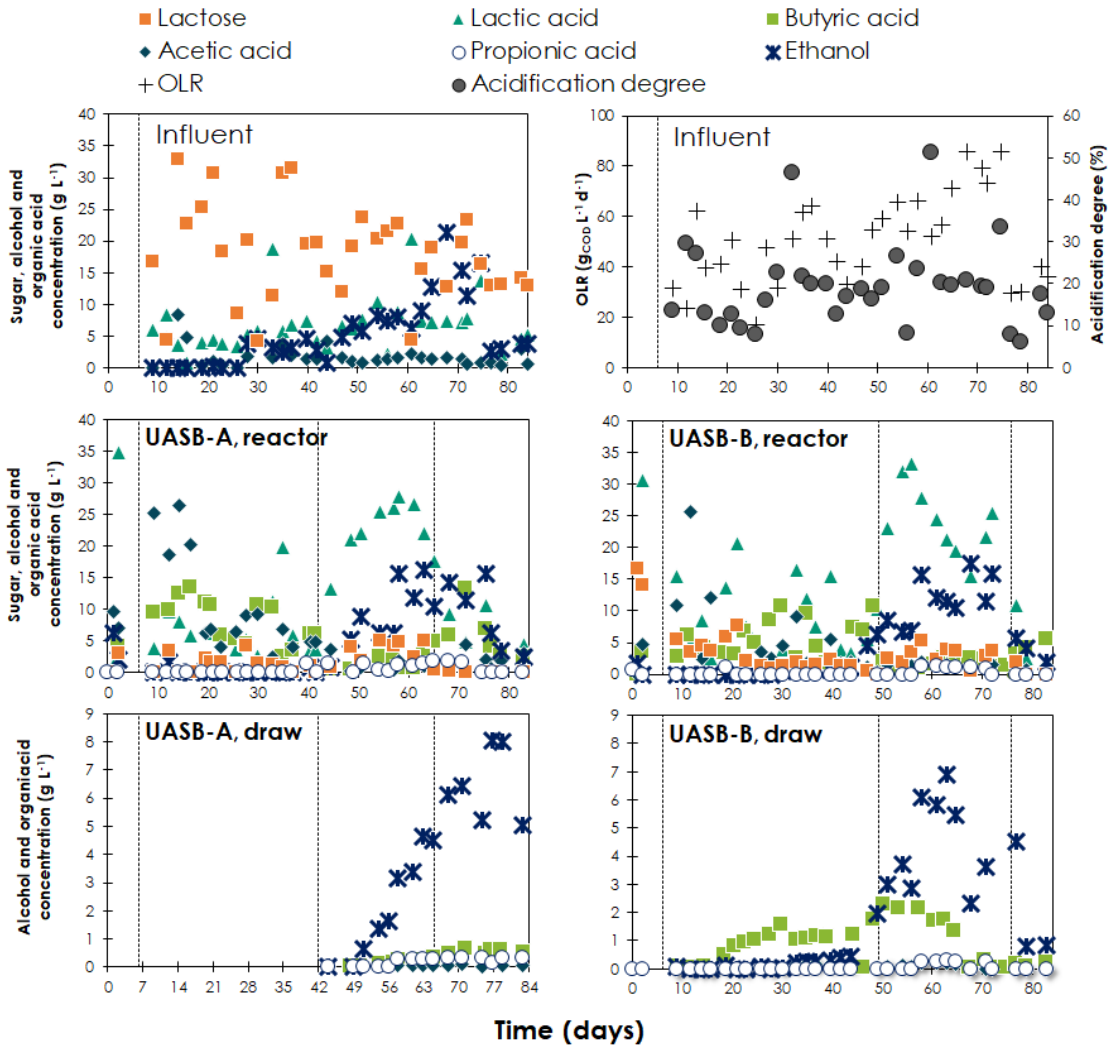


Figure 6. Sugar, alcohol, and carboxylic acid concentration in the influent, the two UASB reactor effluents and the respective draw solutions during the continuous experiment. The organic loading rate (OLR) and acidification degree of the influent are also reported. The experimental stages, separated by the vertical dotted lines, refer to Table 2.

3.3.4 Continuous VFA extraction through silicone membrane

519 In UASB-B, butyric acid was extracted at pH 5.0 up to a concentration of 1.0-1.5 g L^{-1}
520 within 7 days, confirming the results obtained under batch conditions. The extraction of
521 butyric acid benefited from the decrease of fermentation pH down to 4.5, which enabled
522 to reach concentrations up to 2.2 g L^{-1} in the draw solution on days 54-58. However,
523 starting from day 59, the butyric acid extraction was affected by the low production in
524 UASB-B, which caused an inversion of the concentration gradient (Fig. 6). When the
525 UASB reactors were operated at pH 4.5, ethanol was produced in both UASB reactors
526 and extracted up to a concentration of 7 g L^{-1} (Fig. 6), suggesting that non-porous
527 silicone membranes can be used for alcohol extraction as well. Unlike carboxylic acids,
528 alcohols do not dissociate in water, and the extraction is therefore not affected by the
529 pH.
530
531 ***3.4 Off-line VFAs and alcohol extraction from cheese whey fermentate***
532 The effluents of UASB-A and UASB-B were collected on day 71, while the reactors
533 were operated at pH 4.5 and 5.0, respectively, and tested for *off-line* VFAs and alcohol
534 extraction upon acidification (pH 3.0). The fermentate of UASB-B was characterised
535 mainly by the presence of butyric acid (10.8 ± 0.4 g L^{-1}) as well as by lower
536 concentrations of ethanol (5.4 ± 0.3 g L^{-1}) and acetic (7.0 ± 0.2 g L^{-1}), propionic (2.7 ±
537 0.0 g L^{-1}) and lactic (2.9 ± 0.1 g L^{-1}) acid, whereas the fermentate of UASB-A contained
538 mainly lactic acid (19.4 ± 1.1 g L^{-1}) and ethanol (5.9 ± 0.4 g L^{-1}) with lower
539 concentrations of acetic (2.2 ± 0.1 g L^{-1}), propionic (2.2 ± 0.0 g L^{-1}) and butyric (0.8 ±
540 0.0 g L^{-1}) acid.

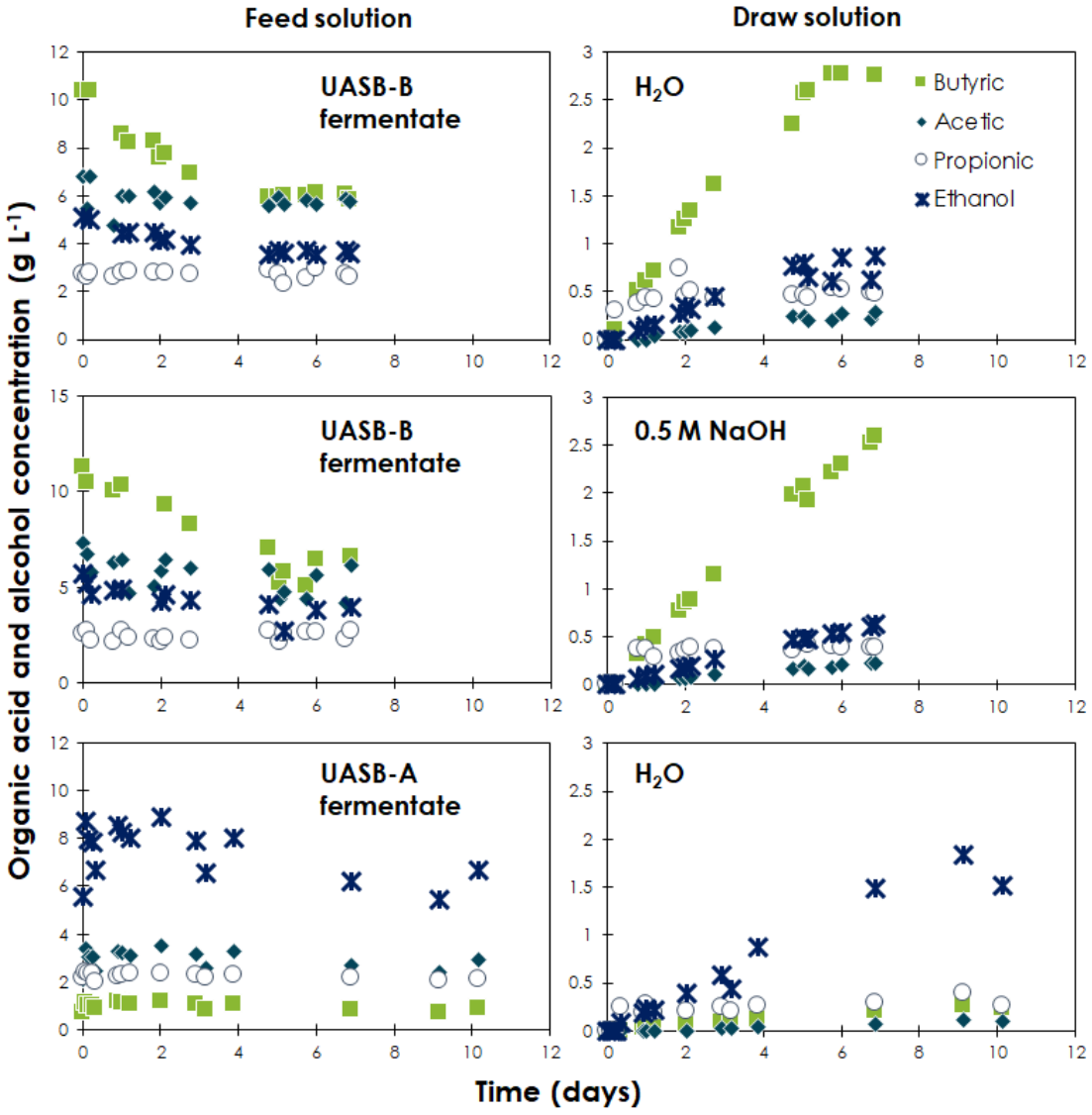


Figure 7. *Off-line* VFAs and alcohol extraction from the UASB reactor effluents, acidified to pH 3.0, via silicone membrane pertraction at 20°C. The UASB-A and UASB-B fermentate was collected while the reactors were operated at pH 4.5 and 5.0, respectively. Lactic acid data are omitted since it was not detected in the draw solutions.

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

550 in the draw solution within 5 days (Fig. 7). Indeed, at pH 3.0, over 99% of the butyric
551 acid is in undissociated form, as compared to the 40% undissociated at pH 5.0. On the
552 other hand, by decreasing the pH the diffusion of acetic and propionic acid is favoured
553 as well, leading to a lower purity of butyric acid in the draw solution (65.4% and 69.2%
554 on carbon content basis, using water and 0.5 M NaOH, respectively, as draw solution).
555 In contrast, over 90% purity was observed in the experiments performed at pH 5.0 and
556 4.5 (Fig. 3).
557
558 Using NaOH as the draw solution resulted in a slower VFAs migration (maximum flux
559 of 0.26 g d^{-1} h^{-1}), but a concentration gradient was maintained (Fig. 7) due to the
560 dissociation of the extracted VFAs, and the subsequent formation of sodium salts. In
561 contrast, a plateau was reached when pure water was used as the draw solution (Fig. 7).
562 The overall mass transfer coefficient (K_{OV}) for butyric acid was 0.109 and 0.101 µm s^{-1}
563 using, respectively, water and NaOH as the draw solution. Those values are lower than
564 the K_{OV} of 0.157 µm s^{-1} obtained for butyric acid extraction from acidified fish
565 fermentate [26], due to the higher initial VFA concentration (15.1 *vs.* 10.8 g L^{-1}) and
566 lower thickness of the silicone tube (0.8 mm *vs.* 1.0 mm) than those in this study.
567
568 Despite the high lactic acid concentration (19.4 g L^{-1}) in UASB-A fermentate at pH 4.5,
569 85% of which in the undissociated form at pH 3, lactic acid was retained by the
570 membrane, resulting in concentrations below the detection limit in the draw solution.
571 This is due to its low volatility (Henry constant 9.6×10^{-9} atm m^{-3} mol^{-1}) and solubility in
572 water, whereas the more volatile (Henry constant 5.0×10^{-6} atm m^{-3} mol^{-1}) ethanol
573 migrated with a K_{ov} of 0.083-0.96 µm s^{-1}.

3.5 Future research directions

Despite the promising results obtained in this study, more research efforts are required to advance the technology readiness level of the integrated process. Further studies should focus on membrane characteristics (material, length, thickness) and process operating parameters (pH, temperature, recirculation flow). A second process to be downstream implemented, e.g. electrodialysis-based technologies [40], is required to concentrate the VFAs extracted and, at the same time, to avoid their accumulation in the draw solution, keeping a sufficient concentration gradient between the fermentation broth and the draw solution to allow VFAs migration. Enhancing *in-line* VFA extraction, in turn reducing their toxic effect on the microorganisms, can also positively affect hydrogen yields [40], resulting in further economic benefits.

4. Conclusions

This study proposes a novel approach for cheese whey valorisation, where dark fermentation is combined to a relatively low-cost extraction process in order to obtain H_2 and high-purity butyric acid from CW. This study showed that:

- HPR up to 2.0 L L^{-1} d^{-1} can be obtained by fermenting cheese whey at pH 5.0,
- A hydrophobic silicone membrane favours extraction of longer chain acids over short-chain acids, which is a unique advantage for any processes generating an acid mixture;
- Up to 3 g L^{-1} of high purity (>90% on carbon content basis) butyric acid can be *in-line* extracted without affecting steady-state hydrogen production, decreasing

the NaOH requirement and saving the energy which is otherwise required for extraction,

- Ethanol can be extracted *via* silicone membrane pertraction, whereas sugars and nutrients are retained,
- Low pH values increase the VFAs extraction rate in silicone membrane pertraction, but drastically decrease the HPR and can negatively affect the selectivity of the extraction process.

Funding

This work was funded by the Science Foundation of Ireland (SFI) Research Professorship Programme on Innovative Energy Technologies for Bioenergy, Biofuels and a Sustainable Irish Bioeconomy (IETSBIO^3, award 15/RP/2763). Fabiano Asunis gratefully acknowledges the Sardinian Regional Government for the financial support of his PhD scholarship (P.O.R. Sardegna F.S.E. - Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2014-2020).

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Dairygold for providing cheese whey and activated and digested sludge, Lisa Guiney and Patrick Maxwell (NUI Galway) for their great contribution to the preliminary experiments, and Carlos Sanchez (NUI Galway) for drawing the reactor scheme (Fig. 1). This research was conducted in the framework of the "Waste

622 Biorefinery" task group of the International Waste Working Group (IWWG),
623 https://www.tuhh.de/iue/iwwg/task-groups/waste-biorefinery.html.
624
625 **References**

626 [1] European Environment Agency, The circular economy and the bioeconomy.
627 Partners in sustainability, EEA Rep. 8/2018. (2018).
628 https://doi.org/10.2800/02937.
629 [2] K. Özdenkçi, C. De Blasio, H.R. Muddassar, K. Melin, P. Oinas, J. Koskinen, G.
630 Sarwar, M. Järvinen, A novel biorefinery integration concept for lignocellulosic
631 biomass, Energy Convers. Manag. 149 (2017) 974–987.
632 doi:10.1016/j.enconman.2017.04.034.
633 [3] S. Venkata Mohan, G.N. Nikhil, P. Chiranjeevi, C. Nagendranatha Reddy, M. V.
634 Rohit, A.N. Kumar, O. Sarkar, Waste biorefinery models towards sustainable
635 circular bioeconomy: Critical review and future perspectives, Bioresour. Technol.
636 215 (2016) 2–12. doi:10.1016/j.biortech.2016.03.130.
637 [4] F. Cherubini, The biorefinery concept: Using biomass instead of oil for
638 producing energy and chemicals, Energy Convers. Manag. 51 (2010) 1412–1421.
639 doi:10.1016/j.enconman.2010.01.015.
640 [5] Eurostat, Agriculture, forestry and fishery statistics - 2018 Edition, 2018.
641 [6] T. Ahmad, R.M. Aadil, H. Ahmed, U. ur Rahman, B.C. V Soares, S.L.Q. Souza,
642 T.C. Pimentel, H. Scudino, J.T. Guimarães, E.A. Esmerino, M.Q. Freitas, R.B.
643 Almada, S.M.R. Vendramel, M.C. Silva, A.G. Cruz, Treatment and utilization of
644 dairy industrial waste: A review, Trends Food Sci. Technol. 88 (2019) 361–372.
645 doi:10.1016/j.tifs.2019.04.003.

[7] K. Valta, P. Damala, A. E, G. Antonopoulou, D. Malamis, K. Haralambous, Current treatment technologies of cheese whey and wastewater by greek cheese manufacturing units and potential valorisation opportunities, Waste and Biomass Valorization. 8 (2017) 1649–1663. doi:10.1007/s12649-017-9862-8.

[8] F. Carvalho, A.R. Prazeres, J. Rivas, Cheese whey wastewater: Characterization and treatment, Sci. Total Environ. 445–446 (2013) 385–396. doi:10.1016/j.scitotenv.2012.12.038.

[9] G. Pagliano, V. Ventorino, A. Panico, I. Romano, F. Pirozzi, O. Pepe, Anaerobic process for bioenergy recovery from dairy waste: Meta-analysis and enumeration of microbial community related to intermediates production, Front. Microbiol. 9 (2019) 3229. doi:10.3389/fmicb.2018.03229.

[10] F. Asunis, G. De Gioannis, M. Isipato, A. Muntoni, A. Polettini, R. Pomi, A. Rossi, D. Spiga, Control of fermentation duration and pH to orient biochemicals and biofuels production from cheese whey, Bioresour. Technol. 289 (2019) 121722. doi:10.1016/j.biortech.2019.121722.

[11] A.R. Gouveia, E.B. Freitas, C.F. Galinha, G. Carvalho, A.F. Duque, M.A.M. Reis, Dynamic change of pH in acidogenic fermentation of cheese whey towards polyhydroxyalkanoates production: Impact on performance and microbial population, N. Biotechnol. 37 (2017) 108–116. doi:10.1016/j.nbt.2016.07.001.

[12] C.S. Murari, D.C.M.N. da Silva, G.L. Schuina, E.F. Mosinahti, V.L. Del Bianchi, Bioethanol production from dairy industrial coproducts, Bioenergy Res. 12 (2019) 112–122. doi:10.1007/s12155-018-9949-5.

[13] V. Luongo, G. Policastro, A. Ghimire, F. Pirozzi, M. Fabbricino, Repeated-batch fermentation of cheese whey for semi-continuous lactic acid production using

mixed cultures at uncontrolled pH, Sustainability. 11 (2019) 3330. doi:10.3390/su11123330.

[14] G. Antonopoulou, K. Stamatelatou, S. Bebelis, G. Lyberatos, Electricity generation from synthetic substrates and cheese whey using a two chamber microbial fuel cell, Biochem. Eng. J. 50 (2010) 10–15. doi:10.1016/j.bej.2010.02.008.

[15] B. Colombo, M. Villegas Calvo, T.P. Sciarria, B. Scaglia, S. Savio Kizito, G. D'Imporzano, F. Adani, Biohydrogen and polyhydroxyalkanoates (PHA) as products of a two-steps bioprocess from deproteinized dairy wastes, Waste Manag. 95 (2019) 22–31. doi:10.1016/j.wasman.2019.05.052.

[16] A.S. Nizami, M. Rehan, M. Waqas, M. Naqvi, O.K.M. Ouda, K. Shahzad, R. Miandad, M.Z. Khan, M. Syamsiro, I.M.I. Ismail, D. Pant, Waste biorefineries: Enabling circular economies in developing countries, Bioresour. Technol. 241 (2017) 1101–1117. doi:10.1016/j.biortech.2017.05.097.

[17] G. De Gioannis, M. Friargiu, E. Massi, A. Muntoni, A. Polettini, R. Pomi, D. Spiga, Biohydrogen production from dark fermentation of cheese whey: Influence of pH, Int. J. Hydrogen Energy. 39 (2014) 20930–20941. doi:10.1016/j.ijhydene.2014.10.046.

[18] M. Akhlaghi, M.R. Boni, G. De Gioannis, A. Muntoni, A. Polettini, R. Pomi, A. Rossi, D. Spiga, A parametric response surface study of fermentative hydrogen production from cheese whey, Bioresour. Technol. 244 (2017) 473–483. doi:10.1016/j.biortech.2017.07.158.

[19] L.M. Ottaviano, L.R. Ramos, L.S. Botta, M.B. Amâncio Varesche, E.L. Silva, Continuous thermophilic hydrogen production from cheese whey powder

694 solution in an anaerobic fluidized bed reactor: Effect of hydraulic retention time
695 and initial substrate concentration, Int. J. Hydrogen Energy. 42 (2017) 4848–
696 4860. doi:10.1016/j.ijhydene.2016.11.168.
697 [20] G. Davila-Vazquez, C.B. Cota-Navarro, L.M. Rosales-Colunga, A. de León-
698 Rodríguez, E. Razo-Flores, Continuous biohydrogen production using cheese
699 whey: Improving the hydrogen production rate, Int. J. Hydrogen Energy. 34
700 (2009) 4296–4304. doi:10.1016/j.ijhydene.2009.02.063.
701 [21] V.M.C. Blanco, G.H.D. Oliveira, M. Zaiat, Dark fermentative biohydrogen
702 production from synthetic cheese whey in an anaerobic structured-bed reactor:
703 Performance evaluation and kinetic modeling, Renew. Energy. 139 (2019) 1310–
704 1319. doi:10.1016/j.renene.2019.03.029.
705 [22] L. Jiang, H. Fu, H.K. Yang, W. Xu, J. Wang, S.-T. Yang, Butyric acid:
706 Applications and recent advances in its bioproduction, Biotechnol. Adv. 36
707 (2018) 2101–2117. doi:10.1016/j.biotechadv.2018.09.005.
708 [23] M. Atasoy, I. Owusu-Agyeman, E. Plaza, Z. Cetecioglu, Bio-based volatile fatty
709 acid production and recovery from waste streams: Current status and future
710 challenges, Bioresour. Technol. 268 (2018) 773–786.
711 doi:10.1016/j.biortech.2018.07.042.
712 [24] F. Valentino, F. Morgan-Sagastume, S. Campanari, M. Villano, A. Werker, M.
713 Majone, Carbon recovery from wastewater through bioconversion into
714 biodegradable polymers, N. Biotechnol. 37 (2017) 9–23.
715 doi:10.1016/j.nbt.2016.05.007.
716 [25] G. Strazzera, F. Battista, N.H. Garcia, N. Frison, D. Bolzonella, Volatile fatty
717 acids production from food wastes for biorefinery platforms: A review, J.

718 Environ. Manage. 226 (2018) 278–288. doi:10.1016/j.jenvman.2018.08.039.
719 [26] V. Outram, Y. Zhang, Solvent-free membrane extraction of volatile fatty acids
720 from acidogenic fermentation, Bioresour. Technol. 270 (2018) 400–408.
721 doi:10.1016/j.biortech.2018.09.057.
722 [27] W.F. Owen, D.C. Stuckey, J.B. Healy Jr., L.Y. Young, P.L. McCarty, Bioassay
723 for monitoring biochemical methane potential and anaerobic toxicity, Water Res.
724 13 (1979) 485–492. doi:http://dx.doi.org/10.1016/0043-1354(79)90043-5.
725 [28] M. Dubois, K. Gilles, J.K. Hamilton, P. Rebers, F. Smith, Colorimetric method
726 for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–
727 356. doi:10.1021/ac60111a017.
728 [29] APHA, Standard Methods for the Examination of Water and Wastewater,
729 twentieth ed. American Public Health Association/American Water Works
730 Association/Water Environment Federation, Washington DC., (1998).
731 [30] R.J. Rouwenhorst, J.F. Jzn, W.A. Scheffers, J.P. van Dijken, Determination of
732 protein concentration by total organic carbon analysis, J. Biochem. Biophys.
733 Methods. 22 (1991) 119–128.
734 [31] S. Bengtsson, J. Hallquist, A. Werker, T. Welander, Acidogenic fermentation of
735 industrial wastewaters: Effects of chemostat retention time and pH on volatile
736 fatty acids production, Biochem. Eng. J. 40 (2008) 492–499.
737 doi:10.1016/j.bej.2008.02.004.
738 [32] N. Venetsaneas, G. Antonopoulou, K. Stamatelatou, M. Kornaros, G. Lyberatos,
739 Using cheese whey for hydrogen and methane generation in a two-stage
740 continuous process with alternative pH controlling approaches, Bioresour.
741 Technol. 100 (2009) 3713–3717. doi:10.1016/j.biortech.2009.01.025.

[33] M.S.A. Tango, A.E. Ghaly, Effect of temperature on lactic acid production from cheese whey using *Lactobacillus helveticus* under batch conditions, Biomass and Bioenergy. 16 (1999) 61–78.

[34] M.A.Z. Bundhoo, R. Mohee, Inhibition of dark fermentative bio-hydrogen production: A review, Int. J. Hydrogen Energy. 41 (2016) 6713–6733. doi:10.1016/j.ijhydene.2016.03.057.

[35] J. Berg, J. Tymoczko, L. Stryer, Biochemistry. 5th edition., New York, 2002.

[36] N.M.C. Saady, Homoacetogenesis during hydrogen production by mixed cultures dark fermentation: Unresolved challenge, Int. J. Hydrogen Energy. 38 (2013) 13172–13191. doi:10.1016/j.ijhydene.2013.07.122.

[37] C. Garrigues, P. Loubiere, N.D. Lindley, M. Cocaign-Bousquet, Control of the shift from homolactic acid to mixed-acid fermentation in *Lactococcus lactis*: Predominant role of the NADH/NAD^+ ratio, J. Bacteriol. 179 (1997) 5282–5287. doi:10.1128/jb.179.17.5282-5287.1997.

[38] M. Pescuma, E. Hébert, F. Mozzi, G. de Valdez, Whey fermentation by thermophilic lactic acid bacteria: Evolution of carbohydrates and protein content, Food Microbiol. 25 (2008) 442–451. doi:10.1016/j.fm.2008.01.007.

[39] R.J. Jones, J. Massanet-Nicolau, A. Guwy, G.C. Premier, R.M. Dinsdale, M. Reilly, Removal and recovery of inhibitory volatile fatty acids from mixed acid fermentations by conventional electrodialysis, Bioresour. Technol. 189 (2015) 279–284. doi:10.1016/j.biortech.2015.04.001.

[40] R.J. Jones, J. Massanet-Nicolau, M.J.J. Mulder, G. Premier, R. Dinsdale, A. Guwy, Increased biohydrogen yields, volatile fatty acid production and substrate utilisation rates via the electrodialysis of a continually fed sucrose fermenter,

766 Bioresour. Technol. 229 (2017) 46–52. doi:10.1016/j.biortech.2017.01.015.
767 [41] H. Duppeti, S. Chakraborty, B.S. Das, N. Mallick, J.N.R. Kotamreddy, Rapid
768 assessment of algal biomass and pigment contents using diffuse reflectance
769 spectroscopy and chemometrics, Algal Res. 27 (2017) 274–285.
770 doi:10.1016/j.algal.2017.09.016.
771 [42] M. Kumar, S. Sundaram, E. Gnansounou, C. Larroche, I.S. Thakur, Carbon
772 dioxide capture, storage and production of biofuel and biomaterials by bacteria:
773 A review, Bioresour. Technol. 247 (2018) 1059–1068.
774 doi:10.1016/j.biortech.2017.09.050.
775 [43] J.B.A. Arends, S.A. Patil, H. Roume, K. Rabaey, Continuous long-term
776 electricity-driven bioproduction of carboxylates and isopropanol from CO_2 with a
777 mixed microbial community, J. CO_2 Util. 20 (2017) 141–149.
778 doi:10.1016/j.jcou.2017.04.014.
779 [44] E. Castelló, C. García y Santos, T. Iglesias, G. Paolino, J. Wenzel, L. Borzacconi,
780 C. Etchebehere, Feasibility of biohydrogen production from cheese whey using a
781 UASB reactor: Links between microbial community and reactor performance,
782 Int. J. Hydrogen Energy. 34 (2009) 5674–5682.
783 doi:10.1016/j.ijhydene.2009.05.060.
784

Supplementary Material

Click here to download Supplementary Material: Supplementary material.doc

***Declaration of Interest Statement**

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: