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Research article

From dairy waste to butyric acid: Scaling the valorization process

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ABSTRACT

To move towards a circular dairy sector and reduce its environmental impact, sustainable management of its by-products is essential. This study presents a novel approach to valorize milk permeate through the production of butyric acid. Key operating parameters, including hydraulic retention time (HRT) and pH, were tested at a laboratory scale and the best conditions were validated at a pre-industrial scale in a dairy factory. A relatively short retention time (2 d) and pH 5.5 was demonstrated to be appropriate for the conversion of milk permeate into volatile fatty acids (58% yield), likely due to the permeate carbohydrate composition, which is primarily lactose. Furthermore, the acidic pH (5.5) promoted a shift in the product spectrum, increasing the molar fraction of butyric acid from 0.2 to 0.6. These operational conditions resulted in butyric acid productivities of 9.0 g HBut/L·d and 3.1 g HBut/L·d at lab- and pilot scale, respectively. This study represents a significant step towards a promising valorization route for milk permeate at a representative scale, while highlighting the need for process optimization to overcome scale-up challenges to ensure industrial applicability.

1. Introduction

In 2022, 930 million tons of milk were produced in the world and this production will continue to grow (Hassoun et al., 2024; AHFES, 2021). Milk processing generates large amounts of residual streams, including wastewater and by-products, which have high levels of organic matter due to their content of lactose, proteins and fats (Ahmad et al., 2019). Cheese whey and milk permeate are two significant by-products produced in the dairy industry. The generation of cheese whey occurs at a rate of approximately 9 L per kilogram of cheese; in the case of milk permeate, it is produced at a rate of 80.0–87.5 L per 100 L of filtered milk (Sar et al., 2022). In this context, meeting the demand for dairy products while finding new ways to reduce the environmental impact associated with the waste streams generated is key to the sustainability of the dairy sector (International Dairy Federation, 2019).

Given its high protein and fat content, cheese whey represents a valuable resource for food product valorization. Therefore, the main valorization pathways for cheese whey include its use as animal feed and the production of protein concentrates (Zotta et al., 2020). In contrast, milk permeate, obtained after the removal of proteins and fats through ultrafiltration or microfiltration (Tsermoula et al., 2024), is mainly composed of lactose, minerals and water. Consequently, milk permeate have very limited applications so far and a major part is dried and sold as bulking agent, ingredient for animal feed or as milk solid replacement in dry soups and sauces (Lamothe et al., 2025). However, this practice is not economically viable due to the high energy costs of the drying process, which also has a negative environmental impact. Indeed, the Best Available Techniques (BAT) Reference Document for the Food, Beverage and Milk Industries identifies evaporation as the most energy-intensive process in the dairy industry (Giner et al., 2019). In this sense, Tanguy et al. (2017) estimate an energy consumption of 5508 $kJ \cdot kg{-}1$ of water extracted, with its consequent environmental impact. Other less explored valorization alternatives include energy drinks production (Berry et al., 2022), purified lactose and lactate derivatives production (eg. galacto-oligosaccharides, Djouab and Aïder, 2019), xanthan gum production (Cancella et al., 2024) or energy production (methane, Tarapata et al., 2022).

Due to its high lactose content (>80%, Oliveira et al., 2019), milk permeate is a potential raw material for the carboxylate platform (Sar et al., 2022), through volatile fatty acids (VFAs) production. However, most studies in this field have focused on cheese whey as a substrate. Delmoitié et al. (2025) studied the optimization of whey permeate for lactic acid production, achieving a yield of 70% with a maximum productivity of 658 g/L·d. Similarly, Bengtsson et al. (2008) conducted a

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study to evaluate the acidogenic fermentation of cheese whey permeate to produce volatile fatty acids (VFAs) as precursors for polyhydroxyalkanoates (PHAs). A maximum acidification of 0.9 g COD of VFAs/g SCOD was achieved at a retention time (RT) of 95 h. In contrast, studies focusing on milk permeate for VFA production are scarce. To the best of our knowledge, only one investigation has explored this substrate under thermophilic conditions (60 °C), demonstrating efficient lactose conversion to acetic acid with a yield of 80% (Talabardon et al., 2000). However, it should be noted that this experiment was based on pure culture, which has limitations, including high costs and the requirement for sterilized and homogeneous substrates, so it can only be used for small-scale production.

Among VFAs, butyric acid is particularly noteworthy due to its extensive applications in the chemical, food and pharmaceutical industries (Xu and Jiang, 2019). Additionally, it commands a considerable market value, reaching USD 1,472 per metric ton (on a Free On Board Houston basis) by the end of the second quarter of 2023. The high carbohydrate content of milk permeate specifically promotes the production of butyric acid over other acids under certain conditions (González-Cabaleiro et al., 2015; Regueira et al., 2020), such as acidic pH and low protein to glucose ratio. The present work investigates the potential valorization of milk permeate, a low-value dairy by-product, to produce butyric acid, a high-added value product, through anaerobic fermentation. The study includes the optimization of operating conditions (hydraulic retention time and pH) at laboratory scale and the process scale up to a pilot demonstration (fermenter of 2 m^3) in a dairy industry. To our knowledge, this is the first study valorizing cheese whey into butyric acid at pilot scale in a dairy industry.

2. Material and methods

2.1. Milk permeate

Milk permeate was supplied by a dairy factory located in Galicia (Northwest Spain) dedicated to transforming cow milk into yoghurt and cheese. During the manufacturing process, the pasteurized milk passes through an ultrafiltration (UF) membrane to concentrate milk proteins and fats, which are sent to the cheese and yoghurt production lines. The UF permeate stream (i.e. the milk permeate) was used in the present study as substrate. A complete physicochemical characterization was conducted in accordance with the analytical methodologies outlined in Section 2.3.

2.2. Scale-up strategy

A scale-up strategy (Fig. 1) was carried out to develop and validate the targeted production of butyric acid from milk permeate. Firstly, batch tests (0.5 L) were conducted to determine the maximum conversion of the substrate (acidification yield). Thereafter, 5-L laboratory continuous stirred tank reactors (CSTR) were operated to identify the optimal conditions (HRT and pH) to maximise butyric acid production (yield and productivity). Finally, a pilot plant was designed, installed in a dairy industry and operated to validate butyric acid production from milk permeate at pre-industrial conditions.

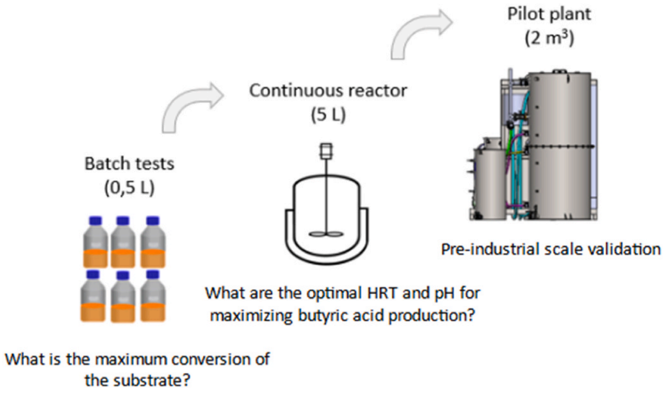

Fig. 1. Scale-up strategy.

2.2.1. Batch test

The batch tests were conducted using an AMPTS II (Automatic Methane Potential Test System) device (BPC Instruments, Sweden), modified to allow liquid sampling during the experiment. The tests were performed in duplicate using 500 mL bottles placed in a thermostatically controlled incubator maintained at 37 ± 1 °C to ensure mesophilic conditions. This temperature range is considered optimal and economically favorable for volatile fatty acid (VFA) production (Atasoy et al., 2018). The initial pH was adjusted to 7.0 using sodium bicarbonate ($NaHCO_3$, 100 g/L). Additionally, sodium bicarbonate was also added to ensure an initial alkalinity of 5 g $CaCO_3$/L, helping to mitigate excessive acidification. Although the pH was monitored daily, it was not actively controlled. The tests were performed at a food to microorganism (F/M) ratio of 4, chosen based on the volatile solids (VS) portion of the inoculum being greater than that of the substrate to minimize acidification or inhibition. This ratio is within the optimal range (2–4) for most applications (Holliger et al., 2016). Since the substrate had a high soluble COD content, an F/M ratio of 4 was selected to avoid rapid VFA accumulation that could inhibit the process. Micro and macronutrients were supplemented (Temudo et al., 2007) (Supplementary Material). The bottles were filled to 500 mL with water after adding the substrate, inoculum, and nutrients. Blank tests containing only the inoculum were included to assess VFA production from the inoculum. The headspace of the bottles was flushed with nitrogen gas for 1 min before the start of the experiment. No selective inhibition of methanogenesis was performed. The average COD concentration in the bottles was 50.7 g tCOD/L and 33.6 g sCOD/L.

Gas production and pH were monitored daily. Liquid samples were also extracted daily to determine soluble COD and VFA concentrations. At the end of the test, pH, total and soluble COD (Chemical Oxygen Demand), TS (Total Solids), VS (Volatile solids), TSS (Total Suspended Solids), VSS (Volatile Suspended Solids), TP (Total Phosphorus), PO_4^{3-}-P (Phosphate), TN (Total Nitrogen), NH_4^+-N (Ammonium) and individual VFA were determined.

2.2.2. Lab scale continuous reactor

A 5.0 L completely stirred tank reactor (CSTR) was operated in continuous mode for 300 days. Three different HRT values (5, 3 and 2 days) were studied at a constant pH of 6.5. Subsequently, the effect of pH (6.5, 6.0 and 5.5) was assessed with a constant HRT of 2 d. The selection of HRTs was made to ensure an adequate duration for the hydrolysis stage while preventing an excessively long period that could promote the growth of the methanogenic population. Likewise, acidic pH values increase butyric acid production from carbohydrates (González-Cabaleiro et al., 2015).

The pH was continuously monitored and controlled by using a solution of Na_2CO_3 (50 g/L) as a weak base and HCl (10%) as acid. Continuous mixing (90 rpm) was provided in the reactor by an electric stirrer (IKA RW Digital 60, Germany). A thermostatic bath was used to maintain the temperature in the mesophilic range (37 ± 1 °C). The redox potential was monitored online and no specific methanogenesis inhibition was applied.

The reactor was inoculated with 3 L of anaerobic biomass (28.4 ± 4.7 VSS/L) from a sewage sludge anaerobic digester. The feeding to the reactor (milk permeate) was supplied weekly by the dairy industry and kept at 6 °C until its use to avoid degradation. Influent and effluent samples were taken three times a week to determine total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total and soluble COD and individual volatile fatty acids (VFAs).

2.2.3. Pilot scale demonstration

A pilot plant (see photo at Supplementary material) was designed and operated onsite at the dairy industry for 160 days. The pilot plant (Fig. 2) consisted of a buffer tank (1 m^3 with a mechanical stirrer (150 rpm)) for homogenization and pH adjustment followed by the fermenter (an upflow anaerobic sludge blanket reactor of 2 m^3). Milk permeate was pumped from the dairy factory to the buffer tank, where two peristaltic pumps (SEKO Kronos 50, Italy) were connected for acid (HCl, 33%) and base (NaOH, 50%) dose for pH adjustment. From the bottom of the buffer tank, milk permeate was pumped to the anaerobic reactor, which had a continuous recirculation from the top to the bottom. The temperature of the reactor was maintained at 37 °C ± 1 °C by water from an electric heater connected to the reactor jacket.

The anaerobic reactor was inoculated with 300 L of anaerobic biomass (33.6 ± 5.2 g VSS/L) from the same sewage sludge anaerobic digester as for the lab-scale reactor. A program logic controller (PLC) (Siemens SISTEMATIC S2-300, Germany) was used to control and monitor the feed flow rate, the temperature and the pH. The redox potential was online monitored and both the buffer tank and the reactor were provided with level sensors and low/high level alarms. The HRT was set at 2 days, with a pH of 5.5. Influent and effluent samples were taken three times per week to determine TS, VS, TSS, VSS, total and soluble COD and individual VFAs.

2.3. Analytical methods

COD, Total Nitrogen (TN) and NH4+-N concentrations were measured using Hach-Lange standard test kits (Hach, Germany). TS, TSS, VS and VSS were determined according to the Standard Methods (APHA-AWWA-WEF, 2017).

Individual VFAs, such as acetic acid (HAc), propionic acid (HPr), isobutyric acid (Hi-but), butyric acid (HBut), iso-valeric acid (Hi-val) and valeric acid (HVal), were determined by high performance liquid chromatography (HPLC) after filtration of the samples through Millipore 0.20 μm filters. A Hewlett Packard chromatograph equipped with a supelcogel C-610 column and two detectors connected in series, an ultraviolet detector and a refractive index detector, was used. A 0.1% phosphoric acid solution was used as mobile phase, with a flow rate of 0.5 mL/min. The column temperature was maintained at 30 °C and detection was performed at a wavelength of 210 nm.

2.4. Statistical analysis

The data obtained from the operation of the lab scale reactor were subjected to statistical analysis by ordinary least squares regression. The objective of these regressions was to ascertain the relationship between a dependent variable (such as acidification yield) and one or more independent variables, related to the process design (such as HRT and/or pH) given their variations during the experimental campaign. The analysis was conducted in MATLAB (version R2023B, MathWorks, USA).

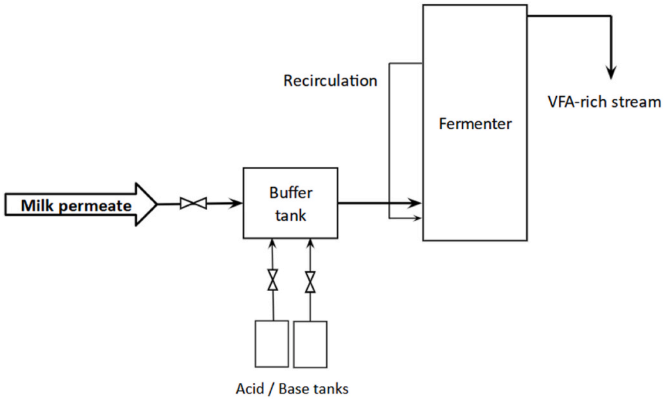

Fig. 2. Diagram of the pilot plant.

2.5. Calculations

The acidification yield was calculated as the amount of VFA produced divided by the amount of substrate fed, in COD basis, according to the following equation (1):

$$\textit{Acidification yield}\ (\%) = \frac{\Sigma\ COD_{VFA}}{COD_{FED}} \cdot 100 \tag{1}$$

where COD_{VFA} is the total VFA concentration expressed in COD units in the effluent (g COD/L) and COD_{FED} is the total COD concentration in the feeding (g COD/L).

Protein concentration (g Pr/L) was estimated by the following equation (2) (Shanthi et al., 2021):

$$Pr = Norg \cdot 6.25 \tag{2}$$

where N_{org} is the organic nitrogen concentration (TN minus N-NH_4^+, NO_2^- and NO_3^-) (g N/L). Nitrite and nitrate concentrations were assumed zero. A conversion factor of 6.25 g Protein/g N was used to convert the nitrogen content into a protein content, assuming an average nitrogen content in the protein of about 16%.

Carbohydrate (Ch) and lipid (Lp) concentrations were estimated by the simultaneous resolution of equations (3) and (4), assuming COD values of 1.1 g COD/g carbohydrate, 1.6 g COD/g protein and 2.9 g COD/g lipid (Henze, 1996; Taboada-Santos et al., 2019).

$$Ch = \frac{COD - (1.6 \cdot Pr) - (2.9 \cdot Lp)}{1.1} \tag{3}$$

$$Lp = VS - Pr - Ch \tag{4}$$

3. Results and discussion

3.1. Milk permeate characterization

Table 1 shows the characterization of the milk permeate used in the different experiments of this study. These values correspond to the initial characterization for batch tests (n = 2), while for lab-scale (n = 70) and pilot plant (n = 68) experiments, they were determined throughout the experimental period. The relatively high variability of some parameters is due to the use of a real industrial effluent. The greatest variability was observed for the VFAs (5.7 ± 2.9), as well as for solids: TSS (7.3 ± 6.4 g/L) and VSS (6.7 ± 5.8 g/L).

pH was 5.88 ± 0.93 during the pilot reactor operation, but lower (average of 4.24 ± 0.37) during lab-scale reactor operation due to the production of VFAs during the storage. This was confirmed by the VFA concentration, which was seven times higher during lab-scale reactor operation (average of 5.9 ± 1.7 g COD/L) compared to the pilot plant tests (0.8 ± 0.2 g COD/L).

Milk permeate had a notable total solids concentration ranging from 42.9 to 59.1 g/L, most of them (88%) organic solids. These solids correspond mainly to the lactose content, which is soluble and permeates through the UF membrane. Soluble COD represented approximately 97% of the total COD, which varies between 55.7 and 76.3 g/L. This organic matter is predominantly composed of carbohydrates (76%), with lower proportions of lipids (16%) and proteins (8%). Overall, milk permeate is an excellent source of lactose (carbohydrates) and some other nutrients that can support microbial growth for butyric acid production.

3.2. Acidification yield of milk permeate

Batch test was performed to evaluate the maximum conversion of the

Table 1
Main characteristics of the milk permeate used in the batch tests and during lab-scale reactor and pilot plant operation.

Parameter	Batch test pH 7.0	Lab-scale					Pilot plant
		HRT 5d pH 6.5	HRT 3d pH 6.5	HRT 2d pH 6.5	HRT 2d pH 6.0	HRT 2d pH 5.5	HRT 2d pH 5.5
pH	4.90 ± 0.01	3.85 ± 0.19	4.07 ± 0.10	4.10 ± 0.39	4.66 ± 0.51	4.60 ± 0.66	5.88 ± 0.93
TS (g/L)	53.7 ± 3.5	59.0 ± 17.7	59.1 ± 7.0	42.9 ± 7.1	58.9 ± 33.3	47.1 ± 5.4	49.6 ± 19.3
VS (g/L)	48.8 ± 3.2	52.1 ± 14.6	52.6 ± 6.4	37.0 ± 7.6	53.0 ± 18.3	38.4 ± 4.8	43.1 ± 18.7
TSS (g/L)	5.1 ± 3.5	7.3 ± 6.4	11.8 ± 0.9	5.5 ± 3.3	3.5 ± 0.7	2.6 ± 0.8	5.2 ± 4.1
VSS (g/L)	4.6 ± 2.7	6.7 ± 5.8	11.5 ± 0.9	5.0 ± 2.9	3.5 ± 0.7	2.3 ± 0.6	4.9 ± 3.8
Total COD (g/L)	62.1 ± 1.8	71.8 ± 15.8	76.3 ± 3.0	55.5 ± 6.9	66.1 ± 19.4	61.0 ± 4.9	55.7 ± 7.3
Soluble COD (g/L)	55.9 ± 1.3	70.8 ± 17.8	74.2 ± 3.2	51.6 ± 7.6	64.7 ± 18.9	59.3 ± 4.6	54.8 ± 5.1
TN (g N/L)	0.3 ± 0.0	0.5 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.5 ± 0.6	0.5 ± 0.2	0.2 ± 0.1
NH_4^+-N (g N/L)	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
VFAs (g COD/L)	1.0 ± 0.1	6.3 ± 0.9	7.3 ± 1.3	4.6 ± 2.5	5.7 ± 2.9	5.4 ± 1.9	0.8 ± 0.2
Lactic acid (g COD/L)	ND	7.5 ± 1.3	4.1 ± 1.2	3.2 ± 1.3	2.4 ± 0.6	2.1 ± 0.5	1.2 ± 0.7
Carbohydrates* (g/L)	42.8 ± 2.9	42.1 ± 16.2	38.7 ± 11.0	26.1 ± 11.1	24.0 ± 16.2	25.7 ± 6.2	35.3 ± 6.7
Proteins* (g/L)	1.9 ± 0.0	2.6 ± 0.9	4.7 ± 1.2	3.9 ± 1.2	4.5 ± 1.4	2.9 ± 1.2	1.6 ± 0.5
Lipids* (g/L)	4.2 ± 0.7	7.5 ± 4.5	9.2 ± 4.5	6.5 ± 3.7	5.6 ± 1.9	9.8 ± 2.2	5.4 ± 2.9

ND: Not determined; *Estimated values (equations (2)–(4); Section 2.5).

milk permeate (Fig. 3). During the initial three days of operation, individual acid concentrations stayed below 2.0 g COD/L, reflecting the acclimatization period of the inoculum to the substrate. The maximum acid concentration was observed on day 7, with a value of 15.7 g COD-VFA/L. It is important to note that the batch test was not extended beyond one week. This was since such a long hydraulic retention time would significantly limit scalability and productivity. This decision was in line with the objective of developing a viable process for the valorization of milk permeate into butyric acid on a pre-industrial scale.

The VFA acidification yield was 31% (expressed as g VFA/g tCOD). However, during the test run, the production of gas was observed, indicating methanation or H_2 production, as no selective inhibition was applied to this step. Assuming that all biogas produced was methane (worst-case scenario), it is estimated that a maximum of 23% additional tCOD to VFA conversion could have occurred if methanation had been inhibited. The VFA acidification yield achieved expressed as g VFA/g SCOD was 47%. This result is consistent with previous studies, such as Atasoy et al. (2020) who used cheese processing wastewater as substrate at pH 10. They observed yields of 36% and 38% (g VFA/g sCOD) with inoculum of small granular sludge and slurry sludge, respectively. Similarly, Molinuevo-Salces et al. (2024) obtained a yield of 37% from cheese whey with an initially adjusted pH of 5.5, using inoculum from a municipal wastewater treatment plant, as is also the case in the present study.

The composition of the VFA obtained revealed butyric acid as the dominant compound, with a production of 12.2 g COD/L. During operation, the pH initially adjusted to 7.0, decreasing to 5.1. This pH drop, when combined with the carbohydrate-rich composition of the milk permeate, resulted in butyric acid being produced as the predominant acid, as expected.

3.3. *Influence of hydraulic retention time on milk permeate acidification and butyric acid production*

Three HRT values (5, 3 and 2 days) were studied at pH 6.5 in 5L continuous reactors to assess the impact of this operating parameter on the VFA production (Fig. 4) (average and standard deviations at Supplementary material). The objective was to identify the shortest HRT that would not compromise acid productivity (g VFA/L·d) and acidification yield. The acidification yield showed almost no variation (5% of maximum variation) for the different retention times tested, with an average value of 60%. This yield was slightly higher than the one achieved in the batch tests, probably due to biomass adaptation to the substrate, and 2-fold higher than the value achieved by Bengtsson et al. (2008), fermenting cheese whey at pH 3.6. However, total VFA concentration was lower at HRT of 2 d (33.5 g COD/L, Fig. 4) due to the lower total COD concentration of the milk permeate used during this period (Table 1).

In contrast, HRT had a significant effect on product spectrum (Fig. 4). The highest production of butyric acid occurred at an HRT of 5 days, with a concentration of 19.1 g COD/L (butyric acid yield: 27%). Reducing HRT to 3 d and 2 d affected negatively butyric acid production in favour of acetic and lactic acid. Regueira et al. (2021) developed a metabolic model to predict and analyse the conditions under which

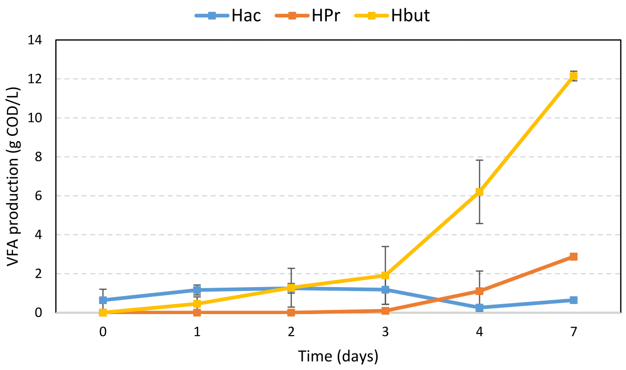

Fig. 3. VFA production over time during batch tests with milk permeate.

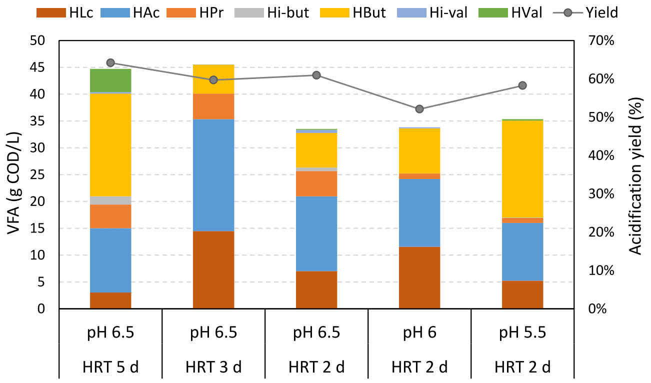

Fig. 4. Acidification yield and product spectrum during milk permeate fermentation at different hydraulic retention times (HRT) and pH values.

lactate production from glucose in a continuous reactor could be beneficial for microorganisms. The findings of this study predicted that lactate production occurs at high dilution rates (low HRT), provided that amino acids are present in the culture medium. In minimal medium and at lower dilution rates (high HRT), butyrate production is predominant, as observed in the present study.

Interestingly, iso-butyric and valeric acids were only present at HRT of 5 d, which is likely related to protein degradation under these conditions. Bevilacqua et al. (2020) showed that the production of iso-butyric acid is linked to the degradation of the amino acid valine. Furthermore, valeric acid is also obtained through the fermentation of specific amino acids instead of sugars (Bevilacqua et al., 2022). Considering that the produced concentration of iso-butyric and valeric acids was 5.9 g COD/L and the protein present in the substrate was 4.1 g COD/L, there was an additional mechanism of acid formation. For example, there may have been an elongation chain of the propionic acid, possibly with lactate as an electron donor, which contributed to the formation of valeric acid. Regarding iso-butyric acid, it could be produced by isomerising n-butyric acid to reduce toxicity in the fermenting environment, as noted by Petrognani et al. (2020).

From an industrial perspective, not only yield matters, but also the productivity. Butyric acid productivity was 3.8, 1.8 and 3.2 g HBut/L·d for HRT 5, 3 and 2 days, respectively (Fig. 5). Taking into account the lower risk of mechanization and the enhanced treatment capacity with a smaller reactor volume, resulting in lower investment, operational and maintenance costs, the HRT of 2 d was selected for the pilot plant.

3.4. *Influence of pH on milk permeate acidification and butyric acid production*

To increase butyric acid production at an HRT of 2 d, lower pH values were tested (6.0 and 5.5) in 5 L continuous reactors, since acidic pH increases butyric acid production from carbohydrates (Garcia-Aguirre et al., 2017). Furthermore, an acidic pH is more economical to control than a basic pH during anaerobic fermentation.

As expected, lower pH values slightly affect VFA yield, which remained at around 55%, but promoted butyric acid production (Fig. 4). Butyric acid production reached 18.1 g COD/L (30%), corresponding to a productivity of 9.0 g HBut/L·d at pH 5.5, almost 3-fold higher than at pH 6.5. Moreover, the butyric acid yield was similar to that obtained at higher HRT, but with much higher productivity.

Overall, both HRT and pH affected significantly butyric acid production from milk permeate. Ordinary least square analyses (Supplementary material) showed that an increase of 1 point of pH is associated with a decrease of 0.27 ($g \cdot L^{-1}$ HBut/$g \cdot L^{-1}$ total VFA) while an increase of 1 d of HRT leads to an increase of 0.07 ($g \cdot L^{-1}$ HBut/$g \cdot L^{-1}$ total VFA). The R^2 of the linear model for these two predictors is close to 0.46, showing that, more than 50% of the variability of the results is due to other mostly unidentified causes.

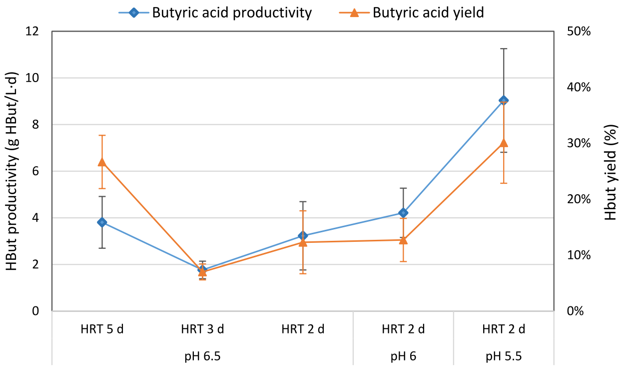

Fig. 5. Butyric acid productivity and yield in the lab reactors (5 L) with different HRT and pH.

3.5. *Butyric acid production from milk permeate at pilot scale*

The designed and constructed pilot plant was installed in a dairy industry and operated for 160 days. The optimal conditions for an increased butyric acid production, both in terms of yield and productivity, achieved in the lab-scale reactor were applied: HRT of 2 d and pH of 5.5. To the best of our knowledge, this is the first experience of milk permeate valorization at pilot plant scale.

The main operational challenge was to control the reactor pH. The rapid acidification, together with the large volume of the reactor (2 m^3) and the low alkalinity of the milk permeate (80 mg $CaCO_3$/L), hindered an effective pH control. Yet, the average value of the entire operation was 5.3 ± 0.3.

Fig. 6 shows pilot plant performance in terms of COD conversion into VFAs. No COD loss occurred throughout the experiment since the total COD in the influent and effluent were similar. The latter indicates that methanization was negligible. After 8 days of operation, the VFA yield started to increase gradually up to 21%. Due to a failure with the internal recirculation pump (days 32–50), biomass was washed-out from the reactor, and consequently, VFA production decreased. The VFA production recovered from day 60 onwards, with average yield and total VFA concentrations of 19 ± 3% and 10.7 ± 1.6 g/L, respectively. These results are significantly lower than those achieved in the lab-scale reactors, and therefore, a detailed analysis is required to determine possible causes. Differences in pH (0.2 units) and OLR (31 g COD/L·d in the lab scale reactors and 28 g COD/L·d in the pilot plant) were not considered relevant. On the contrary, temperature and biomass concentration might explain these results. Due to large variations in the external temperature, the fermenter temperature could be subjected to greater temperature fluctuations. In addition, the biomass concentration in the lab-scale reactor was 2-fold (5.1 g VSS/L) higher than in the pilot reactor (2.6 g VSS/L). The combination of these two factors probably hampered biomass activity, thus affecting the overall performance.

In the pilot plant, despite the lower acidification yield, butyric acid remained dominant in the product spectrum throughout the pilot plant operation (Fig. 7) (average and standard deviations at Supplementary material). At steady state (days 60–160), the average concentration of butyric acid was 6.3 g HBut/L, corresponding to a yield of 11% (COD inlet converted to HBut). This validates the operational conditions for butyric acid production from milk permeate at pilot-scale with to a productivity of 3.1 g HBut/L·d.

4. Conclusions

Butyric acid production from milk permeate is maximized at higher HRT and lower pH values, respectively. From an industrial perspective, balancing both productivity and yield, the highest butyric acid

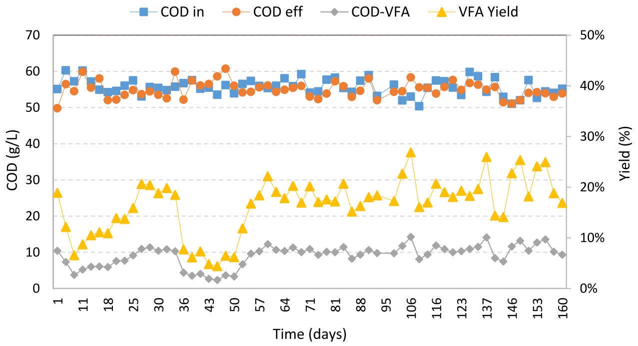

Fig. 6. COD balance and volatile fatty acids production during pilot plant operation.

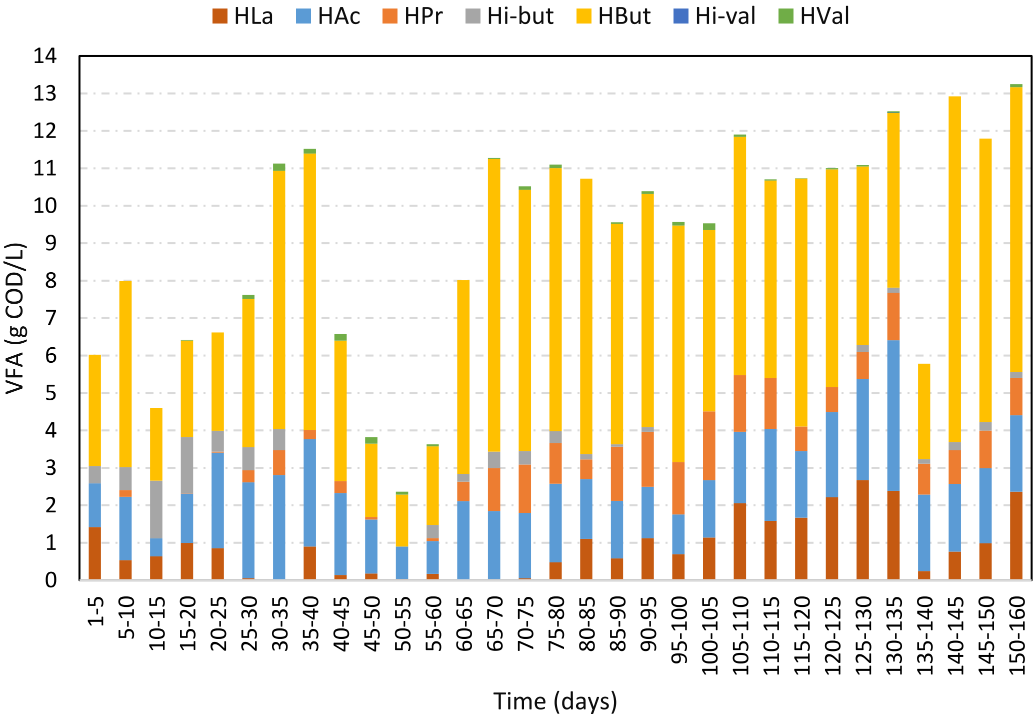

Fig. 7. Evolution (percentage in COD basis) of volatile fatty acids and lactic acid production throughout the pilot plant operation.

production (9.0 g HBut/L·d, 58% yield) was achieved at pH 5.5 and an HRT of 2 d. Yet, some limitations related to the scaleup of the process must still be overcome, such as the exploration of biomass retention strategies, which could help to improve process stability at larger scales. Overall, this study is a further step towards a promising valorization route for dairy by-products on a representative scale, thus contributing to increasing the sustainability and profitability of the sector.

CRediT authorship contribution statement

Tamara Casero-Díaz: Writing – original draft, Visualization, Investigation, Conceptualization. **Celia Castro-Barros:** Writing – review & editing, Project administration, Conceptualization. **Miguel Mauricio-Iglesias:** Writing – review & editing, Supervision. **Marta Carballa:** Writing – review & editing, Supervision.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.124934.

Data availability

Data will be made available on request.

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