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Production of Volatile Fatty Acids from Cheese Whey and Their Recovery Using Gas-Permeable Membranes

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Abstract: The use of anaerobic fermentation to produce volatile fatty acids (VFAs) is an environmentally sustainable alternative for cheese whey (CW) valorization. This study evaluates the effect of pH control on the conversion of organic matter to VFAs from CW and assesses VFA recovery using a novel approach based on gas-permeable membranes. VFA bioconversion and composition were studied with initial and sequential control of pH, both in acidic and alkaline conditions. Bioconversion efficiencies for assays with initial pH control were 36% and 45% for acidic and alkaline conditions, respectively. Sequential control of pH resulted in an increase in bioconversion to 54% under acidic conditions. Under acidic conditions, a variety of VFA was produced (mainly butyric, acetic, and propionic acids), while under alkaline conditions the majority was acetic acid. VFA recovery using a novel system of tubular gas-permeable membranes accounted for 15% and 100% of the total VFA from effluent 1 (butyric, acetic, and propionic acids) and effluent 2 (mainly acetic acid), respectively.

Keywords: cheese whey; anaerobic digestion; volatile fatty acids; kinetic study; gas-permeable membranes

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1. Introduction

Global milk production reached 852 Mt in 2019 and it is expected to grow in the coming years [1]. Cheese whey (CW) is a liquid by-product obtained from cheese production. Approximately 90% of the volume of milk processed for cheese production is converted to CW. This by-product contains lactose, proteins, lipids, and salts [2]. The current uses and management strategies for CW include animal feed, biogas production, and the conversion of CW into valuable products such as whey powder, lactose powder, or protein concentrate [3]. The use of CW to produce novel bioproducts is a green alternative to the proper disposal of this effluent, since innovative and sustainable solutions are needed to ensure the competitiveness of the dairy sector.

Anaerobic digestion (AD) is a widely implemented technology which results in sustainable methane production while stabilizing organic wastes [4]. The AD process consists of four stages, namely hydrolysis, acetogenesis, acidogenesis, and methanogenesis. Traditionally, research has been focused on maximizing methane production. However, due to the value of the intermediate compounds, this technology is shifting to the production of volatile fatty acids (VFAs). VFAs are carboxylic acids with seven or fewer carbon atoms and have a high added value as they are mostly produced by converting petrochemicals. More specifically, VFAs have market values in the range of 450–2800 EUR per ton while biogas is 110 EUR per ton [5]. The use of anaerobic reactors to produce VFAs is a relatively new concept [6], if it is compared to anaerobic digestion to produce methane. The resultant fermentation product can be purified to VFAs, which can be used as building blocks for polyhydroxyalkanoate (PHA) production [7].

In recent years, different types of substrates have been studied for VFA production, including the organic fraction of municipal waste, agricultural by-products, or cheese whey [8–10]. The accumulation and composition of the produced VFAs depend on several factors, such as pH, substrate composition, reactor design, organic loading rate, or the ratio of substrate to inoculum [10–12]. Thus, the results obtained suggest that basic pH leads to increased acetate production, whereas acidic pH leads to increased VFA conversion yields. However, it is still not clear how pH affects VFA production and variability [10,13]. Regarding VFA production, some strategies have been successfully evaluated to maximize production yields, including pH control and inhibition of methanogenic activity with chemicals [8,9,14]. However, more research is needed to maximize the conversion of organic matter to VFA and to understand the composition of the VFA produced. Since anaerobic digestion is directly influenced by its operating conditions, a kinetic study would be able to optimize, predict, and simulate the processes under different conditions. However, there are few studies dealing with the kinetics of VFA production. Some models have been used to predict VFA production, such as the Monod model, a pseudo-first-order model; the Gompertz model; or a combination of different models [15,16].

The recovery of VFAs produced during anaerobic fermentation is still a challenging task because the liquid matrix obtained contains a complex variety of compounds. Different techniques have been proposed for VFA recovery, including solvent extraction, absorption, electrocoagulation, electrodialysis, microfiltration and/or nanofiltration, pervaporation, and ion exchange membranes [17,18]. Gas-permeable membrane (GPM) technology has proven to be an efficient technology for nutrient recovery. The advantages of this technology include low energy consumption, low operating pressure, and lack of pretreatment of effluents, which can be considered as interesting advantages for VFA recovery and concentration [19,20]. The use of GPM to recover and concentrate VFA has been scarcely studied [17,21], so that this work presents a novel and sustainable approach to overcome a bottleneck, as it is the recovery and purification of VFA.

The objectives of this study are to evaluate the effect of pH control on the conversion of organic matter to VFA by anaerobic fermentation of CW and to assess VFA recovery using GPM technology. Initially, two pH levels were tested for VFA production, namely pH 5.5 and pH 10. In the first assay, pH control was performed at the beginning of the experiments. In the second assay, the pH was sequentially controlled, so that it was stable throughout the experiments. In both assays, VFA production efficiency and VFA composition were investigated. Then, two mathematical kinetic models (i.e., first-order and second-order kinetic models) were used to determine the VFA production potential and the maximum VFA production rate. Finally, the VFA recovery yield and the composition of recovered VFA using gas-permeable membranes were studied.

2. Results and Discussion

2.1. Batch Experiments

2.1.1. Bioconversion of Organic Matter to VFA

Cheese whey was anaerobically fermented to produce VFA under different conditions, namely with initial pH control (CW_5.5, CW_10), with 2-bromoethane sulfonate (BES) addition (CW_BES) and with sequential pH control (CW_5.5c, CW_10c). A decrease in pH with time was observed for all experiments that had initial control over pH and BES addition (Table 1). This decrease in pH accounted for 0.4 points, 2.5 points, and 1.6 points for CW_5.5, CW_10, and CW_BES, respectively. The decrease in pH was the result of an accumulation of VFA in the reactor due to the hydrolysis and acidification of organic matter. These VFAs were not converted to methane as they were observed in the periodic gas composition analyses where no methane was detected. Thus, this resulted in the accumulation of VFAs and the acidification of the media. The quick hydrolysis and acidification

of CW resulted in an inhibition of the methanogenic activity. A decrease in pH of approximately 2 points was also observed by Garcia-Aguirre et al. [22], who worked in VFA production under alkaline conditions using different organic wastes as substrate.

The concentration of total chemical oxygen demand (TCOD) and VFA in the media (g COD L^{-1}) and the VFA bioconversion percentage for the different assays are presented in Table 1. The concentration of TCOD was stable during the experiments and a variable proportion of that TCOD was converted to VFA. VFA concentrations accounted for 7.12 ± 0.14, 8.82 ± 0.00, and 8.22 ± 0.27 g COD L^{-1}, for the fermentation experiments with initial pH control (CW_5.5, CW_10, and CW_BES), respectively. In the case of sequential pH control, VFA concentrations were 9.17 ± 0.59 and 6.80 ± 0.94 g COD L^{-1} for CW_5.5c and CW_10c, respectively. In terms of VFA bioconversion efficiencies, the addition of BES resulted in conversion rates like those found with initial pH control, so it was decided not to continue with the BES strategy. This decision was made with the goal of increasing process sustainability and economic viability, since the high cost of BES would increase the price of the process [23].

Table 1. Chemical parameters and bioconversion percentage. Standard deviation is shown in parenthesis.

Experiment	Initial pH	Final pH	TCOD Initial (g L^{-1})	TCOD Final (g L^{-1})	VFA Final (g COD L^{-1})	Bioconversion (%)
CW_5.5	5.50	5.09	19.53 (1.87)	21.24 (5.17)	7.12 (0.14)	36.47
CW_10	10.00	7.52	19.53 (1.87)	17.23 (0.54)	8.82 (0.00)	45.14
CW_BES	7.41	5.85	19.87 (2.29)	19.69 (2.71)	8.22 (0.27)	41.40
CW_5.5c	5.70	6.00	17.07 (0.22)	16.50 (1.00)	9.17 (0.59)	53.72
CW_10c	9.40	9.90	17.66 (0.80)	16.30 (0.92)	6.80 (0.94)	38.45

VFA bioconversion efficiencies for the experiments with initial pH control were 36% and 45%, for acidic and alkaline conditions, respectively. Under acidic conditions, the sequential pH control resulted in a significant increase in TCOD bioconversion to VFA, reaching values of 54%. The difference in TCOD bioconversion between CW_5.5 and CW_5.5c was related to the pH adjustment. In this manner, a stable pH during the whole experimental time (CW_5.5c) resulted in a higher TCOD bioconversion than that obtained in the experiment where the pH was adjusted just at the beginning of the experimental time (CW_5.5). However, the obtained bioconversion values were not as high as those reported by Calero et al. [24], who achieved VFA yields up to 97% working in a pH range between 5 and 6. This was probably due to the fact that these authors supplemented the media with nitrogen and phosphorus sources to achieve a C:P:N ratio of 100:3:1.

In the case of the alkaline conditions, the obtained bioconversion rates were slightly higher than those previously reported by Atasoy et al. [10], using CW as a substrate for VFA production. These authors reported VFA yields in the range of 18–38%, working with initially adjusted alkaline conditions and an inoculum source similar to the one used in the present study. Maintaining the alkaline pH throughout the experiment did not further improve the percentage of bioconversion of TCOD to VFA (Table 1). This finding is contrary to Garcia-Aguirre et al. [22], who reported that alkaline conditions resulted in higher VFA yields than acidic conditions working with different organic waste streams from industry. However, Lu et al. [13] obtained better results with potato peel waste as a substrate and acidic pH (0.27 g VFA-COD g VS^{-1}), when compared to alkaline conditions (0.03 g VFA-COD g VS^{-1}).

2.1.2. VFA Composition

The acidification of the hydrolysis products (i.e., amino acids, sugars, long-chain fatty acids, and glycerol) can be performed by different groups of bacteria using different metabolic pathways, resulting in different products. For this reason, the composition of the VFA produced was determined by the operating conditions. For example, acidic conditions resulted in a variety of volatile fatty acids, while alkaline conditions resulted mostly in acetic acid (Figure 1).

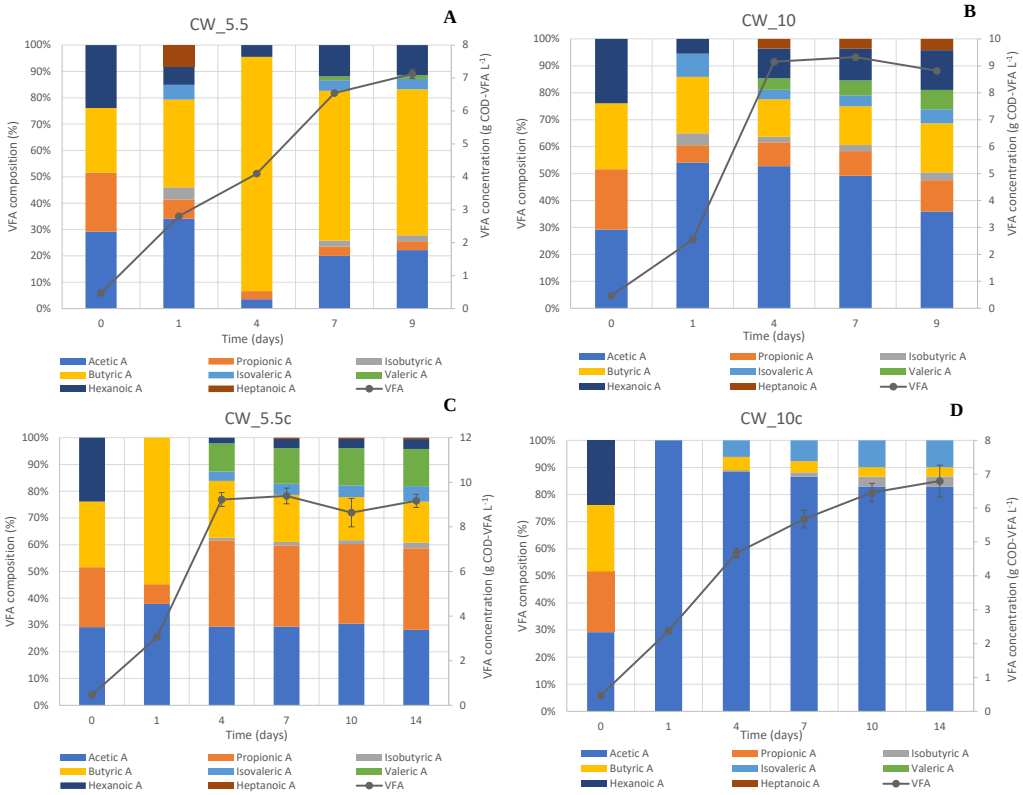

Figure 1. Composition of VFA for experiments with (**A**) acidic conditions with initial pH control (CW_5.5), (**C**) acidic conditions with sequential pH control (CW_5.5c), (**B**) alkaline conditions with initial pH control (CW_10), and (**D**) alkaline conditions with sequential pH control (CW_10c). The black line shows VFA concentration with time. Differences between duplicated assays are shown by error bars.

More specifically, under acidic conditions and initial pH control (CW_5.5), butyric acid represented 55% of the total production of VFA, reaching values up to 89% after 4 days of fermentation (Figure 1a). With a sequential control of pH (CW_5.5c), a higher variety of acids was obtained, with acetic and propionic acids being the most abundant at the end of the experimental time (2.6 and 2.8 g COD L^{-1}, respectively) (Figure 1c). These results could be the result of the type of fermentation pathway. In the first case (CW_5.5), the butyrate fermentation pathway was dominant from the 4th day of the experiment (Figure 1a). Previous studies stated that the butyrate-type fermentation is dominant under uncontrolled pH conditions [14].

However, when the pH was most of the time maintained a 5.5 (CW_5.5c), different metabolic pathways were probably followed simultaneously, one to produce acetate/propionate and another to produce butyrate. At the end of the experiment, acetate, propionate, butyrate, and valerate accounted for 28%, 31%, 15%, and 14% of the total VFA, respectively (Figure 1c). The acetate/propionate pathway was the preferred route at the end of the experimental time, since those two products were the most abundant. Propionate

and acetate production have been associated with anaerobic fermentation of carbohydrates at mesophilic temperatures [22], so in this case this pathway was probably favored due to the composition of CW, which is mainly composed of lactose. In addition, propionic acid production has been previously related to lipid-rich fermentation substrates throughout the glycerol pyruvate fermentation pathway [24]. On the other hand, the butyrate pathway is related to the high presence of carbohydrates in the feedstock [9]. Finally, valerate production started after day 4, indicating that it was probably the result of a lactate-based chain elongation to valerate [25,26].

Alkaline fermentations (i.e., CW_10 and CW_10c) resulted in a high production of acetic acid, in the range of 3.2–5.8 g COD L^{-1}, with productions up to 82% of acetic acid after 14 days of fermentation in CW_10c (Figure 1d). This result is consistent with previous studies that found that alkaline pH favors the metabolic pathway for acetate/ethanol production [13]. If CW_10 and CW_10c are compared, it is clear that the sequential control of pH was a key parameter driving the production of VFAs towards acetic acid, most probably following the acetate ethanol metabolic pathway. This fact was also observed by Cabrera et al. [27], where acetic acid represented up to 79.3% of the total VFAs when they worked at pH 9. Although in CW_10 there was a higher variability of VFAs than CW_10c (Figure 1b,d), this suggests that keeping the basic pH constant favors the production of acetic acid, through the acetate ethanol metabolic pathway. This behavior can be verified by comparing the two samples CW_10 and CW_10c, where it is observed that when the pH was just initially controlled, the production of VFA increased the acidity (ending the fermentation with a pH of 7.52, Table 1) and a higher production of other VFAs than acetic acid. These results fit well with previous studies reporting that anaerobic fermentation at high pH values resulted in a high conversion of organic matter to acetate [13,28].

2.2. Kinetic Study

The behavior of VFA production with respect to the operating pH and the corresponding adjustments of the kinetic models are shown in Figure 2. The parameters of the kinetic models, as well as the configuration data and the results of the Bayesian information criterion (BIC) tests are presented in Table 2. The different operating conditions (with initial and sequential pH control) presented correlation coefficients (r^2) higher than 0.94, indicating that the two models fit with the experimental data well. For BIC values, the lower the value, the better the fit of the data to the model. Thus, a good fit is represented by high values of r^2 and low values of BIC [29].

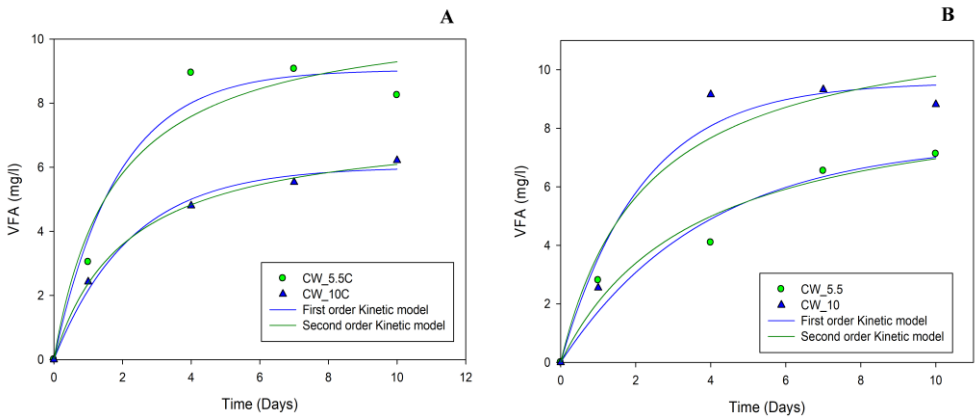

Figure 2. Adjustment of the kinetic model with the experimental data to first and second order: (**A**) with sequential pH control and (**B**) with initial pH control.

Table 2. Parameters of kinetics models.

Experiment	1st-Order Model					2st-Order Model				
	k_1	VFA_{max}	SSE	r^2	BIC	k_2	VFA_{max}	SSE	r^2	BIC
CW_5.5	0.247	7.80	2.10	0.9507	0.2	0.026	9.90	1.53	0.9565	−1.07
CW_10	0.458	9.62	2.77	0.9687	1.3	0.034	12.32	4.11	0.9476	2.88
CW_5.5c	0.475	9.21	2.25	0.9671	0.47	0.051	10.92	3.88	0.9434	2.65
CW_10c	0.338	7.00	0.73	0.9882	−4.03	0.064	7.38	0.04	0.9986	−15.65

The data corresponding to experiment CW_5.5 showed high values of r^2 (0.9565) and the lowest value of BIC (−1.07) for the second-order model, indicating a good fit, so that these data are better described when a second-order model is used (Figure 2b). Conversely, for the experiment with the sequential control of pH under acidic conditions (CW_5.5c), the best fit was obtained when the first order model was used, with a r^2 of 0.9671 and a BIC value of 0.47 (Figure 2a). This finding was also confirmed by the VFA_{max} value of the first order model for CW_5.5c, which also fits well with the experimental data, with a VFA value of 9.17 g COD L^{-1}, and the value predicted by the model, which was 9.21 g COD L^{-1}. The values of k correspond to the hydrolysis rate constant, so that low k values indicate a low biodegradability rate. For VFA production under acidic conditions, k values were higher for sequential pH control than for initial pH control. This finding fits well with the bioconversion data, with values of 36.47 and 53.72% for initial and sequential pH control, respectively (Table 1). The higher hydrolysis rate, the greater COD bioconversion to VFAs.

When analyzing the kinetic parameters for the experiments at alkaline conditions, data corresponding to CW_10 presented BIC values of 2.88 and 1.30, for the second- and first-order models, respectively, indicating that these conditions are the least predictable for these two models. As it can be seen in Figure 2, very similar VFA production takes place during the first days for the initial pH control and the sequential pH control. However, when there is an increase in VFAs in the reactor (Figure 2b), the pH drops, and as it has been observed, an excessively high pH does not favor the production of VFAs from whey. Therefore, for the CW_10 experiment there is a large production of VFAs (8.22 g COD L^{-1}) but a worse fit, since its behavior does not develop as expected.

Meanwhile, the CW_10c experiment resulted in the lowest VFA concentration (6.38 g COD L^{-1}) but a good fit for both the first-order and second-order models (Figure 2a). For both kinetic models, CW_10c presented low BIC values and high r^2 values. For the second order model, it presented better adjustments than for the first order and obtained r^2 values of 0.9986 and BIC values of −15.65.

2.3. VFA-Recovery Experiments

The results corresponding to the VFA recovery experiments using gas-permeable membranes are presented in Figure 3. VFA recovery was performed using VFA-enriched effluents from the anaerobic fermentation of CW (Section 2.2). These effluents were named E1 and E2 and their chemical composition is shown in Table 3. The recovery percentages of total VFAs accounted for 15% and 100% of the VFAs removed from effluents E1 and E2, respectively (Figure 3). In the case of E1, up to 85% of the removed VFAs were volatilized (Figure 3a), while in E2 100% of the removed VFAs were recovered (Figure 3b). This behavior can be related to the composition of the effluents. As it can be seen in Table 3, the VFA composition of the effluents was very different. In the case of E1, a high variability of VFAs was observed, with butyric, acetic, and propionic acids being the most abundant. Conversely, E2 was mainly composed of acetic acid.

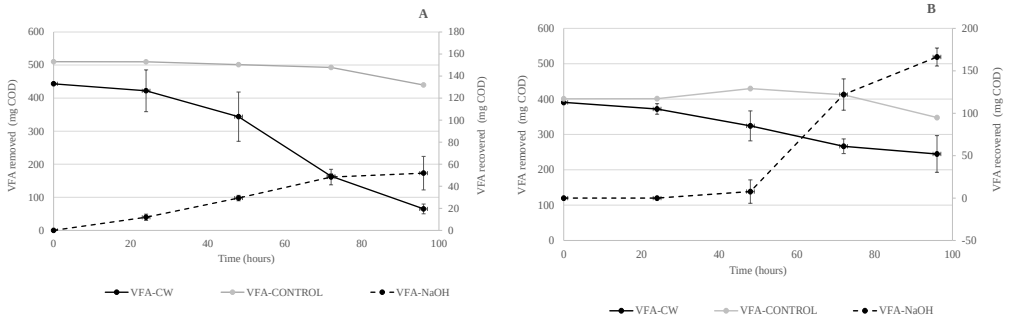

Figure 3. VFA recovery experiments from effluents E1 (**A**) and E2 (**B**). The gray lines correspond to control experiments without membranes. The solid black lines correspond to the removed VFAs in the effluent. The dotted black lines correspond to the recovered VFAs in the trapping solution. The differences between the duplicated assays are shown by error bars.

Regarding the VFA composition of the recovery solution, butyric acid and valeric acids were obtained for E1 in proportions of 85/15. In the case of E2, 86% of the total VFAs corresponded to acetic acid and 14% to valeric acid. These results are much higher than the recovery values reported by Yesil et al. [21], who obtained recovery percentages for acetic, butyric, and valeric acids of 3.3, 1.8, and 10.8%, respectively. The difference was probably due to the initial pH of the VFA effluent. They used an effluent with a pH of 6.6, whereas in our study the pH was adjusted to 4, which increased the protonation of the acids and probably the flux through the membrane. The permeation flux through the membrane after 96 h was calculated for both effluents E1 and E2. For E1, values of 0.104 and 0.002 g m^{-2} h^{-1} were obtained for the butyric and valeric acids, respectively. In the case of E2, the permeation flux values accounted for 0.364 and 0.057 g m^{-2} h^{-1} for the acetic and valeric acid, respectively. These values are in the range of those obtained by Yesil et al. [30], who worked with PTFE membrane contactors integrated in leach bed reactors for the recovery of VFA. These authors also found that the permeation flux decreased as the alkyl group of the VFAs increased. They related this decrease in the permeation flux with the vapor pressures of the different VFAs. In this manner, they found that acetic acid and propionic acid presented the highest vapor pressure, so that they had the highest permeation fluxes, compared to that of other VFAs [30].

To the best of our knowledge, these are the first results obtained using tubular GPM technology for VFA recovery, demonstrating the potential of this novel approach for VFA separation and purification from the fermentation broths. In addition to applications of the separated VFAs as carbon building blocks in a variety of industries, this technology could be used to reduce inhibition due to VFA accumulation in anaerobic digesters.

Table 3. Chemical composition of anaerobic effluents E1 and E2. Standard deviation is shown in parenthesis. n.d. stands for not detected.

Parameter	Unit	Effluent 1 (E1)	Effluent 2 (E2)
Acetic acid	mg COD L^{-1}	368 (45)	1300 (334)
Propionic acid	mg COD L^{-1}	176 (20)	n.d.
Isobutyric acid	mg COD L^{-1}	n.d.	31 (0)
Butyric acid	mg COD L^{-1}	467 (55)	25 (0)
Isovaleric acid	mg COD L^{-1}	32 (3)	48 (11)
Valeric acid	mg COD L^{-1}	31 (2)	n.d.
Hexanoic acid	mg COD L^{-1}	22 (1)	n.d.
Heptanoic acid	mg COD L^{-1}	n.d.	n.d.

3. Materials and Methods

3.1. Origin of Cheese Whey and Inoculum

Cheese whey (CW) was used as the substrate. The CW was obtained from a cheese factory located in Palencia, Castilla y León, Spain, where cheese is produced from pasteurized cow's milk. The CW was frozen until used to avoid degradation. The composition of the CW is shown in Table 4.

Table 4. Chemical composition of cheese whey. Standard deviation is shown in parenthesis.

Parameter	Unit	Value
pH	-	6.31 (0.00)
Conductivity	uS cm^{-1}	5400 (0.00)
TS	%	6.42 (0.16)
VS	%	5.93 (0.14)
Alkalinity	mg L^{-1}	954 (24)
N-NH_4^+	mg L^{-1}	155 (2)
TKN	mg L^{-1}	2781 (38)
TCOD	mg L^{-1}	138,276 (6970)
SCOD	mg L^{-1}	121,950 (3920)

The anaerobic sludge (AS) used as inoculum was collected from the municipal wastewater treatment plant of Valladolid, Castilla y León, Spain. AS with total solid (TS) and volatile solid (VS) concentrations of 18.04 ± 0.05 and 11.81 ± 0.04 g L^{-1}, respectively, was used for the assay with initial pH control. The AS for the assay with sequential pH control presented concentrations of TSs and VSs of 26.55 ± 0.00 and 13.59 ± 0.63 g L^{-1}, respectively. After the collection of AS, it was stored at 4 °C until use.

3.2. VFA Production Experiments

3.2.1. Batch Experiments with Initial pH Control

The batch experiments were carried out in bottles with a total volume of 0.57 L. Three different experiments were carried out, namely CW_5.5, CW_10, and CW_BES. The substrate (So)-to-inoculum (Xo) ratio was 1, expressed as g VS g^{-1} VS. A volume of 0.10 L of AS was used as inoculum in each bottle. The corresponding amount of CW to keep a So/Xo ratio of 1 was added. Finally, distilled water was added up to a final volume of 0.20 L. Concentrated sulfuric acid was used to adjust the initial pH to 5.5 in CW_5.5. A 1 M NaOH solution was used to adjust the initial pH to 10 in CW_10. In CW_BES, 2-bromoethane sulfonate (BES) was added to the bottles at a concentration of 2 g L^{-1} to avoid methanogenesis without pH adjustment [8]. Blank assays were performed to determine the VFA production of the inoculum in the three conditions tested, containing only 0.10 L of inoculum and water. All assays were performed in triplicate. After the set-up of each bottle, the headspace was flushed with N_2 to ensure anaerobic conditions. Then, the bottles were placed in an incubator at 38 ± 1 °C and continuous agitation was provided by a shaker. The incubation time was 9 days. The volume of gas produced by the different substrates was calculated by measuring the pressure of the bottle's headspace. The gas composition was analyzed every two days. TCOD and soluble chemical oxygen demand (SCOD) were measured at the beginning and at the end of the experiments. VFA concentration and composition were determined in the liquid fraction every two days. For this purpose, 2 mL of liquid sample was taken from the bottles with a syringe to maintain anaerobic conditions. The pH was measured in the 2 mL samples.

3.2.2. Batch Experiments with Sequential pH Control

In this case, the batch experiments were carried out in continuously stirred tank reactors (CSTRs) with 1 L of working volume. Two different experiments were carried out,

namely CW_5.5c and CW_10c. The substrate (So) to inoculum (Xo) ratio was 1, expressed as g VS g^{-1} VS. A volume of 0.50 L of anaerobic sludge was used as inoculum in each flask. The corresponding amount of cheese whey to keep a So/Xo ratio of 1 was added. Finally, distilled water was added up to a final volume of 1 L. The experiments were performed in duplicate and the pH was adjusted manually every day. Concentrated sulfuric acid was used to adjust the pH to 5.5 in CW_5.5c. A 1 M NaOH solution was used to adjust pH to 10 in CW_10c. Assays were performed at 38 ± 1 °C with continuous stirring. The temperature was maintained with a water jacket connected to a temperature-controlled water bath. Anaerobic conditions were maintained, and gas production was measured by water displacement. The incubation time was 14 days. The gas composition was analyzed every three days. TCOD and SCOD were measured at the beginning and at the end of the experiments. The VFA concentration and composition were determined in the liquid fraction every three days. For this purpose, 7 mL of liquid sample was removed from the CSTRs.

3.3. VFA Recovery Experiments

The VFA recovery experiments were performed in 0.25 L flasks containing 0.20 L of VFA-enriched effluents obtained from anaerobic fermentation of cheese whey, namely E1 and E2 (Table 3). Effluent E1 was obtained by an acidic fermentation, at pH 5.5, of the CW. Effluent E2 corresponded to a fermentation of the CW carried out at pH 10. These flasks contained a submerged tubular gas-permeable membrane connected to a VFA concentration tank containing 0.20 L of a NaOH solution with a concentration 0.1 M. The membrane was placed in a horizontal configuration and held by plastic connectors to ensure that the membrane was completely submerged in the VFA-enriched effluents throughout the experiments. This solution was recirculated using a peristaltic pump (Pumpdrive 5001, Heidolph, Wood Dale, IL, USA) at a constant rate of 12 L d^{-1}, flowing inside the tubular membranes and returning to the VFA concentration tank to complete a closed loop. The tubular gas-permeable membrane was made of expanded polytetrafluoroethylene (e-PTFE) (ZEUS Industrial Products Inc., Orangeburg, SC, USA) with a length of 30 cm, an outer diameter of 5.2 mm, and a wall thickness of 0.64 mm and a density of 0.95 g cm^{-3}.

Two identical experiments were performed, corresponding to effluents with different VFA compositions obtained after anaerobic fermentation of cheese whey. Both experiments were performed in duplicate for 96 h. In each experiment, a control flask without membrane was carried out. The pH in the effluents and the control flasks was adjusted at the beginning of the assays to ensure that the VFA were in unionized (volatile) form [17]. Samples of VFA effluents and NaOH solutions were daily taken to measure pH, VFA composition, and VFA concentration.

3.4. Analytical Methods and Yields

Analyses of pH, N-NH_4^+, TKN, VS, TS, TCOD, and SCOD were performed in duplicate in accordance with APHA [31].

Gas composition was analyzed using a gas chromatograph (Agilent 7890A, Santa Clara, CA, USA) with a thermal conductivity detector, provided by a HP-Plot column (30 m 0.53 mm 40 μm) followed by a HP-Molesieve column (30 m 0.53 mm 50 μm). Helium (7 mL min^{-1}) was used as the carrier gas. The injection port temperature was set at 250 °C and the detector temperature was 200 °C. The temperature of the oven was set at 40 °C for 4 min and thereafter increased to 115 °C.

The concentrations of acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, hexanoate, and heptanoate were determined by using a gas chromatograph (Agilent 7890A, USA) equipped with a Teknokroma TRB-FFAP column of 30 m length and 0.25 mm i.d. followed by a flame ionization detector (FID). The carrier gas was helium (1 mL min^{-1}). The temperature of the detector and the injector was 280 °C. The oven temperature was set at 100 °C for 4 min, then increased to 155 °C for 2 min, and then to 210 °C. Total

volatile fatty acids (VFAs) were calculated as the sum of these acids after applying the appropriate COD conversion factor.

VFA bioconversion was calculated according to Equation (1):

$$\% \text{ VFA bioconversion} = (\text{VFA (g COD/L)})/(\text{TCOD in (g COD/L)}) * 100 \quad (1)$$

where VFA and TCOD correspond to the concentration of VFAs at the end of the experiment and the initial TCOD in the cheese whey, respectively.

Two types of kinetic models (first-order and second-order) were selected to predict the maximum production of VFA.

$$\text{First-Order model: } VFA = VFA_{max} * [1 - \exp(-kt)] \quad (2)$$

$$\text{Modified Gompertz model: } VFA = [k_2 * (VFA_{max})^2 * t]/[1 + k_2 * VFA_{max} * t]. \quad (3)$$

where VFA_{max} is the maximum VFA yield (g COD L^{-1}) with respect to time t (day), k is the hydrolysis rate constant for first-order model (1 day^{-1}), k_2 is the hydrolysis rate constant for second-order model (1 day^{-1}), and t is the time (day).

The Bayesian information criterion (BIC) test shown in Equation (4) was used to compare the models and determine which model is more likely to be correct.

$$BIC = N \ln(SSE/N) + K \ln(N) \quad (4)$$

where N is the number of data points, K is the number of parameters fitted by the regression model, and SSE is the squared estimate of errors.

Parameter values for k, k_2 and VFA_{max} were estimated using the curve fitting of the experimental data by the SigmaPlot program.

4. Conclusions

Up to 54% of the bioconversion of organic matter to VFAs was obtained at acidic conditions under sequential control of pH. However, under alkaline conditions, the sequential control of pH resulted in a decrease in the bioconversion to VFAs, when compared to initial pH control. A variety of VFAs was obtained under acidic conditions while acetic acid was the predominant VFA under alkaline conditions. The tubular gas-permeable membranes successfully recovered VFAs, accounting for 15% and 100% of the total VFAs from effluent 1 (composed of butyric, acetic, and propionic acids) and effluent 2 (mainly composed of acetic acid), respectively. These results demonstrate that it is possible to recover and concentrate VFAs using a novel approach based on gas-permeable membranes.

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