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ORIGINAL ARTICLE

Optimizing volatile fatty acids production from fish canning wastewater: the role of feeding strategies and retention time

Tamara Casero-Díaz[1,2] · Celia Castro-Barros[1] · Marta Carballa[2] · Miguel Mauricio-Iglesias[2] · M. Salomé Duarte[3,4]

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Abstract

The transition to a circular economy requires innovative strategies for wastewater valorization, particularly in water-intensive sectors, such as fish canning. Anaerobic fermentation of fish canning wastewater to produce volatile fatty acids (VFAs) is a promising alternative to reach this goal. This study evaluated the impact of two bioreactor feeding strategies (continuous (UASB) and sequential batch (SBR)) and two hydraulic retention times (HRT) (6 and 3 days) on VFA yield and product spectrum. The results showed that both feeding modes resulted in high VFA production, exceeding 30 g VFA-COD/L. It can be concluded that the feeding mode has no impact on VFA yield. However, it does exert a significant influence on the product spectrum. Sequential feeding was found to favor butyric acid production, with a 15% higher yield compared to continuous feeding. In contrast, continuous feeding promoted the formation of propionic and valeric acids, with yields 5% and 4% higher, respectively, than those observed under sequential feeding. In contrast, variations in HRT demonstrated no significant impact on either yield or product spectrum. These findings suggest that, for the scaling up of this valorization, the selection of feeding mode should be tailored to the desired objective. Furthermore, a lower HRT (3 days) may lead to financial savings and enhanced productivity without any detrimental impact on yield or product spectrum.

Keywords Anaerobic fermentation · Carboxylate platform · Industrial wastewater · Resource recovery

1 Introduction

The transition to a circular economy has become a fundamental objective of sustainable development in the twenty-first century. In March 2020, the European Commission adopted the Circular Economy Action Plan, which aims to enhance the resilience and environmental sustainability of the European Union's economy [1]. A central aspect of this plan is the efficient use of resources and improved waste management practices. It is estimated that, globally, the municipal sector alone generates approximately 380 billion cubic meters of wastewater annually, with significant contributions from industrial activities as well [2]. Given the substantial volumes of wastewater generated and its potential for resource recovery, circular economy strategies are increasingly being implemented in wastewater management [3–5].

The fish canning industry is a major water consumer, producing considerable volumes of wastewater. On average, about 11 m3 of water are used per ton of raw material processed [6]. The composition of this wastewater varies depending on the type of raw material and specific processing activities. Typically, canning factories produce two main segregates wastewater streams: washing and cooking streams. Carrera et al. [7] characterized these streams in detail. Washing streams, which account for approximately 95% of the total volume, contain low to moderate concentrations of organic matter, nutrients, and suspended solids (1 g COD/L; 21 mg TN/L; 3 mg $P\text{-}PO_4^{-3}$/L; 0.3 g TSS/L) [7]. In contrast, cooking streams (approximately 5% of the total volume) are generated during the cooking of raw material and liquid by-products, resulting in effluents rich in fats and oils, with high levels of organic matter and nutrients (11–29 g COD/L;

✉ Tamara Casero-Díaz
tamara.casero@rai.usc.es

1 Water Technology Centre, CETAQUA, A Vila da Auga, José Villar Granjel 33, 15890 Santiago de Compostela, Spain

2 Department of Chemical Engineering, CRETUS, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

3 CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710 - 057 Braga, Portugal

4 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

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1025–1839 mg TN/L; 1–193 mg TP/L; 2.4–27 g TSS/L) [7, 8]. Elevated salt concentrations (600–30,000 mg NaCl/L) are also characteristic of both streams [9]. Washing streams are traditionally treated using physicochemical methods [10]. In contrast, cooking streams are typically treated using biological methods, both aerobic and anaerobic [11]. Emerging approaches, such as electrochemical oxidation, are also being explored as potential treatment options [12, 13].

The canning industry is confronted with two significant challenges: the effective management of effluents and the exploration of valorization strategies. One valorization strategy is water recovery, which reduces both water demand and costs [14]. Anaerobic digestion, primarily used for energy recovery, has also been explored as it allows the transformation of waste streams into biogas, which can be used for electricity or heat generation [15, 16]. However, sulfates present in canning wastewater pose a challenge by increasing hydrogen sulfide levels in digesters, requiring biogas purification [9]. In this context, volatile fatty acid (VFA) production presents an appealing alternative to biogas. VFAs, which are currently produced mainly through petroleum-based processes, have numerous applications, including biodegradable plastics production, biological nutrient removal, and as chemical reagents in the cosmetics industry [17]. Considering the increasing costs of oil and the necessity for sustainable alternatives, there has been a notable surge in interest surrounding the field of biological VFA production [18].

The conditions of anaerobic fermentation, such as pH, temperature, food to microorganism ratio (F/M), organic loading rate (OLR), and hydraulic retention time (HRT), have a strong influence on the yield and product spectrum of VFAs obtained [19, 20]. In a previous study [21], the potential of fish canning wastewater to produce volatile fatty acids (VFA) was analyzed by evaluating the F/M ratio and hydraulic retention time (HRT). The F/M ratio did not significantly affect yield, while HRT influenced the outcome depending on the water composition. The highest yield (12.6 g COD_{VFA}/L, 51%) was achieved with carbohydrate-rich wastewater (mussel cooking). Fra-Vázquez et al. [22] investigated the potential of mussel cooking wastewater for generating a VFA-rich effluent, with a particular focus on the influence of pH on both VFA yield and selectivity. The findings of this study revealed a substantial production rate of 0.72 ± 0.07 g COD VFA/L. The production of VFA from solid waste has also been studied. Bermúdez-Penabad et al. [23] investigated the effect of pH (5–10) and solid content in tuna waste, finding that alkaline conditions significantly enhanced VFA production. At pH 8, a production of 30 g COD/L was achieved, with the best results observed at 2.5% total solids (TS). These previous studies support the feasibility of using fish canning wastewaters as a source for VFA production. However, no research has yet explored the effect of the feeding mode.

Once the feasibility of VFA production from fish canning wastewaters has been demonstrated, further research is needed to optimize and scale up the process. The feeding mode, along with HRT, are crucial parameters for scaling and industrializing this valorization process as they have a direct impact on both the infrastructure costs (CAPEX) and operating expenses (OPEX). Therefore, the objective of this study was to evaluate the impact of these two operational parameters on VFA yield and product spectrum during the fermentation of fish canning wastewater.

2 Material and methods

2.1 Industrial wastewater and inoculum

Fish canning wastewater from a medium-sized fish canning industry (Galicia, Spain), which processed different raw materials (tuna, mussels and octopus, among others), was used as substrate. A large volume of wastewater was collected (around 60 L) and characterized (Table 1). Wastewater was stored at low temperature (3 °C) to prevent the degradation of the organic matter. The inoculum consisted of a microbial mixed culture of anaerobic granular sludge collected from a brewery treatment plant (Porto, Portugal), previously used to produce VFAs from wastewater containing high salinity levels [24]. The same inoculum was used for the two reactors, which were inoculated with a concentration of 17.1 ± 0.4 g VS/L.

2.2 Experimental set-up

Two twin reactors with a working volume of 2.2 L were used (Fig. 1). A double plexiglass wall allowed them to maintain the temperature at 37 °C ± 1 °C by circulation of water from a thermostatic bath. An external liquid recirculation promoted the sludge bed expansion, and therefore, a better mixing and contact between sludge and wastewater. Both reactors had a gas sample bag with N_2 at the top of the reactor to maintain anaerobic atmosphere. pH was not controlled. One of the reactors was fed in continuous mode (CR), while the other reactor was fed in sequential mode (SBR). The CR was an upflow anaerobic sludge blanket (UASB) reactor, which was fed from a glass bottle containing the substrate with constant magnetic stirring and refrigeration (4 °C). The feed and recycle flow were pumped from bottom to the top of the reactor to ensure an upflow. The SBR cycle included a feeding step (20 min), followed by anaerobic reaction (34–70 h) (recirculation active), a settling period (90 min), and the effluent discharge (5 min). The total duration of each cycle at HRT of 3 days was 36 h, while the duration of each cycle at HRT of 6 days was

72 h. The volume exchange ratio was 50%. The selection of UASB and SBR bioreactors was based on their shared configuration, enabling a direct comparison of continuous and sequential batch operation strategies. In contrast to a CSTR, these reactors require lower HRT and do not need additional sedimentation units.

Two different hydraulic retention times (HRT) of 6 and 3 days were tested in each reactor. These HRTs were selected with the aim of ensuring a sufficient period for the hydrolysis stage, while avoiding an excessively long period that could favor the methanogenic population. In the CR, the HRT was decreased by increasing the feeding flowrate, whereas in the SBR, the HRT was decreased by decreasing the reaction time, maintaining the volume exchange ratio. Four operational stages were distinguished in each reactor: adaptation, stage I (operation at HRT of 6 days), transition (from HRT 6 to 3 days), and stage II (operation at HRT of 3 days). It is important to highlight that, due to the typology of both reactors, the solids retention time (SRT) remained independent of the HRT. Samples of the influent and effluent were collected and analyzed for VFA, total and soluble chemical oxygen demand (COD), pH, and salinity, throughout the experiment. In the case of CR, samples were taken every other day, whereas for SBR, samples were taken before starting the cycle and after discharge (end of the cycle), respectively.

2.3 Analytical methods

The pH was measured with a pH-meter Consort C5020 (Consort, Belgium). Conductivity and salinity were measured using a Consort multi-parameter analyzer C3010 (Consort, Belgium). Total (tCOD) and soluble (sCOD) COD, total nitrogen (TN), and ammonium (NH_4^+-N) were determined with the spectrophotometric system Hach Lange DR 2800 using cuvette kits (Hach, Germany). The method used by these cuvettes for COD was oxidation with dichromate; for TN, it was peroxydisulfate digestion, and for NH_4^+-N, it was colorimetric detection with sodium salicylate. TN and tCOD concentrations were determined in the sample without filtering, whereas sCOD and NH_4^+-N concentrations were measured in samples filtered through 0.2-µm pore size nylon filters. Total (TS), volatile (VS), and suspended (TSS and VSS) solids were determined according to the Standard Methods (APHA, 2017). VFA samples were centrifuged for 15 min at 15,000 rpm and filtered through a 0.2-µm nylon filter. Individual VFA was analyzed using a Jasco (Jasco, Japan) HPLC equipped with a PU880 JASCO isocratic pump (Jasco, Japan), a 7971 Jones Chromatography (Jones Chromatography Limited, UK) oven at 60 °C, an AS- 2057 Plus automatic injector (Jasco, Japan), and a UV- 2070 plus detector (Jasco, Japan). The mobile phase was composed of a 2.5 mmol/L H_2SO_4 solution and was pumped at a flowrate of 0.6 ml/min.

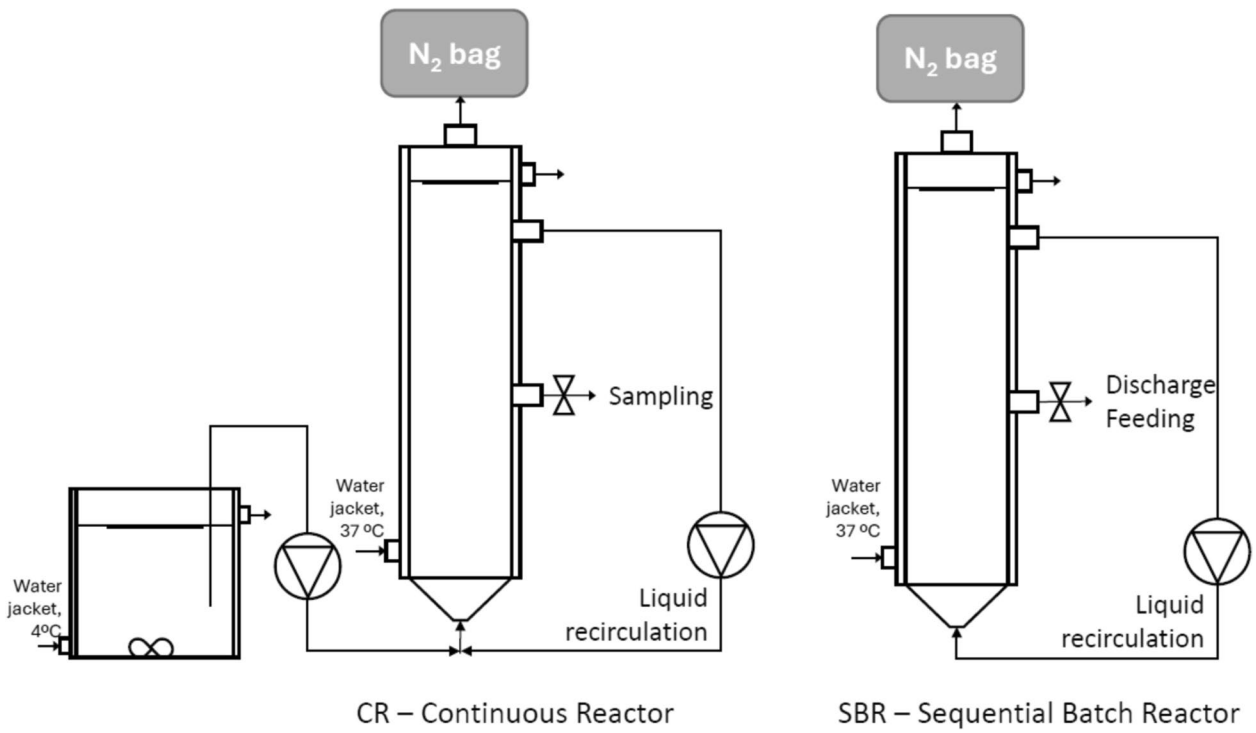

Fig. 1 Schematic diagram of the experimental set-up

2.4 Statistical analysis

Student's *t*-test for independent samples of equal variance with $\alpha = 0.05$ was used to compare the yield of total and individual acids obtained with both feeding regimes. Student's *t*-test is a statistical method for determining whether data from two independent groups are significantly different. This method assumes that the data being analyzed follow a normal distribution and the variances of the two compared groups are equal.

2.5 Calculations

The conversion of each individual VFA concentration (g/L) to COD units was done using the corresponding coefficients: 1.07 g COD/g lactic acid (HLc), 0.35 g COD/g formic acid (HFr), 1.07 g COD/g acetic acid (Hac), 1.51 g COD/g propionic acid (HPr), 1.82 g COD/g iso-butyric acid (Hi-but), 1.82 g COD/g n-butyric acid (HBut), 2.04 g COD/g iso-valeric acid (Hi-val), and 2.04 g COD/g n-valeric acid (HVal)[25].

The acidification yield was calculated as the amount of VFA produced divided by the amount of substrate fed, according to the following Eq. (1):

$$Yield(\%) = \frac{COD_{VFA\,EFF}}{COD_{INF}} \cdot 100 \quad (1)$$

where $COD_{VFA\ EFF}$ is the total (sum of) VFA concentration expressed in COD units in the effluent (g COD/L) and COD_{INF} is the tCOD concentration in the influent (g COD/L).

The protein concentration (g Pr) was estimated according to the following equation (2) [26]:

$$Pr = N_{org} \cdot 6.25 \quad (2)$$

where N_{org} is the organic nitrogen (TN minus N-NH_4^+, as nitrite and nitrate were considered zero)(g N/L). To transform the nitrogen content into protein content, an average nitrogen (N) content in the protein of about 16% was considered, resulting in the conversion factor of 6.25 (1/0.16 ≈ 6.25 g protein/g nitrogen).

Carbohydrate (Ch) and lipid (Lp) concentrations were estimated by simultaneously solving Eqs. (3) and (4), assuming COD values of 1.1 g COD/g carbohydrate and 2.9 g COD/g lipid [27, 28].

$$COD = 1.6 \cdot Pr + 1.1 \cdot Ch + 2.9 \cdot Lp \quad (3)$$

$$VS = Pr + Ch + Lp \quad (4)$$

3 Results and discussion

3.1 Fish canning wastewater characterization

The characterization of the fish canning wastewater used in this work is shown in Table 1. Although it is real industrial effluent, the characterization showed minimal standard deviations since the wastewater used was collected on a single time point. Except for pH, salinity, and COD, all values in Table 1 correspond to the initial characterization ($n = 2$ samples). These latter variables were determined throughout the experimental time ($n = 43$ samples).

The pH was found to be nearly neutral. The COD concentration was high (32.9 ± 1.4 g/L) with a soluble fraction of 92%. In addition, it also contained a high concentration of nitrogen (2.7 ± 0.1 g/L), which correlates with its predominant protein content (estimated value: 16.9 g/L; 75% on COD basis). The observed characteristics are consistent with previous studies of fish cannery effluents, which typically have high concentrations of organic matter (11.4–90.0 g COD/L) and nutrients (0.8–2.0 g TN/L) [7, 11]. It is worthy of note that the concentration of total suspended solids is relatively low, with a recorded value of 2.4 ± 0.6 g/L. Regarding the concentration of the total solids, the large difference between the TS and VS is likely correlated with the salt levels. This was confirmed by the values for conductivity (20.1 ± 0.0 mS/cm) and salinity (10.6 ± 1.1 g NaCl/L). Nevertheless, the salt concentration represents only half of the total inorganic solids present, suggesting the potential presence of additional inorganic substances in the wastewater, such as sand. The high concentration of salt is common in the effluent of fish canneries, which typically use seawater

Table 1 Characterization of the fish canning wastewater

Parameter	Unit	Value
pH	-	6.09 ± 0.19
Conductivity	mS/cm	20.1 ± 0.0
Salinity	g NaCl/L	10.6 ± 1.1
Total COD	g/L	32.9 ± 1.4
Soluble COD	g/L	30.2 ± 1.5
TS	g/L	45.5 ± 0.5
VS	g/L	22.5 ± 0.1
TSS	g/L	2.4 ± 0.6
VSS	g/L	0.4 ± 0.2
TN	g N/L	2.7 ± 0.1
NH_4^+-N	g N/L	0.1 ± 0.0
Carbohydrates*	g/L	1.3 ± 0.2
Lipids*	g/L	4.2 ± 0.0
Proteins*	g/L	16.9 ± 0.2

*Estimated values (Eqs. 2, 3, and 4; Sect. 2.5)

in their production processes [6]. Salt concentrations above 10 g NaCl/L can inhibit anaerobic processes [29]. To avoid this, an optimal strategy would be the previous adaptation of anaerobic biomass to high salt concentrations [30]. The anaerobic sludge used as inoculum in this study had been previously adapted to salinity and methanogens had been inactivated in a previous study [24].

The composition of the substrate influences both the yield and composition of VFAs produced [17]. In general, carbohydrates and proteins are more readily fermentable than lipids, as lipid hydrolysis is slower and generates long-chain fatty acids and glycerol, which hinder VFA production by acidogenic microorganisms [18]. Furthermore, although proteins are more biodegradable than lipids, their degradation is slower than that of carbohydrates due to their complex structure, which can make hydrolysis the rate-limiting step in VFA production from protein-rich substrates [31]. In a previous study [21], the VFA production from various fish canning wastewaters with different compositions was evaluated. Mussel cooking wastewater, characterized by a high carbohydrate content, yielded the highest VFA production (70–80%). In contrast, protein-rich wastewaters, such as mix and tuna cooking wastewater, exhibited slower fermentation, resulting in lower VFA yields (50–60%).

3.2 Influence of hydraulic retention time on VFA production and spectrum

HRT is a key parameter in anaerobic fermentation for VFAs production as it influences both the substrate degradation and the composition of the microbial community [32]. A longer HRT provides extended contact time between biomass and substrate, which can enhance hydrolysis and, consequently, acidogenesis, particularly for complex substrates such as fats and proteins. However, excessive HRT may promote to further conversion of VFAs into methane if methanogenic microorganisms are present. In addition, a higher HRT requires a larger reactor, increasing capital expenditure (CAPEX). Therefore, optimizing HRT is essential to balance substrate degradation efficiency, microbial dynamics, and economic feasibility. In the present study, two HRT values, 6 and 3 days, were investigated for continuous and sequential reactor feeding. These values were selected to ensure sufficient time for hydrolysis, as similar retention times have been identified as optimal in previous studies [21, 22].

3.2.1 Continuous operation

The continuous reactor (CR) was operated for 52 days (Fig. 2). After the adaptation period, an average VFA yield of 86% (28.7 ± 2.1 g COD_{VFA}/L) was reached at HRT of 6 days (stage I). In a study conducted by Fra-Vázquez et al. [22], the maximum VFA yield achieved from anaerobic fermentation of mussel wastewater in a continuous reactor at HRT of 6 days was 43%. Despite the absence of pH control in the present study, the pH was monitored throughout the operation, with an average value of 7.01 ± 0.17. Fra-Vázquez et al. [21] conducted their experiment at pH values below 4.5, which appeared to influence protein degradation and, consequently, the yield. In acidic conditions, protein conversion is reduced, potentially due to a decline in enzymatic activity [20]. Duong et al. [20] investigated the impact of pH on a protein-rich stream, observing that protein degradation was inhibited when the pH decreased from 7 to 5.

Regarding the product spectrum, formic acid and isocaproic acid concentrations remained below 1 g/L throughout the entire operation and were, therefore, excluded from Fig. 2. Butyric (7.7 ± 0.9 g COD_{VFA}/L), acetic (7.4 ± 1.1 g COD_{VFA}/L), and propionic (6.3 ± 1.6 g COD_{VFA}/L) were the dominant acids maintaining concentrations above 5 g COD_{VFA}/L each. This agrees with the literature, since

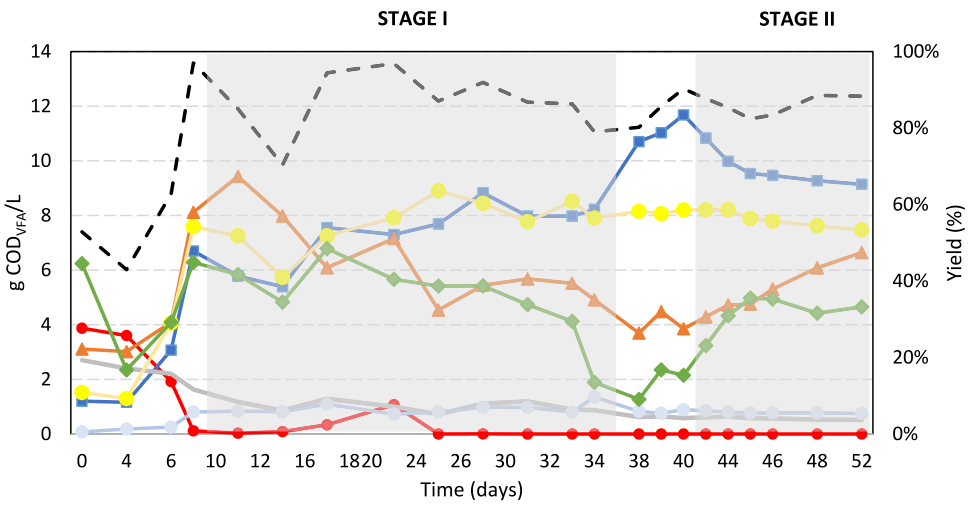

Fig. 2 VFA concentration over time during continuous reactor operation. Stage I: Hydraulic Retention Time of 6 days. Stage II: Hydraulic Retention Time of 3 days. HLac (red line with circle); HAc (blue line with square); HPr (orange line with triangle); Hi-But (gray line); HBu (yellow line with circle); Hi-val (light blue with circle); HVal (green with diamond); Yield (black broken line). Yield as COD VFA EFF/COD NF expressed as percentage. To be printed in color

acetic acid is usually positioned as the dominant acid in the anaerobic fermentation of waste streams [33]. The concentration of valeric acid (5.0 ± 1.4 g COD_{VFA}/L) must also be highlighted. The production of this acid is typically related to protein-rich substrates, as the canning effluent used in this work (75% protein). Regueira et al. [34] established a mathematical model for anaerobic fermentation and demonstrated a correlation almost linearly between n-valerate yields and protein concentration in the substrate. This outcome has also been documented with other studies, which investigated the fermentations of protein-rich substrates with a valeric acid composition of up to 25% [31, 35]. In the present study, the average yield of valeric acid reached 17%. The concentrations of propionic and valeric acids presented different profiles over stage 1: from days 10–28, propionic acid decreased progressively from 9 to 5 g COD_{VFA}/L, and valeric acid remained between 6 and 5 g COD_{VFA}/L (average 5.6 ± 0.6 g COD_{VFA}/L); from days 28–35, propionic acid remained practically constant (average of 5.7 ± 0.7 g COD_{VFA}/L) and valeric acid decreased from 5 to 2 g COD_{VFA}/L. The variation on propionic and valeric profiles during the operation, conducted at a constant pH (7.01 ± 0.17) and an HRT (6 days), can be attributed to the progressive increase in solids retention time (SRT). Between days 10 and 28, the SRT was 28.9 days, while between days 28 and 35, it increased to 33.5 days. Duong et al. [20] investigated the impact of SRT in a continuous reactor with gelatin as a substrate, achieving a maximum SRT of 30 days. They observed that increasing the SRT from 12 to 30 days resulted in a slight elevation in the concentrations of propionic and valeric acids. However, it is important to note that the study was conducted at pH 5, where hydrolysis was more than 12 times slower compared to pH 7. Therefore, it is possible that the differences in pH between studies could explain the observed variations.

After a transitional period during which the HRT was reduced, the reactor was operated at an HRT of 3 days for 12 days (stage II). The reduction in HRT did not impact the VFA yield, which also demonstrated an average VFA yield of 86% (28.8 ± 0.4 g COD_{VFA}/L). In terms of the product spectrum, there were no notable alterations. The average production of acetic acid increased by 2.3 g COD_{VFA}/L, reaching 9.7 g COD_{VFA}/L, thus being the predominant acid at an HRT of 3 days. The increase in acetic acid at shorter hydraulic retention times has been supported by several studies. Khan et al. [19] attributed this increase to enhanced acetogenesis at shorter retention times during glucose fermentation. Similarly, Houbron et al. [36] observed that low HRT favors the growth of acetogenic bacteria. Additionally, acetic acid is a crucial intermediate in anaerobic metabolism, meaning it can be a common product of various metabolic pathways [37], leading to greater accumulation at shorter residence times.

3.2.2 Sequential operation

Following an adaptation period, the sequential batch reactor (SBR) was operated for five cycles with HRT of 6 days and five cycles with HRT of 3 days. The results showed an average VFA yield of 90% with a VFA concentration of 25.3 ± 2.1 g COD_{VFA}/L for HRT of 6 days. When HRT was decreased to 3 days, the average VFA yield dropped to 79% (25.1 ± 4.0 g COD_{VFA}/L), which may indicate that a shorter retention time (3 days) was not enough to degrade the same protein concentration as compared to HRT of 6 days. Ziganshin et al. [32] investigated the effect of HRT on the microbial activity at ASBR with simulated thin stillage. However, in contrast to the current study, the authors reported that the shortening of HRT (from 6 to 3 days) affected the microbial community structure which resulted in higher VFA concentration. The discrepancy in the results observed may be attributed to the dissimilar composition of the substrates employed. The composition of thin stillage is primarily comprised of non-fermented sugars (carbohydrates), in contrast to the protein-based composition of canning wastewater. However, the yields observed in this study align with those reported by Atasoy et al. [33] who operated an SBR for VFA production from cheese production wastewater (also rich in proteins and fats). In their study, at an HRT of 3.5 days at pH 10, they achieved an average yield of 81% (based on soluble COD), with a VFA concentration of 12.78 g COD/L. Similarly, Calero et al. [38] assessed the performance of an anaerobic sequential batch reactor (ASBR) for VFA production using cheese whey. In their study, HRT for 2 days achieved a maximum VFA yield of 87%. From an industrial perspective, the HRT of 3 days is a more advantageous option, as its reduced processing time allows for a higher overall yield and potentially lower operating costs, despite the slight decrease in VFA production.

The VFA spectrum was not significantly influenced by the hydraulic retention time (HRT). Figure 3 shows the spectrum of acids produced at each HRT with their respective standard deviations. Butyric acid dominated the spectrum at HRT of 6 days, presenting a maximum concentration of 11.6 g COD_{VFA}/L. Santiago et al. [39] discovered a direct correlation between HRT and the microbial community composition of fermentative bacteria in a SBR. A prolonged HRT (48 h) was observed to favor the growth of *Clostridium* bacteria and the production of butyric acid, as in the present study. Acetic acid was the second predominant acid with a maximum concentration of 5.8 g COD_{VFA}/L. When the HRT was reduced to 3 days, the VFA spectrum was not significantly affected, as the profile of the acids remained consistent. Atasoy et al. [33] operated an SBR for VFA production from cheese production wastewater at pH 10, identifying propionic acid as the primary acid, followed by butyric and acetic acids. The observed differences can be attributed to

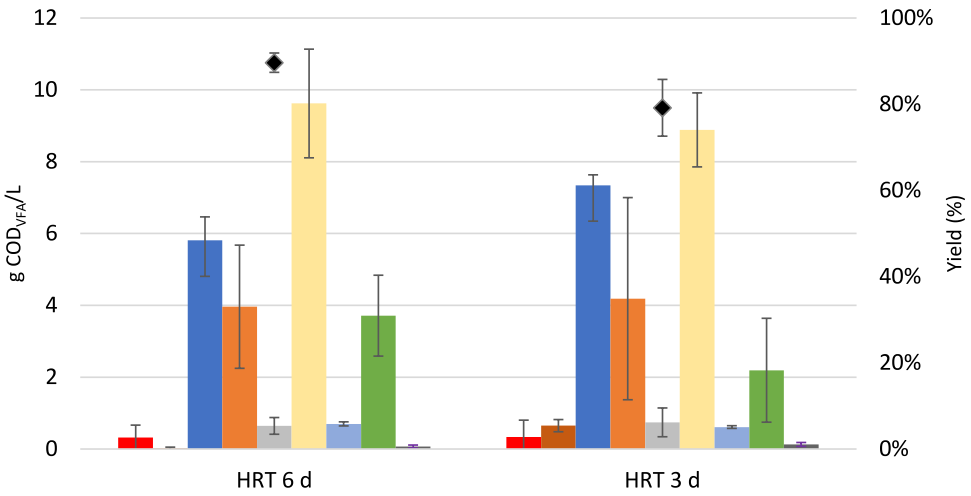

Fig. 3 VFA spectra and deviations at different hydraulic retention time (HRT) at the end of each cycle in sequential batch reactor (SBR). HLac (red square); HFor (brown square); HAc (blue square); HPr (orange square); Hi-But (gray square); HBut (yellow square); Hi-Val (light blue square); HVal (green square); Hi-Cap (dark gray square); yield (black diamond). Yield as $COD_{VFA\ EFF}/COD_{NF}$ expressed as percentage. *To be printed in color*

variations in pH between the studies (pH 10 vs. approximately 7) and the substrate composition (cheese wastewater vs. fish canning wastewater). Furthermore, the present study revealed a reduction of 1.5 g COD_{VFA}/L in valeric acid, linked to protein degradation, when HRT decreased to 3 days. This decrease aligns with the hypothesis presented in the yield discussion, suggesting that an HRT of 3 days was not enough to degrade the same amount of protein as an HRT of 6 days.

3.3 Influence of feeding strategies on VFA production and spectrum

To the best of our knowledge, the effect of feeding mode on the production and composition of volatile fatty acids (VFAs) during the fermentation of fish wastewater has not been previously studied. The most common reactor to produce VFAs from organic waste streams is the continuous stirred tank reactor (CSTR) [40]. While anaerobic sequential batch reactor (ASBR) is one of the most common reactor types for biogas production, in recent years, it has also been commonly employed for VFA production [38, 41]. This study aimed to evaluate how this feeding modes affects the VFA yield and spectrum. Figure 4 shows the VFA yield and product spectrum for each of the feeding strategies under investigation, continuous and sequential. Furthermore, the impact of HRT (6 and 3 days) on these two feeding strategies is also shown.

Both feeding modes achieved high yields (79–90%), regardless of HRT variation. The slight differences between feeding mode and HRT values might be explained by the SRT. At HRT of 6 days, the SBR allowed a higher solids retention resulting in an average SRT of 48.1 days, considerably higher than that of the CR (31.2 days). This indicates that the sludge in the SBR had a longer period to grow and adapt, potentially leading to a higher quantity of

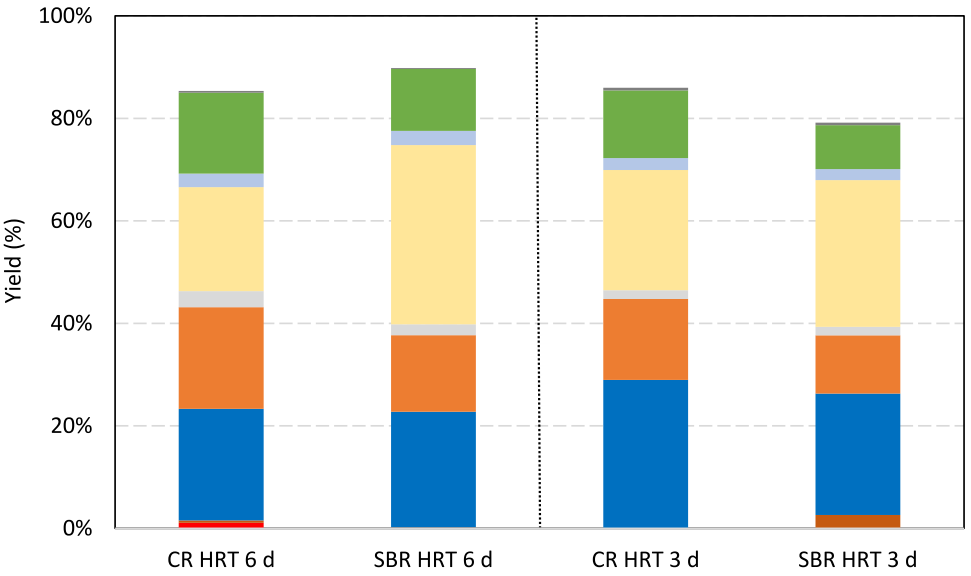

Fig. 4 VFA yield and spectrum of the two reactors at different hydraulic retention times. HLac (red square); HFor (brown square); HAc (blue square); HPr (orange square); Hi-But (gray square); HBut (yellow square); Hi-Val (light blue square); HVal (green square); Hi-Cap (black square).Yield as $COD_{VFA\ EFF}/COD_{NF}$ expressed as percentage. *To be printed in color*

microorganisms and enhanced VFA yield. At an HRT of 3 days, the average SRT in the CR was 16.6 days, while that of the SBR reactor was 14.6 days due to a poorer sedimentation of the biomass. This may be attributed to the higher organic loading rate (OLR) applied. Ruiz et al. [42], treating food industry effluent in an SBR, observed that OLR above 6 g COD/L·d resulted in reactor operation difficulties due to poor sedimentation. Additionally, the effect of organic loading rate (OLR) on a sequencing batch reactor (SBR) treating cheese whey was evaluated by Calero et al. [38]. Their results showed that the highest acidification (98%) was achieved at a lower OLR (2.7 g COD/L d). Furthermore, Simonetti et al. [43] revealed in their study that SBR results obtained at the same HRT but at different SRTs indicate that it is the SRT, rather than the HRT, that determines product yield. Statistical analysis was performed to determine if this difference in yield between the two feeding modes, at two HRT, was significant, and the results showed that it was not (HRT 6 days: p-value $=0.29>0.05$; HRT 3 days: p-value $=0.08>0.05$). Therefore, it can be concluded that the feeding mode does not appear to affect yield, based on the available data.

In terms of product spectrum for HRT 6 days, butyric and propionic acids showed the greatest variation between the two feeding strategies. Butyric acid was 15% higher in sequential mode compared to continuous mode. However, propionic acid was 5% lower in the VFA spectrum in the sequential reactor compared to the continuous reactor. pH is an essential operational parameter that may influence the metabolic pathways and, consequently, the composition of the final VFAs [44]. However, the average pH values in the reactors were found to be highly comparable, with a mean of 7.01 ± 0.17 and 6.97 ± 0.09 for the CR and SBR, respectively. The Student's t-test indicated that feeding strategy significantly impacted butyric acid ($p = 0.004$) and propionic acid production (p-value $=0.001$), with the SBR reactor favoring butyric acid over propionic acid. During HRT of 3 days, the product spectrum pattern remained consistent with that observed during the HRT of 6 days. There was a 6% increase in butyric acid in the sequential mode in comparison to the continuous mode. Therefore, as was observed in the case of HRT of 6 days, a relationship between SBR and butyric acid production was identified. Conversely, propionic acid was 5% lower in the VFA spectrum of the sequential reactor than in the continuous reactor. Furthermore, the concentrations of acetic and valeric acids were observed to decrease slightly in the sequential reactor, with reductions of 5% and 4%, respectively. Regarding pH, at HRT of 3 days, the mean pH at which both reactors were operated was neutral, at 6.92 ± 0.39 and 7.09 ± 0.02 for the CR and SBR, respectively. Therefore, as was observed in the case of HRT of 6 days, a relationship between SBR and butyric acid production was identified. To confirm this hypothesis, a Student's t-test was performed. However, this statistical analysis showed that there was no significant effect of the feeding strategy on the production of butyric acid (p-value $=0.32>0.05$), but there was a significant effect on the production of acetic acid (p-value $=0.00002<0.05$).

4 Conclusions

The results demonstrated that neither the hydraulic retention time (HRT) nor the feeding strategies influenced the VFA yield. Nevertheless, the VFA spectrum was influenced by the feeding strategy, but not by the HRT. These findings suggest that, while the yield remains independent of both operational parameters, adjusting the feeding strategy allows the targeted production of specific VFA. The sequential feeding mode resulted in the preferential production of butyric acid, whereas the continuous feeding mode favored the formation of propionic and valeric acids. Additionally, a low HRT (3 days) has been identified as the most efficient and scalable option, as it reduces costs and increases productivity without compromising the yield or altering the product spectrum.

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Data availability Data will be made available on request.

Declarations

Competing interests The authors declare no competing interests.

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