



## Turning fish canning wastewater into resources: Effluents and operational conditions selection for volatile fatty acids production

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### ABSTRACT

The potential of three fish canning wastewaters to produce volatile fatty acids (VFA) was evaluated using a stepwise procedure at laboratory scale. Batch tests (0.5 L), at different food to microorganism ratios (F/M), presented tuna and mussel cooking wastewaters as the highest yield substrates (60 % and 80 %, respectively). F/M ratios tested did not significantly influence the yield or the VFA spectrum. Continuous reactors (5 L) were operated with these two wastewaters at different hydraulic retention times (HRT). The shortest HRT studied (2 d) favoured the yield and the production of longer-chain VFA (butyric and valeric acids) in tuna wastewater. However, for mussel wastewater, the yield decreased 2-fold and the production of short-chain VFA (acetic acid) was favoured. This study offers an overview of the best fish canning wastewater, from a point of view of maximum VFA yield, and its optimal operational conditions for VFA production.

### 1. Introduction

Fish canning industry is a sector that generates enormous amounts of waste, which include both solid discards as well as wastewaters [1]. The quantity of solid discards can reach 50 % by weight of the total waste, consisting of muscle trimmings, skin and fins, bones, heads and viscera [2]. To date, solid fish waste is partly valorised to produce fishmeal, fertilizers, fish oil or bio-flocculant [3,4]. Anaerobic digestion (AD) to produce biogas or intermediate products was studied as well for the valorisation of these solid wastes [5]. Regarding the fish canning wastewater, previous studies focused on the treatment but not in its valorisation [6]. In general, the management of wastewater from fish canning industry is challenging given its high organic matter (chemical oxygen demand, COD) and nitrogen concentrations (1.14–90 g COD/L; 20–2000 mg TN/L) and eventually high salt concentration (2–36 g NaCl/L) [6]. For the regions with high economy dependency on fish canning industry, a good waste stream management is key to keep the sustainability of the sector and preserve the aquatic and natural ecosystems. In the region of Galicia (NW of Spain), where this study was carried out, the production of canned fish reached approximately 260,000 tons in 2021 which led to the production of >2.1 million m<sup>3</sup> of

wastewater.

Anaerobic processes were explored to valorise industrial wastewaters [7,8], but recently, other higher value products are pursued, such as volatile fatty acids (VFA) [9]. VFA are short-chain fatty acids of six or fewer carbon atoms (acetic, propionic, butyric, isobutyric, valeric and isovaleric acids) produced as intermediates in the conversion of organic matter to biogas. These acids can have a wide range of uses in the pharmaceutical and food industries as preservatives [10], as well as in chemical industries to produce esters or polymers, among others [10,11]. The total global market demand for VFA is increasing with an annual growth rate (CAGR) for acetic, propionic and butyric acids of 5.8 %, 6.8 % and 3.5 %, respectively [14].

Anaerobic fermentation conditions are highly influential on the yield and product spectrum of VFA obtained. Many studies have investigated the effect of operation parameters, such as pH, temperature, organic composition, hydraulic retention time (HRT) and organic loading rate (OLR) [15,16]. Yu et al. [17] studied the effect of pH during anaerobic fermentation of food waste and achieved a maximum VFA yield (0.79 mg COD/mg COD) at pH 6.5. Ma et al. [18] investigated the effect of co-fermentation of sewage sludge and chicken manure to produce VFA, with an increase in production. VFA production from different food

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industry wastewaters has been also previously studied. Gouveia et al. [19] evaluated the acid production from cheese whey and Tamis et al. [20] from candy bar factory wastewater. Nevertheless, the literature on VFA production from fish canning wastewaters is scarce. Fish cannery effluents often differs from that of other food industries in its high salt (NaCl) content. To the best of our knowledge, only Fra-Vázquez et al. [21] studied the suitability of mussel cooking wastewater to produce a VFA-rich effluent, analysing the effect of pH on VFA yield and selectivity. That study showed a significant production of  $0.72 \pm 0.07$  g COD<sub>VFA</sub>/L·d, demonstrating the feasibility of fish canning wastewaters to produce VFA.

Once the feasibility of VFA production from fish canning wastewaters has been demonstrated, the objective of this study was to develop a methodology to select the most favourable substrates (in terms of maximum VFA yield) and operational variables to scale up the process. In a first step, a screening of three wastewaters to select the most favourable to produce VFA was carried out through batch tests (0.5 L). In addition, in this step the influence of food to microorganism ratio (F/M) on the yield and product spectrum was evaluated. In the second step, the two wastewaters with the highest potential for VFA production were treated in continuous reactors (5 L) to evaluate the impact of hydraulic retention time (HRT) on the yield and spectrum of the product.

## 2. Materials and methods

The potential of VFA production from industrial wastewater was assessed by a scale up methodology from laboratory batch tests to continuous bioreactor experiments.

### 2.1. Selection of wastewater streams and inoculum

Three types of wastewater from fish canning industry were selected: (i) mix cooking wastewater from a traditional medium-sized fish canning plant that processes different raw materials (sardine, calamari and octopus, among others), (ii) mussel cooking wastewater collected from a cooking and processing of mussels factory; (iii) tuna cooking wastewater from the cooking ponds of different species of tuna. The three fish canning factories selected are in the Arousa estuary (Galicia, Spain), where 44 % of the Galician fish canning companies are located.

The three wastewater streams were selected due to their representativeness of the fish canning sector, including different organic matter (COD) concentration and composition (carbohydrates and proteins). Wastewaters were refrigerated at 6 °C from collection until their use in the tests.

The inoculum used in all experiments was collected, before each experimental assay, from the anaerobic digester of the urban wastewater treatment plant of Pontevedra (Spain).

### 2.2. Batch tests

Batch tests were performed to assess the VFA yield of each fish canning wastewater (Section 2.1) at two food to microorganism (F/M) ratios: 2 and 4. These ratios were established by keeping the amount of inoculum constant (g SV) and varying the substrate (g COD) (doubling for F/M 4). The selection of the F/M ratios was based on the VS portion of the inoculum being greater than that of the substrate, to minimize acidification or inhibition problems. Consequently, the optimal F/M ratio based on VS for most applications is between two and four [22]. Since the substrates had a high soluble COD content, an F/M ratio of 4 was selected to avoid the rapid accumulation of VFA, which could potentially inhibit the process. Furthermore, in case the substrates were not easily biodegradable, an F/M 2 ratio was also selected as a more conservative approach. The batch tests were carried out in an AMPTS (Automatic Methane Potential Test System) II (BPC Instruments, Sweden), adapted to take liquid samples during the tests. Each tested condition was performed in duplicate by using 500 mL bottles. The bottles

were placed in a thermostatic incubator to keep mesophilic conditions ( $37 \pm 1$  °C). The mesophilic temperature is the optimal and economically favourable condition for VFA production [23]. pH was adjusted to 7 with NaHCO<sub>3</sub> (100 g/L) at the beginning of the tests and it was monitored, but not controlled, along the experiments. As the pH would not be controlled during the process, it was decided to adjust the pH to a neutral value, close to that of the substrates. This was done assuming a slight pH decrease during acidogenesis. Calcium carbonate (5 g CaCO<sub>3</sub>/L, [24]) was added as a buffer to avoid a significant pH drop during the tests. Micro and macronutrients were supplemented according to Temudo et al. [24] (Supplementary materials Table S1) and the bottles headspace was flushed with nitrogen gas during at least a minute before the start of the experiments to remove any residual oxygen. The empty volume remaining after adding substrate, inoculum, micro- and macronutrients was filled with water to 500 mL. Blank tests (just inoculum without any substrate addition) were included to evaluate the production of VFA from the inoculum. No selective inhibition of methanogenesis was performed.

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), TN (Total Nitrogen), NH<sub>4</sub><sup>+</sup>-N (Ammonium), TP (Total Phosphorus), PO<sub>4</sub><sup>3-</sup>-P (Phosphate) and individual VFA were determined.

### 2.3. Continuous experiments

Two completely stirred tank reactors (CSTR) of 5 L (diameter of 15 cm) were operated in continuous mode for 60 and 80 days from mussel and tuna wastewater, respectively, to assess the effect of HRT in the production of VFA. Two HRT were tested: 2 and 5 days. The selection of these HRT was carried out with the objective of ensuring a sufficient period for the hydrolysis stage, while avoiding an excessively long duration that might favor the methanogenic population. Continuous shaking (90 rpm) was provided by an electric stirrer (IKA RW Digital 60, Germany) over the top (blade diameter of 12.5 cm). The temperature was maintained at  $37 \pm 1$  °C by using a thermostatic bath through a double glass wall. The redox potential was online monitored and the pH was controlled at 6.5 by using Na<sub>2</sub>CO<sub>3</sub> (50 g/L) and HCl (10 %) solutions. The pH value was lowered by half a point in comparison with the value set at the beginning of the batch tests. This value is based on the average pH at which the highest yields were obtained in the batch tests. No selective inhibition of methanogenesis was induced.

Samples were collected three times a week for the analysis of the pH, TS, VS, TSS, VSS, total and soluble COD, TN, NH<sub>4</sub><sup>+</sup>-N and individual VFA.

### 2.4. Analytical methods

pH was measured by using an electrode Sension<sup>+</sup> CAT, 5014 T (Hach, Germany). The concentrations of COD, TN, NH<sub>4</sub><sup>+</sup>-N, TP and PO<sub>4</sub><sup>3-</sup>-P were measured with the standard test kits Hach-Lange (Hach, Germany). TS, TSS, VS and VSS were determined according to the Standard Methods (APHA, 2017).

Individual VFA (HAc, HPr, Hi-But, HBut, Hi-Val and HVal) were determined after the filtration of the samples through Millipore 0.20 µm filters by a High Efficiency Liquid Chromatography (HPLC) system. A Hewlett Packard chromatograph equipped with supelcogel C-610, a column and two detectors connected in line, an ultraviolet (UV) detector and a refractive index (RI) detector, was used.

### 2.5. Calculations

The concentrations of HAc, HPr, Hi-But, HBut, Hi-Val and HVal were transformed to COD units by the application of the corresponding coefficients: 1.07 g COD<sub>HAc</sub>/g HAc, 1.51 g COD<sub>HPr</sub>/g HPr, 1.82 g COD<sub>Hi-</sub>

But/g Hi-But, 1.82 g COD<sub>HB</sub>But/g HBut, 2.04 g COD<sub>Hi-Val</sub>/g Hi-Val and 2.04 g COD<sub>HVal</sub>/g HVal.

The yield of volatile fatty acids was calculated according to Eq. (1):

$$\text{Yield (\%)} = \frac{\sum \text{COD}_{\text{VFA}}}{\text{COD}_{\text{FED}}} \cdot 100 \quad (1)$$

where COD<sub>VFA</sub> is the total VFA concentration expressed in COD basis in the fermentation liquor (g COD/L) and COD<sub>FED</sub> is the total COD concentration in the feeding (g COD/L).

The productivity of volatile fatty acids was calculated according to Eq. (2):

$$\text{Product} = \frac{\sum \text{COD}_{\text{VFA}}}{\text{HRT}} \quad (2)$$

where COD<sub>VFA</sub> is the total VFA concentration expressed in COD basis in the fermentation liquor (g COD/L) and HRT is the hydraulic retention time in the reactor (days).

Lipid concentration was assumed negligible since the wastewaters were taken in the factories after a dissolved air flotation system (DAF) to remove the fat, oil and grease from the wastewater. Carbohydrates (Ch) and proteins (Pr) concentrations were estimated by the simultaneous resolution of Eq. (3) and Eq. (4), assuming COD values of 1.6 g COD/g protein and 1.1 g COD/g carbohydrate [25] [26]. These concentrations were expressed as percentages in the document.

$$\text{COD} = 1.6 \cdot \text{Pr} + 1.1 \cdot \text{Ch} \quad (3)$$

$$\text{VS} = \text{Pr} + \text{Ch} \quad (4)$$

To evaluate the changes in the product spectrum from short-chain VFA (acetic and propionic) to longer-chain acids (butyric and valeric, i.e., more reduced VFA), the degree of reduction proposed by Bevilacqua et al. [27] was used (Eq. 5).

$$\text{Degree of reduction (gCOD/gVFA)} = \frac{\sum \text{COD}_{\text{VFA}}}{\sum C_{\text{VFA}}} \quad (5)$$

where COD<sub>VFA</sub> is the total VFA concentration expressed in COD basis and C<sub>VFA</sub> is the total concentration of the measured VFA expressed in g/L in the fermentation liquor. For VFA with 2–5 carbon atoms, this degree varies between 1.07 g COD/g VFA corresponding to pure acetic acid, to 2.04 g COD/g VFA corresponding to pure valeric acid.

### 3. Results and discussion

#### 3.1. Wastewaters and inoculum

The characterization of the three types of industrial fish canning wastewater used is shown in Table 1. The three streams had a pH close to neutral with a range from 6.13 to 6.78. COD concentrations varied depending on the type of raw material processed. Mussel cooking wastewater had the highest total COD (19.7 ± 0.8 g/L), in accordance with other studies [21,28]. The COD concentration for the tuna cooking wastewater was lower than the values previously reported in literature (7.8 g/L vs. 11.0 g/L reported by Achour et al. [29]). The soluble COD fraction was always >80 %, which is consistent with a low concentration of suspended solids (<1.5 g/L). Regarding the TS and VS concentrations, the lower difference between both parameters was obtained for mix and tuna wastewater (TS-VS = 5.1 g/L and 8.1 g/L, respectively) and the highest difference for mussel wastewater (TS-VS = 33.5 g/L). This difference is likely correlated with the salinity levels. The concentrations of nitrogen and phosphorus and their species were in the same range for the three wastewaters (TN: 1.3 ± 0.4 g N/L; NH<sub>4</sub><sup>+</sup>-N: 26.7 ± 11.6 mg N/L; TP: 0.2 ± 0.1 g P/L; PO<sub>4</sub><sup>3-</sup>-P: 0.1 ± 0.1 g P/L). Nevertheless, COD/TN ratio differed between the mix and tuna cooking wastewaters to the mussel wastewater. While the COD/TN ratio was 9.1 and 7.8 for mix and

**Table 1**

Characterization of the different fish canning wastewaters and inoculum used in the batch tests.

Parameter	Unit	Mix cooking	Tuna cooking	Mussel cooking	Inoculum
pH	–	6.13 ± 0.01	6.16 ± 0.01	6.78 ± 0.00	8.35 ± 0.11
TS	g/L	16.0 ± 0.9	13.5 ± 0.2	49.1 ± 2.3	27.7 ± 5.0
VS	g/L	10.9 ± 0.7	5.4 ± 0.2	15.6 ± 1.1	11.7 ± 1.2
TSS	g/L	1.2 ± 0.5	0.1 ± 0.0	0.5 ± 0.2	27.5 ± 3.2
VSS	g/L	1.1 ± 0.3	0.1 ± 0.0	0.4 ± 0.1	11.5 ± 2.7
Total COD	g/L	15.4 ± 0.5	7.8 ± 0.2	19.7 ± 0.8	22.8 ± 2.4
Soluble COD	g/L	12.5 ± 0.2	7.7 ± 0.1	17.5 ± 0.4	0.3 ± 0.1
VFA	g COD/L	1.2 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.2 ± 0.0
NH <sub>4</sub> <sup>+</sup> -N	mg N/L	40.0 ± 0.0	20.0 ± 10.0	20.0 ± 0.0	0.63 ± 0.01
TN	g N/L	1.7 ± 0.2	1.0 ± 0.3	1.2 ± 0.2	1.9 ± 0.1
PO <sub>4</sub> <sup>3-</sup> -P	g P/L	0.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
TP	g P/L	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.1
Carbohydrates*	% (VS basis)	37.6 ± 16.5	31.5 ± 5.6	67.3 ± 17.3	ND
Proteins*	% (VS basis)	62.4 ± 7.3	68.5 ± 1.9	32.7 ± 7.7	ND

ND: Not determined.

\* Estimated values (Eqs. 3 and 4, Section 2.5).

tuna cooking wastewaters, respectively, the COD/TN for mussel cooking wastewater was 16.4. Carbohydrates contribute to increasing the COD/TN ratio, while proteins tend to decrease it (Table 1). Thus, mussel wastewater resulted in a lower content in proteins (33 %) than mix and tuna wastewaters (62 % and 69 %, respectively).

The wastewaters used for the 5 L reactors continuous operation (mussel and tuna wastewaters) were provided weekly by the fish canning industries along the experiments. Table 2 shows the average values for the wastewater characterization during the continuous reactor experiments. It is well-known that the composition of the wastewater for fish canning industries can vary remarkably, even within the same factory, depending on the raw material used, the season and the type of processing [30]. For mussel cooking wastewater, there was no significant variation between the wastewater used in the continuous reactors compared to batch tests. The mussel factory processes a single raw material, which favoured a relatively stable composition over time. However, there were more significant differences in the composition of tuna cooking wastewater. It is worth mentioning the higher TS concentration for the tuna wastewater used in the continuous experiments.

**Table 2**

Average values (n = 30) of the different fish canning wastewaters used in continuous reactors operation and inoculum.

Parameter	Unit	Tuna cooking	Mussel cooking	Inoculum
pH	–	8.04 ± 0.67	6.15 ± 0.99	7.82 ± 0.76
Conductivity	mS/cm	40.3 ± 13.9	27.6 ± 4.4	ND
TS	g/L	34.6 ± 13.7	37.6 ± 4.2	26.4 ± 1.9
VS	g/L	5.0 ± 1.0	10.2 ± 3.2	13.6 ± 1.3
TSS	g/L	4.7 ± 1.5	6.1 ± 2.3	ND
VSS	g/L	2.1 ± 0.9	4.6 ± 1.8	ND
Total COD	g/L	7.2 ± 1.2	13.7 ± 5.5	29.0 ± 8.8
Soluble COD	g/L	6.1 ± 1.7	11.7 ± 3.2	0.9 ± 0.4
VFA	g COD/L	0.8 ± 0.6	1.2 ± 0.4	0.2 ± 0.1
NH <sub>4</sub> <sup>+</sup> -N	g N/L	0.6 ± 0.1	0.3 ± 0.1	ND
TN	g N/L	1.5 ± 0.2	1.1 ± 0.1	ND
Carbohydrates*	% (VS basis)	33.3 ± 25.5	51.0 ± 31.4	ND
Proteins*	% (VS basis)	66.7 ± 17.6	49.0 ± 23.5	ND

\* Estimated values (Eqs. 3 and 4, Section 2.5); ND: Not determined.

The VS/TS ratio changed from 40 % (batch tests) to 14 % (continuous experiments), indicating a higher inorganic compound content, likely salt. The higher conductivity (40.3 mS/cm), which can be related to salinity, was consistent with the previous result. This variation in composition may be related to the type of tuna processed, as the factory processes several species. Moreover, the wastewater from the tuna cooking ponds is reused several times, which gradually increases its salt content. Therefore, the salinity of the wastewater was higher or lower depending on the time of collection.

The content of carbohydrates and proteins were estimated (Tables 1 and 2). Two types of substrates can be distinguished according to the results obtained: carbohydrate-rich wastewater with a higher proportion of carbohydrates than proteins (mussel cooking wastewater, with 60 % carbohydrates (on COD basis)) and protein-rich wastewater with a higher proportion of proteins than carbohydrates (mix and tuna cooking wastewater, with >60 % (on COD basis)).

The inoculum for both batch and continuous experiments was taken from the sludge thickener of the same municipal wastewater treatment plant before each experiment. Although they were collected at different times, the results of their characterization were similar (Tables 1 and 2).

### 3.2. Screening through batch tests

The effect of the F/M ratio on the VFA production from three types of wastewater (mix, mussel and tuna cooking wastewater) was assessed in batch tests (Fig. 1).

The mix cooking wastewater reached the maximum acid

concentration on day six of the batch test for both F/M ratios: 3.04 g COD/L (Fig. 1A) and 6.44 g COD/L (Fig. 1B), respectively. The maximum VFA concentration decreased to 1.87 g/L and 5.42 g/L on day nine, pointing out the occurrence of methanization since no selective inhibition of this step was induced. For both assays, the highest VFA productivity occurred during the first day, with 1.88 g COD<sub>VFA</sub>/L·d for F/M 2, and double (3.87 g COD<sub>VFA</sub>/L·d) for F/M 4. The acid production continued to rise from the first to the sixth day of operation, but slower. For the F/M 2 ratio, the increase in production between days three and six of operation was only 0.1 g/L. A similar pattern occurred for tuna cooking wastewater (Fig. 1C and D) with the highest productivity on the first day of operation (1.08 g COD<sub>VFA</sub>/L·d (F/M 2) and 2.42 g COD<sub>VFA</sub>/L·d (F/M 4)) and the highest VFA concentration being achieved on day six (1.81 g COD<sub>VFA</sub>/L for F/M 2 and 3.87 g COD<sub>VFA</sub>/L for F/M 4). Regarding mussel cooking wastewater, the maximum concentration of VFA was reached on day 3, 5.32 g/L and 8.91 g/L for the F/M ratios 2 (Fig. 1E) and 4 (Fig. 1F), respectively. In this case, the concentration of acids did not double in the assay with high F/M ratio due to the methanization produced. The methanization for the F/M 4 ratio was doubled compared to the F/M 2 (Supplementary materials Fig. S1). Once again, the greatest VFA productivity occurred on the first day for both F/M ratios (2.90 and 5.25 g COD<sub>VFA</sub>/L·d for F/M 2 and 4, respectively). The mussel wastewater suffered the greatest decrease in pH during the test (0.99 and 1.53 for F/2 and F/M 4, respectively) (Supplementary materials Fig. S1). This was since it was the one that suffered the greatest acidification, reaching the highest concentration of VFA.

As expected, the fermentation of protein-rich substrates (mix and

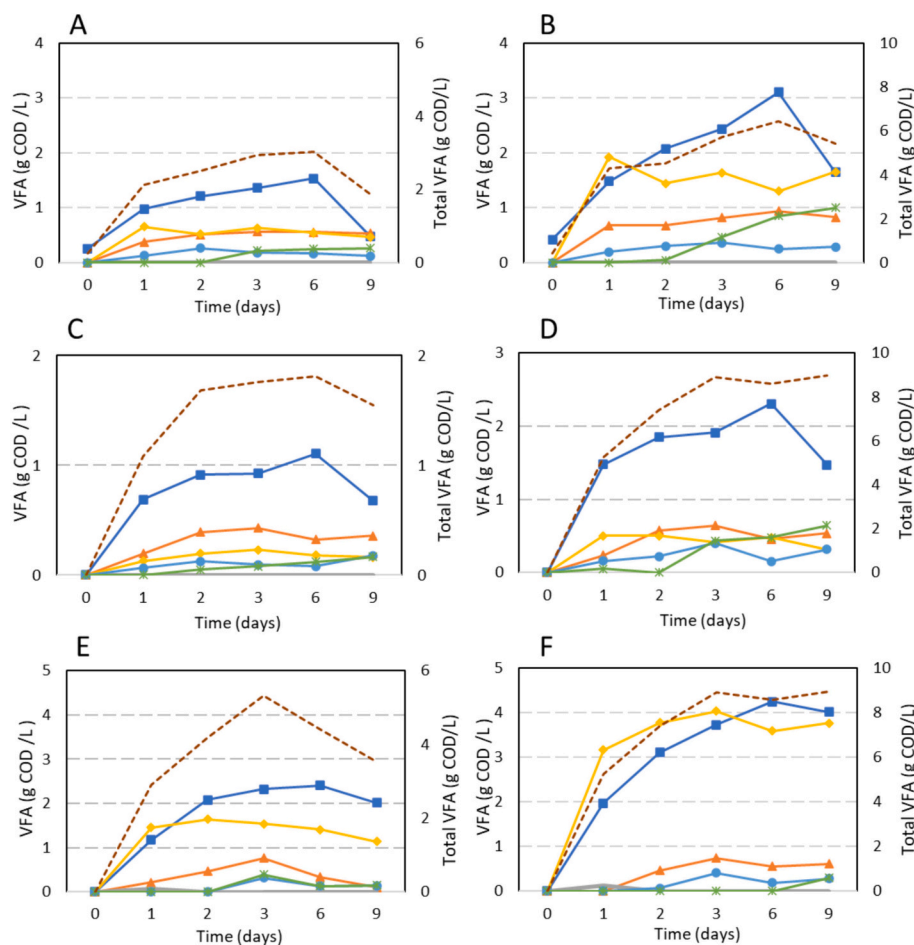


Fig. 1. VFA production over time. (A) Mix cooking wastewater at F/M 2; (B) Mix cooking wastewater at F/M 4; (C) Tuna cooking wastewater at F/M 2; (D) Tuna cooking wastewater at F/M 4; (E) Mussel cooking wastewater at F/M 2; and, (F) Mussel cooking wastewater at F/M 4. HAC (—■—); HPr (—▲—); Hi-But (—●—); Hi-Val (—◆—); HVal (—▼—); Total VFA-COD (---).

tuna cooking) was slower than the fermentation of carbohydrate-rich substrates (mussel), since proteins are less easily biodegraded due to their more complex structure [31–33]. Protein-rich substrates doubled their VFA concentration with a doubling of the F/M ratio, in contrast to the carbohydrate-rich substrate. In the latter case, a higher F/M ratio provides more food available for microorganisms, allowing them to convert a larger amount of organic matter into VFA, and subsequently, promoting higher rates of methanation. Considering that all the biogas produced during the tests was methane (the worst scenario), a maximum of 8 % more COD to VFA conversion could have happened for F/M 2 and 14 % for F/M 4, if methanization had been inhibited. Methanisation was lower for mixed and tuna effluents. The maximum conversion to acids would only increase by an average of  $2.8 \pm 0.8$  %.

Acetic, propionic and butyric acids were the dominant compounds produced during the anaerobic fermentation of the three fish canning wastewaters (Fig. 2). Many authors [34–36] have positioned acetic acid as the main acid in the spectrum after anaerobic fermentation of waste streams, as was observed in the present work for mix and tuna cooking wastewaters. Butyric acid was dominant in the mussel wastewater product spectrum. Butyric acid production is favoured in carbohydrate-rich waste streams at pH lower than 7 [37]. The VFA spectrum produced was influenced by the composition of the substrate used, highlighting the production of valeric acid for protein-rich substrates. An increase in the concentration of valeric acid was observed from the second day until the end of the operation in mix and tuna wastewaters (Fig. 1). Tuna wastewater reached the maximum percentage of valeric acid with 20 % (0.64 g COD/L at F/M 4). This percentage rises to 30 % considered the production of its isomer, isovaleric acid. However, the maximum concentration was reached by the mix wastewater with 1.0 g COD/L (18 % at F/M 4) at the end of the operation. Regueira et al. [38] has linked the yield of isovalerate and n-valerate with the protein concentration of the substrate through a mathematical model for anaerobic fermentation. This result has also been reported experimentally in fermentations of protein-rich substrates with up to 25 % valeric acid composition [39,40].

The highest VFA yield was obtained for mussel cooking wastewater (70–80 %), followed by the tuna cooking (60 %) and the mix cooking (50–55 %) wastewaters (Fig. 2). No effect of F/M ratio was observed, except for the mussel wastewater, where the yield was improved at low F/M ratio. As previously mentioned, this difference in yield was partly due to the methanization produced for the F/M 4 ratio. Neither significant difference were observed between the product spectrum distribution for the different F/M ratios studied. In contrast, wastewater

composition affected significantly the VFA obtained, since longer-chain acids (butyric and valeric), and therefore, a higher degree of reduction (1.38 g COD/g VFA) was achieved when fermenting carbohydrate-rich wastewater (mussel). It should be noted that in the mussel wastewater, valeric acid was only produced in F/M 2. As mentioned above, valeric acid is associated with protein-rich substrates [41] and the mussel wastewater did not have high protein content. However, during the F/M 2 test there was valeric acid production. Therefore, it was possible that at F/M 2, carbohydrates were converted more rapidly, followed by the conversion of proteins to valeric acid. In the case of F/M 4, protein degradation occurred later because a greater amount of carbohydrate had to be converted first (Fig. 1F). However, another hypothesis comes from what was demonstrated by Bevilacqua et al. [27] Valeric acid could also be produced by chain elongation from propionic acid in the absence of an external electron donor. During anaerobic fermentation, electron-donating compounds, such as ethanol and/or lactate, are produced.

Taking into account that the F/M ratio did not affect significantly the VFA yield and spectrum, from an industrial perspective, it is advantageous the operation of anaerobic fermenters at high F/M ratios due to the lower investment costs. The possibility of treating a greater organic load in the reactor leads to a reduction in reactor size, which generally means a reduction in infrastructure costs (CAPEX, Capital Expenditures) and therefore a reduction in OPEX (Operation Expenditures). When the F/M ratio is doubled from 2 to 4, the OLR and therefore the net production of VFA is also doubled. In the case of tuna water, for example, the OLR for F/M 2 was 71.4 g COD/L-d, which increased to 142.8 g COD/L-d for F/M 4. Therefore, the net production also doubled and was 0.3 g COD<sub>VFA</sub>/L-d for F/M 2 and 0.6 g COD<sub>VFA</sub>/L-d for F/M 4. This means that with a smaller reactor size, higher production can be achieved by using a higher F/M ratio.

### 3.3. Influence of operational conditions on the VFA spectrum during continuous operation

Based on the results of the batch tests (Section 3.2), mussel and tuna cooking wastewaters were selected to evaluate their technical feasibility in continuous reactors and at a higher scale. More specifically, the influence of HRT on the yield and VFA product spectrum was studied.

The reactor fed with mussel cooking wastewater was operated at an HRT of 5 days for 20 days (Fig. 3A). The average yield during this period was 51 %, with a maximum VFA concentration in the effluent of 12.6 g COD<sub>VFA</sub>/L. The concentration obtained by Fra et al. (2020) (the only study, to our knowledge, that produces VFA with canning industry effluent) during anaerobic fermentation of mussel water reached a concentration of 4.54 g COD<sub>VFA</sub>/L. This is 64 % lower than that obtained in the present test. Acetic was the main acid (55 %) during this period (Fig. 3B), increasing its presence in the spectrum compared to the results obtained in the batch tests. Butyric acid, which dominated the spectrum in batch tests, decreased in continuous reactors to only 18 %. The difference in carbohydrate and protein composition of mussel wastewater between the batch tests and the continuous reactors explained these spectrum differences. The wastewater used for the continuous reactors resulted in 20 % more protein compared to the batch tests and, as previously mentioned, butyric acid production is favoured in carbohydrate-rich waste streams.

After a transition period, where HRT was decreased (Fig. 3A, shaded area), the reactor was operated at HRT of 2 days for 25 days (from day 35 to 60). During this period, a significant decrease in wastewater COD occurred, due to the already commented composition variation of real wastewaters. However, the organic loading rate (OLR) was still higher (5.3 g COD/L-d) than in the operation with an HRT of 5 days (3.9 g COD/L-d). The average yield during this stage was 24 %, with a maximum effluent concentration of 3.8 g COD<sub>VFA</sub>/L. These poorer results were attributed to the different quality of the wastewater treated. Regarding the product spectrum, acetic acid share increased notably

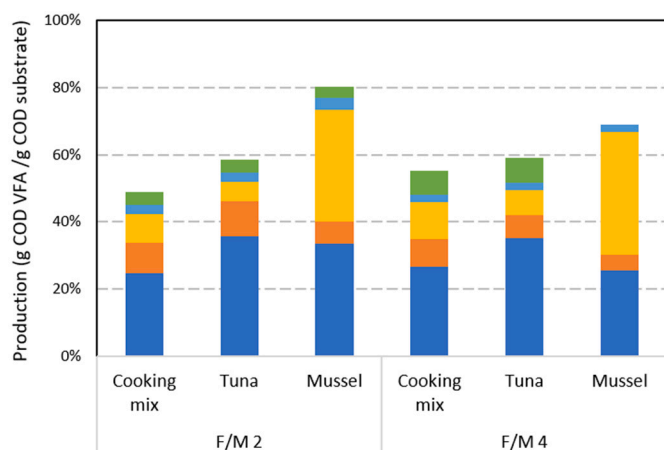
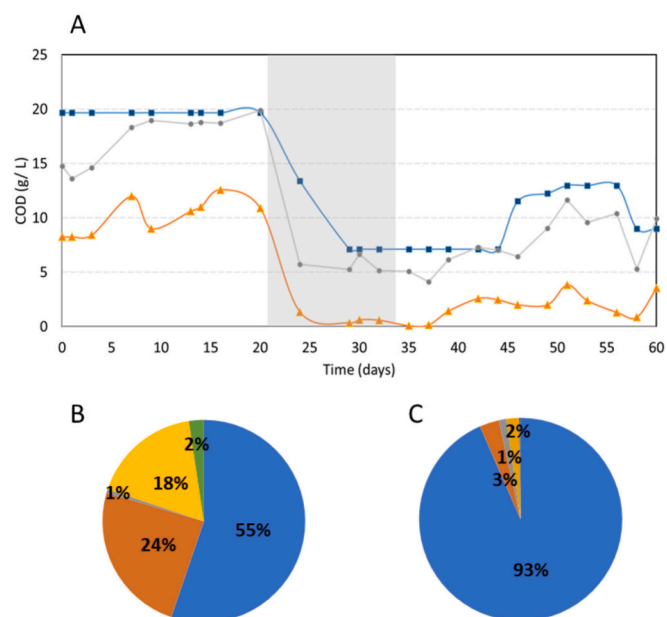


Fig. 2. VFA yield and product spectrum on day 3 for mussel wastewater and on day 6 for mix cooking and tuna wastewaters of the batch tests at two different F/M ratios: HAC (■); HPr (■); Hi-But (■); HBut (■); Hi-Val (■); HVAl (■).

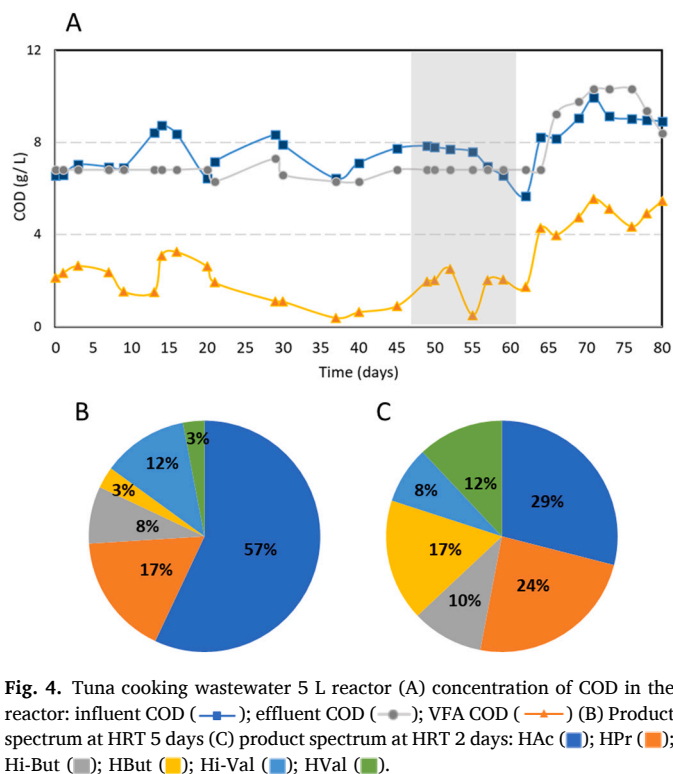


**Fig. 3.** Treatment of mussel cooking wastewater in a continuous reactor. (A) COD concentration in the reactor influent (—■—), effluent (—●—) and as VFA in the effluent (—▲—). Product spectrum at HRT of 5 days (B) and 2 days (C). HAC (■); HPr (■); Hi-But (■); HBut (■); Hi-Val (■); HVal (■).

accounting for 93 % of the VFA COD in the effluent (Fig. 3C), resulting in a lower degree of reduction (1.1 g COD/g VFA) compared with the operation at an HRT of 5 days (1.3 g COD/g VFA). This increase in acetic acid might be explained by an enhanced acetogenesis at shorter HRT, as previously observed by Khan et al. [42] during glucose fermentation. Low HRT favours the population of acetogenic bacteria [43]. In addition, acetic acid is a key intermediate in anaerobic metabolism [44], which means that it can be a common product of several metabolic pathways. This may lead to greater accumulation with short residence times.

The reactor with tuna cooking wastewater was operated at an HRT of 5 days for 47 days (Fig. 4A). An average yield of 25 % was reached with a maximum acid concentration in the effluent of 3.24 g COD<sub>VFA</sub>/L. During this period of operation there was a difference between the input and output COD of 9 %, showing a reduced degree of possible methanization or H<sub>2</sub> production. Previous results in literature have shown that a value higher than 30 mS/cm in salinity resulted in a complete inhibition of methanogenesis [45,46]. Considering that the salinity of the tuna cooking wastewater reaches  $40.3 \pm 13.9$  mS/cm, it is possible that this led to the elimination of methanogenesis. Regarding to product distribution, as in the batch tests, acetic acid dominates the spectrum of tuna wastewater with 57 % of the total VFA (Fig. 4B).

After a transition period to decrease the hydraulic retention time (shadowed area), the reactor was operated at an HRT of 2 days (Fig. 4A) for 20 days. It should be noted that the COD output from the effluent began to increase during this period. The decrease in retention time, together with the high salinity of the tuna cooking wastewater, favoured the washing of the biomass, which increased the VSS concentration of the effluent from an average of 1.4 g/L (HRT 5 d) to 2.2 g/L. The presence of high concentrations of Na<sup>+</sup> ions has been described in the literature that affects the structure of the sludge [47]. The monovalent cations (Na<sup>+</sup>) can exchange with the divalent cations (Ca<sub>2</sub><sup>+</sup> and Mg<sub>2</sub><sup>+</sup>) present in the extracellular polymeric substances structure of sludge, promoting the disintegration of the biomass structure [48]. However, the decrease in biomass concentration in the reactor did not affect the



**Fig. 4.** Tuna cooking wastewater 5 L reactor (A) concentration of COD in the reactor: influent COD (—■—); effluent COD (—●—); VFA COD (—▲—) (B) Product spectrum at HRT 5 days (C) product spectrum at HRT 2 days: HAC (■); HPr (■); Hi-But (■); HBut (■); Hi-Val (■); HVal (■).

yield that reached the double (51 %) than the one achieved at an HRT of 5 days, with a maximum concentration of acids of 5.54 g COD<sub>VFA</sub>/L. This suggests that the biomass activity was sufficient to sustain the production of VFA. The product distribution (Fig. 4C) varied with respect to HRT 5 days. Acetic acid (29 %) was not predominant, sharing position with propionic acid (24 %). Other notable changes in the spectrum were the increase of 14 % butyric and 9 % valeric acid. As a result, the degree of reduction increased from 1.3 g COD/g VFA to 1.5 g COD/g VFA.

#### 4. Conclusions

Batch tests allowed a rapid screening of different fish canning effluents, with the highest VFA yield being obtained for mussel and tuna cooking wastewater. Yet, the results of the continuous reactors demonstrated that wastewater composition has a greater influence on the yield and product spectrum than the operational conditions. The highest VFA concentration and yield (12.6 g COD<sub>VFA</sub>/L, 51 %) was achieved with carbohydrate-rich wastewater (mussel). Based on the average wastewater production from mussel processing, which is estimated at 10 m<sup>3</sup>/t, around 0.13 t VFA could be produced per t or processed mussel. Overall, this study shows the potential of VFA production from fish canning wastewaters, but further investigation is required to optimize and scale up the process.

#### CRedit authorship contribution statement

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

## 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jwpe.2024.105738>.

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