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Research Paper

Optimization of methane production from solid tuna waste: Thermal pretreatment and co-digestion

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ABSTRACT

Fish canning industries generate large amounts of solid waste during their processing operations, creating a significant environmental challenge. Nonetheless, this waste can be efficiently and sustainably treated through anaerobic digestion. In this study, the potential of biogas production from anaerobic digestion of thermally pretreated and co-digested solid tuna waste was investigated. The thermal pretreatment of raw fish viscera resulted in a 50 % increase in methane yield, with a production of 0.27 g COD-CH_4/g COD added. However, this pretreatment did not lead to a significant increase in biogas production for cooked tuna viscera. When non-thermally pretreated raw viscera was tested, a large accumulation of volatile fatty acids and long chain fatty acids was observed, with levels reaching 21 and 6 g COD/L, respectively. On the other hand, anaerobic co-digestion of cooked tuna viscera with fat waste significantly enhanced methane production, achieving 0.87 g COD-CH_4/g COD added. In contrast, co-digestion of cooked tuna viscera with dairy waste and sewage sludge resulted in notably lower yields of 0.36 and 0.46 g COD-CH_4/g COD added, respectively. These results may be related to the C/N ratio, which was found to be within the optimal range for anaerobic digestion only in the tuna and fat waste co-digestion assay.

1. Introduction

Sustainable management of organic solid wastes can be achieved through anaerobic digestion since it generates biogas as a renewable energy source and allows the recycling of wastes and by-products. The anaerobic degradation pathway of organic matter involves a series of interconnected steps, comprising four distinct stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. This intricate process is the result of complex interactions among different groups of micro-organisms, which transform complex organic matter into methane, carbon dioxide, ammonium, hydrogen sulphide, and water as end products (Chen et al., 2020).

The waste generated in the tuna canning industry is characterized by a high organic content, making it a good candidate for the anaerobic digestion process. Currently, this type of waste is used to obtain animal feed products, mostly by fishmeal production (Ivanovs et al., 2018). Nevertheless, over the past several years, anaerobic digestion has appeared as a promising alternative. In this context, other authors have discussed the transformation of fish waste into different value-added products, such as volatile fatty acids (VFA), proteins and oils (Bermúdez-Penabad et al., 2017; Coppola et al., 2021). Furthermore, biomethane production from fish waste can contribute significantly to the attainment of the European Union's goal of reducing greenhouse gas emissions by at least 55 % by 2030. The promotion of biomethane utilization within the EU is steered by the Renewable Energy Directive (Directive (EU) 2018/2001), which sets a renewable energy target of 32 % (European Commission, 2023).

Tuna is mainly composed of proteins with a high nitrogen content, which is transformed into ammonia via the biological breakdown of the nitrogenous matter. Although nitrogen plays a crucial role as a nutrient for the growth of bacteria, it can have an inhibitory effect on methanogenesis when present in high concentrations (1.5–3 g $N\text{-}NH_4^+$/L) (Chen et al., 2008; Wang et al., 2011). Consequently, ammonia is considered to be a prospective inhibitor of the anaerobic digestion process (Yulisa et al., 2022). In addition, fish waste contains a high percentage of fat (5–55 %) (Cadavid-Rodríguez et al., 2019). The

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degradation of lipids theoretically yields a greater amount of biogas (1.42 L CH_4/g waste) compared to proteins or carbohydrates (0.92 and 0.83 L CH_4/g waste, respectively), making them an optimal substrate for methane production (Alves et al., 2009). However, lipid-rich residues tend to produce a greater amount of VFA, which could inhibit the anaerobic process (Cadavid-Rodríguez et al., 2019). These possible inhibitions could be avoided: (a) by diluting the digester contents with water and (b) by modifying the C/N ratio through a process of co-digestion with another type of waste that presents a lower amount of nitrogen.

Anaerobic co-digestion involves the simultaneous digestion of two or more different substrates. This process has attracted growing interest in recent years due to its numerous advantages over mono-digestion. One of its key benefits is the improved balance of nutrients and C/N ratio, which effectively mitigates the inhibitory effects caused by toxic substances and other inhibitors through dilution (Mata-Alvarez et al., 2014; Choi, 2020; Iglesias-Iglesias et al., 2020). By combining various substrates, anaerobic co-digestion harnesses synergistic effects, leading to enhanced biodegradation rates and overall process efficiency. Additionally, it contributes to the stabilization of the digestion process, reducing operational risks and minimizing digester upsets (Rabii et al., 2019). Moreover, this process enables the utilization of diverse organic wastes, promoting waste valorization and resource recovery. Consequently, co-digestion not only maximizes methane yield (Salama et al., 2019; Solé-Bundó et al., 2019), but also facilitates the management of challenging waste streams, ultimately improving the overall economics of anaerobic digestion systems.

On the other hand, there is a growing interest in pretreatment methods, as they have shown promising results in modifying biomass structure and composition, ultimately leading to improvements in the anaerobic digestion process. Pretreatment techniques are used to optimize the conversion of the biomass into energy by accelerating the hydrolysis step and thus allowing the appropriate by-products to be obtained. In this way, poor digestion performance frequently found in industrial-scale applications can be avoided (Hajji and Rhachi, 2022). Despite the significant energy cost associated with pretreatments, the potential for increased biogas production justifies its application. The major advantages of waste pretreatment also include pathogen removal, reduced digestate volumes, and shorter retention times. The efficacy of pretreatment methods in enhancing substrate utilization is influenced by both the nature of the pretreatment applied and the characteristics of the substrates. Mechanical, thermal, chemical, and biological methods, either alone or in combination, represent the main categories of pretreatment (Carrere et al., 2016; Carlini et al., 2018; Gunes et al., 2019; Atelge et al., 2020).

Mechanical pretreatments increase the surface area, enhancing the contact between anaerobic bacteria and the substrate, and therefore promoting biodegradation. Additionally, it may accelerate the hydrolysis and acidogenesis steps, considered limiting stages in the digestion process, favoring mass transfer and food availability for microorganisms. Although it is possible that the production of VFA increases, which could lead to their accumulation causing inhibition (Chairattanawat et al., 2022).

The effect of thermal pretreatments is influenced by the nature of waste and the temperature range applied. High solubilization is achieved by applying low temperatures (100 °C) for long periods of time, while high temperatures (>170 °C) could result in the formation of chemical bonds, causing agglomeration of the particles (Ariunbaatar et al., 2014). Several authors have reported the positive effect of thermal pretreatment of food waste in advance of anaerobic digestion as it significantly increases methane yield (Ariunbaatar et al., 2015; El Gnaoui et al., 2020; Liu et al., 2020; Gianico et al., 2021).

The objective of this work was to evaluate the methanation potential of solid tuna waste through the application of a thermal pretreatment and a co-digestion process in order to optimize the C/N ratio and, consequently, reduce the possible inhibitory effect of VFA and ammonia. The study was carried out on a laboratory scale, and it could be of significant interest in assessing the feasibility of a pilot-scale system.

2. Material and methods

2.1. Substrates and inoculum

Solid tuna waste, composed of raw fish viscera (RFV) and cooked fish viscera (CFV), was obtained from a cannery industry in Galicia (Spain). Both CFV and RFV were divided into two fractions. In order to assess the effect of a thermal pretreatment, one fraction was subjected to a heat treatment (stove at 100 °C for 36 h) to eliminate its water content, whereas the other fraction did not undergo this conditioning. For the co-digestion assays, three types of residues were selected to co-digest with tuna waste: wastewater from dairy industry (WDI), secondary sludge from an aerobic wastewater treatment plant treating canned tuna effluents (SS) and fat separated from the canned tuna effluents (FW). All the samples, for both thermal pretreatment and co-digestion tests, were subjected to a mechanical pretreatment, consisting of disintegrating the substrates to a particle size smaller than 2 mm. The main characteristics of these residues are summarized in Table 1.

The inoculum used was collected from an acidogenic reactor, which operated with tuna waste. The total solids (TS) and volatile solids (VS) concentrations were 108.1 g/L and 79.3 g/L, respectively, with a VS/TS ratio of 0.7 in the co-digestion assays. For the thermal pretreatment assays, TS and VS concentrations were 86.9 g/L and 65.2 g/L, respectively, and the VS/TS ratio was 0.8.

2.2. Methane production assay

The biodegradability assays were conducted following the methodology outlined in Eiroa et al. (2012). Glass flasks of 600 mL, containing 50 mL of liquid volume and sealed with butyl rubber stoppers and aluminum crimps, were used. All assays were performed in triplicate. Suitable alkalinity was achieved by adding bicarbonate (5 g/L). Once the ground waste, medium and inoculum were added, the headspace was purged with N_2/CO_2 (80/20, v/v), while Na_2S (1 mM) was introduced as a reducing agent. Subsequently, the vials were incubated at

Table 1
Composition of residues: cooked fish viscera (CFV), raw fish viscera (RFV), wastewater dairy industry (WDI), secondary sludge (SS) and fat waste (FW).

Parameters	RFV	CFV	SS	FW	WDI
g COD/g dried waste	1.5 ± 0.2	1.1 ± 0.2	0.8 ± 0.1	0.4[1]	35.3 (±0.1) g COD/L
g N/g dried waste	0.12 ± 0.02	0.12 ± 0.02	0.03[2]	0.04[2]	3.01[2] g N/L
g protein/g dried waste	0.73 ± 0.04	0.74 ± 0.04	0.21 ± 0.01	0.22 ± 0.02	18.81 (±0.03) g protein/L
g SO_4^{2-}/g dried waste	0.09 ± 0.01	0.27 ± 0.02	0.11 ± 0.01	0.07[2]	6.80 (±0.01) g SO_4^{2-}/L
g PO_4^{3-}/g dried waste	0.01 ± 0.01	0.01[2]	0.01[2]	0.04[2]	0.53[2] g PO_4^{3-}/L[1]
g fat/g dried waste	0.11 ± 0.01	0.17[2]	0.04[2]	0.40 ± 0.01	0.002[2] g fat/L
g Cl^-/g dried waste	0.04[2]	0.04[2]	ND	ND	ND
Moisture (%)	80.2 ± 0.6	70.2 ± 0.2	93.1[1]	72.4 ± 0.2	ND
VS (%)	90.2 ± 0.6	91.7 ± 0.2	85.6 ± 0.4	94.7 ± 0.1	15.4[1] g VS/L[3]
C/N	5.1[1]	5.1[1]	5.5 ± 0.1	45.5 ± 0.3	4.9[1]

Note ND, not determined.
Data are mean values ± standard deviation of three replicates.
[1] Standard deviation < 0.1.
[2] Standard deviation < 0.01.
[3] VS concentration represented 69.8% of TS.

37 °C and a handheld pressure transducer was employed to control the pressure increase. The methane concentration in the biogas was assessed at regular time intervals, and the volume of methane produced was adjusted for standard temperature and pressure conditions. The medium without substrate was run as a blank control. The methane production from the degradation of biomass and residual substrates was calculated by the methane production from the blank controls, and the resulting values were subtracted from those in the treatments. Methane production was determined as the maximum plateau attained in the methane production curves after adjustment for the residual substrate content in the inoculum. Methane yield was expressed as the mass of COD converted into methane relative to the initial mass of COD added to the batch vials (g COD-CH_4/g COD added).

Both thermal pretreatment and co-digestion tests were conducted with a TS concentration of 2.5 % (w_{waste}/v), except for the experiments involving WDI and CFV + WDI, where concentrations of 1.6 % and 2.9 % (w_{waste}/v) were used, respectively. In the case of co-digestion tests, a mixing ratio of 50:50 was employed for CFV + SS and CFV + FW experiments, while a ratio of 43:57 was applied to the CFV + WDI assay. The $VS_{waste}/VS_{inoculum}$ ratio was maintained within the range of 0.7–1.4 for all the experiments.

The duration of the thermal pretreatment assays was 136 days for non-thermally pretreated samples and 139 days for thermally pretreated samples. Regarding the co-digestion tests, they were conducted over 108, 144, and 158 days for SS, WDI, and FW assays, respectively.

2.3. Analytical methods

Total solids (TS), volatile solids (VS), ammonia, total Kjeldahl nitrogen (TKN), and total and soluble chemical oxygen demands (COD and sCOD, respectively) were determined following the procedures described in Standard Methods (APHA, 2012). The protein content was measured using the Bradford method (Bradford, 1976), and the lipid content was assessed following the Folch method (Folch et al., 1957). All analyses were carried out in triplicate.

VFA (formic, acetic, propionic, butyric, n-valeric, i-valeric acids) and lactic acid were analyzed by high performance liquid chromatography (HPLC) employing a Hewlett- Packard 1100 Series chromatograph (Agilent Technologies, Spain) with a Supelcogel C-610 column (Supelco, Spain) and two detectors in series: an ultraviolet detector (UV) and a refractive index detector (RI). A conversion factor was used to express VFA concentration as chemical oxygen demand (COD) (Gujer et al., 1995). A mobile phase with 0.1 % phosphoric acid was employed, and the flow rate was set at 0.5 mL/min. The temperature of the column was programmed at 30 °C and detection was performed at a wavelength of 210 nm. Prior to analysis, all samples were centrifuged at 10,000 rpm for 10 min and filtered through a 0.22 μm nylon membrane filter (Scharlab, Spain).

Long chain fatty acids (LCFA) (caproic, C6:0; caprylic, C8:0; capric, C10:0; lauric, C12:0; myristic, C14:0; palmitic, C16:0; palmitoleic, C16:1; stearic, C18:0; oleic, C18:1; and linoleic, C18:2, acids) analyses were carried out as described in Neves et al. (2009). LCFA were assessed in both the liquid and solid matrices due to their adsorption onto the solid matrix. Esterification of free fatty acids was carried out using HCl:1-propanol, followed by extraction with dichloromethane (DCM). LCFA were chromatographically resolved employing a TRB-WAX CP-Sil 52 CB column (Teknokroma Analítica, Spain) with helium as the carrier gas, operating at a flow rate of 1 mL/min, and a flame ionization detector. The injector and detector temperatures were set at 220 and 250 °C, respectively. The oven was initially set at a temperature of 50 °C, sustained for 2 min, then followed by a 10 °C/min ramp up to 225 °C and finally kept under isothermal conditions for 10 min. The identification of the compounds was carried out based on the respective retention times and the quantification was done with a calibration curve, using synthetic compounds as standards of each of the acids in DCM solution. Pentadecanoic acid (C15:0) was used as internal standard LCFA (IS).

The methane content of the biogas was assessed using a Hewlett-Packard 5890 Series II gas chromatograph (Agilent Technologies, Spain) containing a Porapak-Q column (Sigma-Aldrich, Germany) and a thermal conductivity detector (TCD). Helium was employed as the carrier gas at a flow rate of 15 mL/min, while the injector, oven, and detector were maintained at 100, 30, and 100 °C, respectively. Methane concentration was expressed in terms of COD using a conversion factor (1 mol CH_4 = 64 g COD) (Heidrich et al., 2011).

3. Results and discussion

3.1. Effect of thermal pretreatment on the anaerobic digestion of solid tuna waste

The influence of a heat pretreatment on the methane production from solid tuna waste was studied in batch assays. Two different substrates were chosen for this study: raw fish viscera (RFV) and cooked fish viscera (CFV) (Table 1).

The results of methane production for the RFV and CFV tests are shown in Fig. 1 and detailed in Table 2. The effect of the thermal pretreatment varied depending on the type of waste. In the case of RFV, methane production increased by 50 % after the thermal pretreatment, reaching 0.18 and 0.27 g COD-CH_4/g COD added for non-pretreated (RFV_{NP}) and pretreated (RFV_P) samples, respectively. However, the

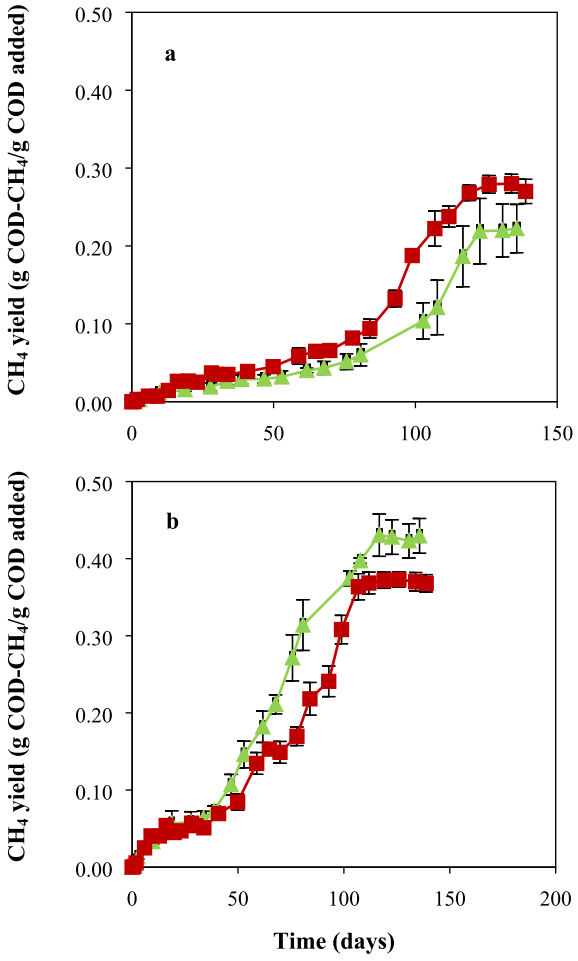

Fig. 1. Methane production in the anaerobic digestion tests of (a) raw fish viscera (RFV), (b) cooked fish viscera (CFV): (▲) non-subjected to thermal pretreatment, (■) subjected to thermal pretreatment. Data are mean values ± standard deviation of three replicates. Vertical bars are ± SD of the mean.

Table 2
Effect of thermal pretreatment on the anaerobic digestion of solid tuna waste: raw fish viscera (RFV) and cooked fish viscera (CFV), subjected to thermal pretreatment (P) and non-subjected to thermal pretreatment (NP).

Experiment	TS removal (%)	VS removal (%)	N-NH_4^+ (g/L)	sCOD (g/L)	CH_4 yield (g COD-CH_4/g COD added)
RFV_{NP}	38.2 ± 1.9	62.6 ± 3.9	1.3 ± 0.1	15.5 ± 0.9	0.18 ± 0.03
RFV_P	68.3 ± 4.4	70.9 ± 6.2	1.1 ± 0.1	10.9 ± 0.6	0.27 ± 0.02
CFV_{NP}	49.2 ± 3.1	71.3 ± 5.3	1.0 ± 0.1	4.6 ± 0.2	0.36 ± 0.02
CFV_P	68.7 ± 4.8	72.4 ± 5.6	0.5[1]	9.3 ± 0.4	0.36 ± 0.01

Data correspond to samples collected at the end of each assay, except for CH_4 yield, which represents the experiment's maximum values.
Data are mean values ± standard deviation of three replicates.
[1] Standard deviation < 0.1.

thermal treatment did not improve methane production from CFV, obtaining 0.36 g COD-CH_4/g COD added for both CFV_{NP} and CFV_P. Thus, CFV produced a higher methane yield than any of the RFV samples (Table 2).

These results cannot be attributed to the composition of the residues, as RFV had a considerably higher COD (1.5 g COD/g dried residue) compared to CFV (1.1 g COD/g dried residue), and the fat content was similar for both substrates (Table 1). Therefore, the differences observed in methane production might be related to the thermal pretreatment applied. Besides, it must be taken into account that CFV had already been subjected to a previous heat treatment during the manufacture of canned tuna. The high temperature reached during the fish cooking could have a similar effect to the thermal pretreatment used in this study. Temperatures close to 110 °C do not cause degradation of the molecular complexes but allow the solubilization of the proteins due to their denaturation and an increase in the elimination of particulate carbohydrates (Ariunbaatar et al., 2014). This could explain why a subsequent thermal pretreatment did not increase the methane production and, instead, significant differences were seen in the RFV tests.

Another aspect to be considered is the possible loss of volatile organic compounds from readily biodegradable substrates when the sample is subjected to thermal pretreatment (Kor-Bicakci and Eskicioglu, 2019). Regarding the percentage of volatile solids (VS) removal, both CFV_{NP} and CFV_P as well as RFV_P presented a higher VS removal (about 70 %) than the RFV_{NP} sample (63 %) (Table 2).

On the other hand, soluble COD (sCOD) was higher in the RFV_{NP} sample (15.5 g COD/L), which suggests that a high percentage of solubilized COD was not transformed into methane. This would indicate that methanogenesis was the rate-limiting step in the anaerobic process and not the hydrolysis step, suggesting that an inhibition by the accumulation of VFA could have happened. Effectively, the highest concentration of VFA (21.13 g COD/L) was found in RFV_{NP}. The main VFA produced were acetic (10.37 g COD/L), propionic (4.36 g COD/L) and i-valeric acids (5.12 g COD/L) (Table 3). It is known that the major inhibitory compounds in the anaerobic processes are ammonia, sulfate, heavy metals and other organic inhibitors such as VFA (Chen et al., 2008; Kangle et al., 2012). High concentrations of VFA may cause inhibition of the methanogenic step, due, among other causes, to a decrease of pH in the medium (Bücker et al., 2020). Thus, a high VFA accumulation in the RFV_{NP} assay could explain the low methane production (0.18 g COD-CH_4/g COD added) (Table 2).

Pretreatment processes are known to accelerate the hydrolysis and acidogenesis stages, favoring a satisfactory development of anaerobic digestion. Moreover, thermal pretreatments transform substrates with a high lipid concentration into LCFA, which subsequently are converted into fatty acids of lower molecular weights (Appels et al., 2010). In an anaerobic environment, lipids are hydrolyzed into glycerol and LCFA by extracellular lipases (Masse et al., 2003; Palatsi et al., 2009). Glycerol undergoes additional oxidation in the acidogenesis step to generate volatile fatty acids. In contrast, LCFA are metabolized through the β-oxidation pathway during the acetogenesis stage, leading to the production of short-chain fatty acids (SCFA) (Elsamadony et al., 2021).

Tuna waste contains a high concentration of fat, 0.17 g fat/g dried residue (Table 1), which could be converted to LCFA in the biodegradation process. Other authors have reported LCFA accumulation in the anaerobic digestion of fish waste (Eiroa et al., 2012; Neves et al., 2009), which has been associated to a possible decrease in bacterial activity (Zonta et al., 2013). Hence, the stability of the anaerobic digestion of lipid-rich substrates represents a great challenge due to potential LCFA-induced inhibition (Jannat et al., 2022). The inhibition is mainly related to the adsorption of these acids on the microbial surface, affecting cellular nutrient transport mechanisms (Neves et al., 2009; Palatsi et al., 2009; Jannat et al., 2022). Several authors have proposed different approaches aiming to mitigate LCFA-caused inhibition, evaluating reactor configurations, operational conditions, and feeding strategies (Alves et al., 2009; Dasa et al., 2016; Elsamadony et al., 2021). In this study, the highest LCFA concentration (6.23 g COD/L) was observed in the RFV_{NP} test, being the major acids in order: C8:0, C18:0, C16:0 and C6:0 (Table 3). This LCFA accumulation, along with VFA, could have led to a lower methane production. Furthermore, in the assays conducted with RFV samples, a lower degradation rate was observed compared to those performed with CFV samples (Fig. 1), probably related to the accumulation of LCFA and VFA during the anaerobic digestion process.

Total nitrogen concentration was very similar between RFV and CFV (0.12 g N/g dried residue) (Table 1). However, it was observed a higher ammonia concentration in those samples not subjected to thermal pretreatment, 1.3 and 1.0 g N-NH_4^+/L, for RFV_{NP} and CFV_{NP} respectively,

Table 3
(a) Volatile f atty acids (VFA) and (b) long chain fatty acids (LCFA) concentration (g COD/L) at the end of the anaerobic digestion assays of solid tuna waste: raw fish viscera (RFV) and cooked fish viscera (CFV), subjected to thermal pretreatment (P) and non-subjected to thermal pretreatment (NP). Data are mean values ± standard deviation of three replicates. When not specified, standard deviation < 0.01.

(a)

Experiment	HLac	HFor	HAc	HPr	HBu	i-HVal	HVal	Total VFA
RFV_{NP}	0.16 ± 0.01	0.01	10.37 ± 0.45	4.36 ± 0.24	0.80 ± 0.10	5.12 ± 0.24	0.31 ± 0.03	21.13 ± 0.83
RFV_P	1.01 ± 0.10	0.03 ± 0.01	0.35 ± 0.09	0.20 ± 0.07	–	1.68 ± 0.06	–	3.27 ± 0.04
CFV_{NP}	0.01 ± 0.01	0.01 ± 0.01	0.45 ± 0.03	2.97 ± 0.39	0.07 ± 0.06	3.73 ± 0.52	0.02 ± 0.02	7.26 ± 0.18
CFV_P	–	0.02 ± 0.01	0.08 ± 0.01	0.03 ± 0.01	–	–	–	0.13 ± 0.01

(b)

Experiment	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	Total LCFA
RFV_{NP}	1.06	1.39 ± 0.01	0.54 ± 0.01	0.20	0.09	1.21 ± 0.08	0.29	1.34 ± 0.01	0.11	6.23 ± 0.23
RFV_P	–	–	–	–	0.04 ± 0.01	0.34	0.33 ± 0.01	0.26	0.07	1.04 ± 0.01
CFV_{NP}	–	–	–	–	–	0.13	0.04	0.06	0.06	0.28 ± 0.01
CFV_P	–	–	–	–	–	0.12 ± 0.01	–	0.16	0.10	0.38

compared to 1.1 and 0.5 g N-NH_4^+/L for RFV_P and CFV_P, respectively. These concentrations, although high, are not within the range of 1.5–3 g N-NH_4^+/L which are considered inhibitory (Chen et al., 2008; Wang et al., 2011). Thus, it can be assumed there was not inhibition by ammonia.

In addition, salt concentration was very similar for both RFV and CFV (0.04 g Cl^-/g dried residue) (Table 1). Those salt concentrations are moderate, which can stimulate the bacterial growth, not inhibiting the anaerobic process (Choudhury et al., 2022).

3.2. Effect of co-digestion on the anaerobic digestion of tuna waste

A series of experiments were performed in order to study the influence of co-digestion on the production of methane from solid tuna waste. CFV was selected based on the results obtained in the tests described above, where a higher production of methane was observed (0.36 g COD-CH_4/g COD added). Also, three different substrates were chosen for the co-digestion assays: wastewater from dairy industry (WDI), secondary sludge from an aerobic wastewater treatment plant treating canned tuna effluents (SS) and fat waste separated from the canned tuna effluents (FW). Fig. 2 shows methane production as a function of time for individual and co-digested residues.

Despite the fact that WDI individual digestion showed a noteworthy methane yield (0.49 g COD-CH_4/g COD added), co-digestion of CFV and WDI did not favor biogas production from tuna waste, since methane yield (0.36 g COD-CH_4/g COD added) was similar to that observed with CFV alone (Table 4). Similarly, methane yield for co-digestion test of CFV and SS decreased in relation to individual SS fermentation, reaching 0.46 and 0.72 g COD-CH_4/g COD added, respectively. Although, it did improve when compared to CFV individual assay (0.36 g COD-CH_4/g COD added). These results could be explained by a higher accumulation of VFA in those assays where CFV was used (Table 5). Anaerobic digestion of CFV lead to a VFA concentration of 7.28 g COD/L. A high concentration of organic matter, mainly lactose, fat and proteins, is a defining feature of dairy wastewater, which can be easily degraded by acidogenic bacteria, generating VFA (Charalambous et al., 2020). Nevertheless, VFA concentration did not exceed 0.07 g COD/L by the conclusion of the WDI individual assay, which increased up to 3.74 g COD/L when combined with CFV residue. Additionally, SS digestion reached a VFA concentration of 0.36 g COD/L, increasing to 3.14 g COD/L in the CFV + SS co-digestion test.

On the contrary, with respect to the co-digestion assay of CFV + FW, methane yield (0.87 g COD-CH_4/g COD added) was significantly higher than the ones obtained in the respective individual assays: 0.36 g COD-CH_4/g COD added for CFV and 0.55 g COD-CH_4/g COD added for FW (Table 4). Lipids fermentation produces more methane (0.99 L CH_4/g) than protein (0.63 L CH_4/g) or carbohydrates (0.42 L CH_4/g) (Wu and Song, 2021). Thus, co-digestion of CFV + FW was expected to enhance methane production. The highest VFA concentration (21.90 g COD/L) was reached during FW individual test, with a significant concentration of acetic acid, which represented 86 % of the total acids. Contrary to previous cases, VFA accumulation decreased to 4.42 g COD/L after its co-digestion with CFV.

Table 5 also summarizes LCFA concentration upon completion of the anaerobic digestion experiments. In the case of FW, anaerobic digestion led to the highest LCFA concentration (20.36 g COD/L), while low concentrations were obtained in the rest of the tests performed (0.28–1.42 g COD/L). The LCFA profile was similar in all assays, except for the FW test where C16:0 represented 62 % of the total LCFA, reaching 12.57 g COD/L. The presence of C6:0 and C12:0 was also observed but in lower concentrations. Since SS and WDI have a much lower lipid content than FW (0.04 g/g dried waste, 0.40 g/g dried waste and 0.002 g/L, respectively) (Table 1), it was expected to find lower accumulation of LCFA in those assays.

In addition, differences in methane yield between WDI and SS versus FW co-digestion assays could be due to C/N ratios (Table 1). CFV exhibits a low C/N ratio of 5.1, comparable to other C/N ratios previously reported for fish waste (5.7–6.5) (Cadavid-Rodríguez et al., 2019; Bücker et al., 2020; Ingabire et al., 2023). It is known that anaerobic digesters perform optimally with a C/N ratio ranging from 20 to 35

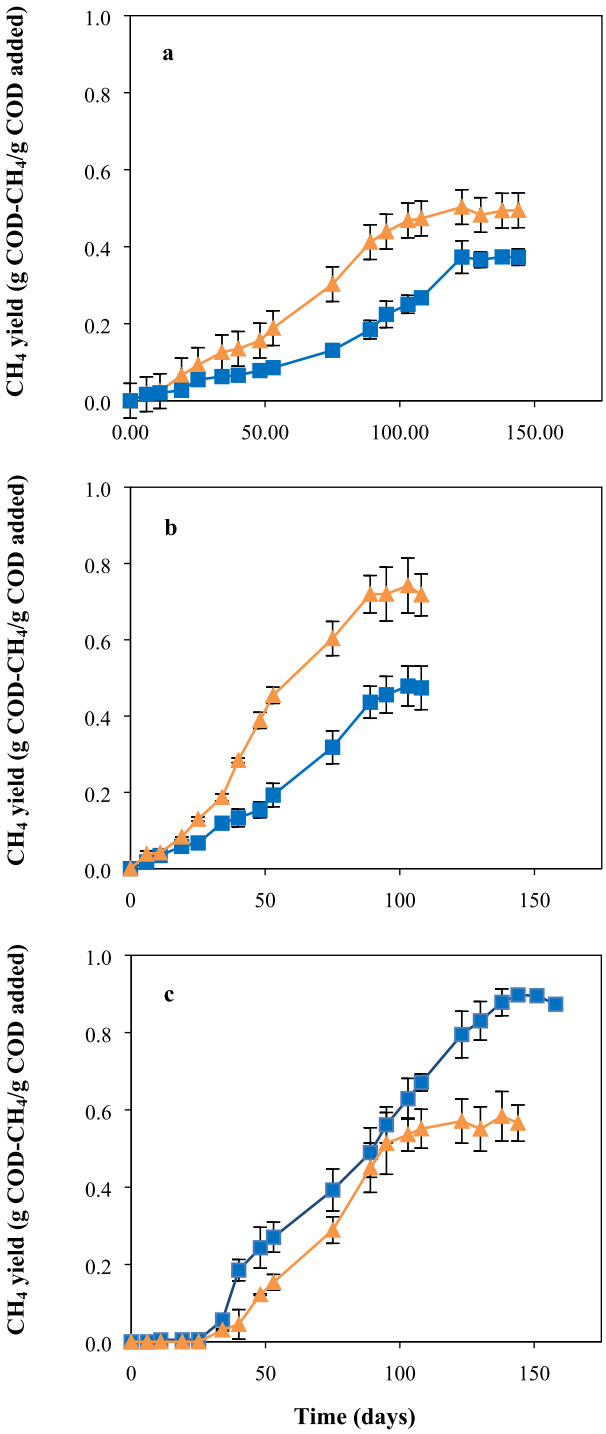

Fig. 2. Methane production in the anaerobic digestion tests of (a) wastewater from dairy industry (WDI) and WDI co-digested with cooked fish viscera (CFV), (b) secondary sludge (SS) and SS co-digested with CFV, (c) fat waste (FW) and FW co-digested with CFV: (▲) individual substrate assay, (■) co-digestion assay. Data are mean values ± standard deviation of three replicates. Vertical bars are ± SD of the mean.

Table 4
Effect of co-digestion on the anaerobic fermentation of cooked fish viscera (CFV) with wastewater from dairy industry (WDI), secondary sludge (SS) and fat waste (FW).

Experiment	TS removal (%)	VS removal (%)	N-NH_4^+ (g/L)	sCOD (g/L)	CH_4 yield (g COD-CH_4/g COD added)
CFV	49.2 ± 3.1	71.3 ± 5.3	1.0 ± 0.1	4.6 ± 0.2	0.36 ± 0.02
WDI	58.4 ± 2.9	62.1 ± 7.7	1.2 ± 0.1	6.1 ± 0.2	0.49[2]
CFV + WDI	39.1 ± 2.7	75.2 ± 7.9	1.7 ± 0.1	11.8 ± 0.9	0.36 ± 0.02
SS	70.4 ± 4.3	43.3 ± 5.6	1.6[1]	8.1 ± 0.1	0.72 ± 0.06
CFV + SS	64.7 ± 7.5	66.5 ± 4.0	1.9 ± 0.4	13.4 ± 1.2	0.46 ± 0.05
FW	46.3 ± 5.2	44.1 ± 3.4	1.1 ± 0.1	44.3 ± 1.7	0.55 ± 0.05
CFV + FW	58.8 ± 6.8	39.9 ± 5.0	1.2 ± 0.1	6.8 ± 1.0	0.87 ± 0.01

Data correspond to samples collected at the end of each assay, except for CH_4 yield, which represents the experiment's maximum values.
Data are mean values ± standard deviation of three replicates.
[1] Standard deviation <0.1.
[2] Standard deviation <0.01.

(Habiba et al., 2009). However, during the co-digestion with WDI and SS, achieving this ratio becomes a challenge due to their limited carbon content (with C/N ratios of 4.9 and 5.5, respectively). Consequently, this carbon deficiency results in reduced methane production during the digestion process (Choe et al., 2020). On the other hand, FW shows a very high C/N ratio of 45.5, obtaining a C/N ratio of 25.3 for combined CFV + FW. An optimal C/N ratio in co-digestion assays could favor anaerobic digestion and avoid accumulation of LCFA. In fact, despite the high lipid concentration in CFV + FW co-digestion assay, no accumulation of LCFA was observed (Table 5).

Another aspect to consider is the degree of solubilization and hydrolysis. In the CFV individual assay, soluble organic matter was 4.6 g sCOD/L, increasing up to 11.8, 13.4 and 6.8 g sCOD/L for WDI, SS and FW co-digestion assays, respectively. Regarding FW digestion, the concentration obtained was much higher, 44.3 g sCOD/L. This indicates that part of the organic matter was not converted to methane, which points out that the hydrolysis was not the limiting step in this case and suggests a possible inhibition in the methanogenic step. This may be due to an imbalance between the microorganisms that produce acid and those that consume it (Eiroa et al., 2012).

Moreover, CFV presented a TS removal of 49 %, which increased to 59 and 65 % when co-digested with SS and FW, respectively (Table 4). Nonetheless, co-digestion with WDI lead to a lower TS removal (39 %), despite having a higher solids concentration (2.9 %) than the individual residues. Solid removal has a direct relationship with methane yield at different TS levels (Rubio et al., 2022).

Finally, ammonia concentration was higher in the co-digestion assays than in the WDI, SS and FW individual tests, with an increase of 19–39 %, due to the higher initial nitrogen concentration of tuna residue (Table 5). As previously discussed, ammonia is considered a potential inhibitor in the anaerobic digestion process when present in high concentrations (1.5–3 g N-NH_4^+/L) (Chen et al., 2008; Wang et al., 2011). However, since the amounts obtained in all these assays are much lower, ammonia concentration may have not affected the results.

4. Practical applications and future research perspectives

The findings of this study highlight the potential of pretreatment methods and co-digestion strategies to enhance the biogas production efficiency of solid tuna waste. Anaerobic co-digestion, especially with lipid-rich waste streams like fat waste, demonstrated substantial improvements in methane yield. This insight can guide the design and optimization of anaerobic digestion processes for treating organic solid wastes from various sources, contributing to sustainable waste management and renewable energy production.

Future steps in our research must involve a thorough exploration of the microbial structure and metabolic pathways implicated. Uncovering the mechanisms governing the microbial community dynamics during the anaerobic digestion of renewable substrates for biogas production holds the key to potentially improving the process efficiency. In a similar study focusing on biogas production from fish waste, Bücker et al. (2020) identified different groups of Bacteria, Fungi, and Archaea. The prevalent bacterial classes were Clostridia and Synergistia, while Methanosaeta and Methanosarcina were the main representatives of the Archaea group.

Moreover, investigating the viability of VFA recovery within the anaerobic digestion process for methane production holds considerable promise. Despite the potential inhibitory effects of their accumulation, these compounds also present a valuable opportunity for utilization, leading to the production of various bioproducts, including biofuels and bioplastics (Sekoai et al., 2021). Notably, several recovery methods have

Table 5
(a) Volatile fatty acids (VFA) and (b) long chain fatty acids (LCFA) concentrations (g COD/L) at the end of the anaerobic digestion assays of cooked fish viscera (CFV), wastewater from dairy industry (WDI), secondary sludge (SS) and fat waste (FW), as well as their respective co-digestion assays (CFV + WDI, CFV + SS and CFV + WDI). Data are mean values ± standard deviation of three replicates. When not specified, standard deviation <0.01.

(a)

Experiment	HLac	HFor	HAc	HPr	HBu	i-HVal	HVal	Total VFA
CFV	0.01 ± 0.01	0.01 ± 0.01	0.45 ± 0.03	2.97 ± 0.39	0.07 ± 0.06	3.73 ± 0.52	0.02 ± 0.02	7.26 ± 0.18
WDI	–	0.01	0.06 ± 0.04	–	–	–	–	0.07 ± 0.04
CFV + WDI	–	0.01	0.21 ± 0.08	3.52 ± 0.42	–	–	–	3.74 ± 0.35
SS	–	0.01	0.10 ± 0.03	0.04 ± 0.02	–	0.04	0.17 ± 0.06	0. 36 ± 0.03
CFV + SS	–	0.02 ± 0.01	0.21 ± 0.07	1.54 ± 0.09	–	1.78 ± 0.31	0.16 ± 0.01	3.71 ± 0.51
FW	–	–	18.78 ± 4.02	0.82 ± 0.30	2.18 ± 0.14	0.88 ± 0.24	0.12 ± 0.01	22.78 ± 4.27
CFV + FW	–	0.14 ± 0.01	0.19 ± 0.19	2.61 ± 0.06	0.10 ± 0.02	1.38 ± 0.09	–	4.42 ± 0.19

(b)

Experiment	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	Total LCFA
CFV	–	–	–	–	–	0.13	0.04	0.06	0.06	0.28 ± 0.01
WDI	–	–	–	–	–	0.26 ± 0.03	0.06 ± 0.01	0.31	0.11	0.74 ± 0.02
CFV + WDI	–	–	–	–	0.08 ± 0.02	0.47	0.24	0.45	0.18 ± 0.01	1.42 ± 0.02
SS	–	–	–	–	–	0.14	–	0.16	0.09	0.40
CFV + SS	–	–	–	–	–	0.14	0.20	0.06 ± 0.01	0.09 ± 0.01	0.50 ± 0.01
FW	2.48 ± 0.04	0.30 ± 0.01	0.70	2.85 ± 0.04	0.74 ± 0.01	12.57 ± 0.02	0.23 ± 0.01	0.16 ± 0.01	0.33	20.36 ± 0.10
CFV + FW	–	–	–	–	–	0.43 ± 0.01	0.21	0.63 ± 0.01	0.12	1.39 ± 0.01

been explored, such as adsorption, solvent extraction, electrodialysis, reverse osmosis, and nanofiltration (Atasoy et al., 2018). Furthermore, research into in-situ recovery techniques has shown promising outcomes, enhancing recovery efficiency while simultaneously alleviating their potential inhibitory impact within the anaerobic process (Roume et al., 2016).

Lastly, the scale-up of laboratory findings to pilot-scale systems represents a critical step in determining the practical viability of the studied processes. Initially, it's essential to demonstrate the reproducibility of results observed on a larger scale, ensuring that the processes maintain their efficiency and efficacy. The transition to pilot-scale systems requires rigorous monitoring and optimization of operational parameters, such as temperature, retention times and organic loading rates. Furthermore, careful evaluation of factors like reactor design, waste availability and consistency, or heat management is imperative to ensure a cost-effective scale-up. Taking into consideration the potential challenges that may arise during the transition from the laboratory scale will be crucial for assessing the environmental and economic feasibility of the proposed co-digestion and pretreatment strategies.

5. Conclusions

The thermal pretreatment of raw fish viscera resulted in increase of 50 % in methane yield. However, this pretreatment did not increase biogas production for cooked viscera, probably due to the fact that it had already been subjected to a previous heat treatment during the manufacture of tuna. Anaerobic co-digestion of cooked viscera with fat waste significantly increased methane yield (0.87 g COD-CH_4/g COD added), while co-digestion with dairy waste and sewage sludge lead to notoriously lower yields (0.36 and 0.46 g COD-CH_4/g COD added, respectively). This result may be related to C/N ratio, which was within optimal range for anaerobic digestion in the first case.

CRediT authorship contribution statement

Noela Bermúdez-Penabad: Writing – original draft, Investigation, Formal analysis. **Andrea Rodríguez-Montes:** Writing – review & editing. **Madalena Alves:** Methodology. **Christian Kennes:** Writing – review & editing, Funding acquisition. **María C. Veiga:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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

The authors are unable or have chosen not to specify which data has been used.

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