



Impact of food waste composition on acidogenic co-fermentation with waste activated sludge

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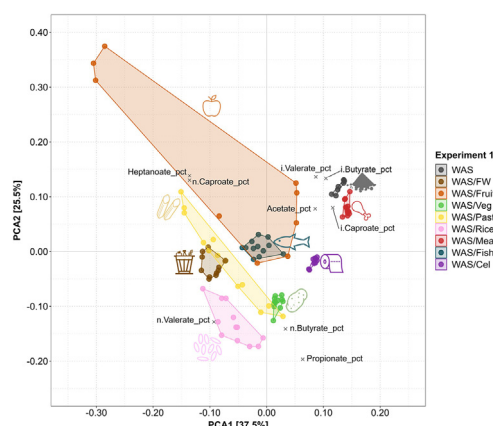
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HIGHLIGHTS

- Each FW component had a distinct VFA yield and profile.
- Protein-rich components showed higher VFA yields than carbohydrate-rich ones.
- Positive interaction on VFA yield when mixing protein and carbohydrate co-substrates.
- The interaction was not proportional to the co-substrates proportion in the mixture.
- FW composition can be used to drive the VFA profile to a certain extent.

GRAPHICAL ABSTRACT



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ABSTRACT

The impact of food waste (FW) composition on co-fermentation performance was studied to elucidate if adjusting FW composition can be used to drive the fermentation yield and profile, which is relevant for biorefinery applications. First, the impact of individual FW components (i.e., fruit, vegetables, pasta, rice, meat, fish, and cellulose) was assessed. Subsequently, the effect of mixing a protein-rich component and a carbohydrate-rich component was studied (i.e., fish/fruit and fish/cellulose, and meat/rice and meat/vegetable). All experiments were carried out in mesophilic batch assays using waste activated sludge (WAS) as main substrate, the same mixture ratio (70 % WAS + 30 % FW on VS basis), and no pH control. Results showed that each FW component had a distinct effect on VFA yield and profile, with protein-rich components reaching the highest VFA yields; 502 and 442 mgCOD/gVS for WAS/Fish and WAS/Meat, respectively. A positive interaction on VFA yield was observed when mixing a protein-rich and a carbohydrate-rich component. This interaction was not proportional to the co-substrates proportion in the mixtures. On the other hand, the VFA profile was clearly driven by the components in the mixture, including both WAS and FW composition. Overall, these results indicate that predicting the VFA yield of WAS/FW co-fermentation is not just related to FW composition, but FW composition could be used to adjust the VFA profile to a certain extent.

1. Introduction

The European Union Bioeconomy Strategy aims to manage natural resources sustainably and reduce the dependence on non-renewable and

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unsustainable resources (Directorate-General for Research and Innovation (European Commission), 2018a). The action plan of this strategy remarks the importance of developing and implementing sustainable biorefineries to substitute fossil-based materials for bio-based, recyclable and biodegradable materials using organic wastes, residues and side streams (Directorate-General for Research and Innovation (European Commission), 2018b).

Fermentation is a key biotechnology in most microbial-driven biorefineries due to its capability to transform organic waste into easily assimilable organic compounds such as volatile fatty acids (VFAs), lactic acid and alcohols (Battista et al., 2022; Vázquez-Fernández et al., 2022). These fermentation products can be subsequently utilised as organic platform chemicals to produce biopolymers (Fradinho et al., 2019; Valentino et al., 2018), single cell protein (Allegue et al., 2022; Capson-Tojo et al., 2020), medium chain fatty acids (Carvajal-Arroyo et al., 2021; Roghair et al., 2018), or bioenergy (Abreu et al., 2019; Dahiya et al., 2015).

Co-fermentation is an emerging strategy to increase the yield of fermenters and valorise several waste in a single facility (Perez-Esteban et al., 2022). Co-fermentation improves the fermentation yields by (i) increasing the organic loading rate, (ii) balancing macro- and micro-nutrients, (iii) diluting potential inhibitory compounds, (iv) increasing the buffer capacity, (v) improving rheological properties, and/or (vi) promoting an active microbial community (Fang et al., 2020; Peces et al., 2020; Perez-Esteban et al., 2022).

Wastewater treatment plants (WWTP) are pioneering the paradigm change from treatment to resource recovery. WWTP generate large amounts of waste activated sludge (WAS) that is commonly diverted to anaerobic digestion. However, biogas and digestate have a relatively low market value and a lower range of applications than fermentation products (Dahiya et al., 2018). The acidogenic fermentation of WAS could be implemented in WWTP to produce VFA for biological nutrient removal as well as to support other more advanced and profitable biotechnologies (Puyol et al., 2017). However, WAS is characterised by low fermentation yields due to its poor biodegradability and low hydrolysis rate (Gonzalez et al., 2018; Gou et al., 2014; Peces et al., 2020; Xu et al., 2020).

Food waste (FW) is the most studied co-substrate for WAS co-fermentation (Perez-Esteban et al., 2022). FW is a suitable co-substrate for WAS due to its high organic content and biodegradability, while WAS is a suitable main substrate for FW due to its water content and buffer capacity. Most WAS/FW co-fermentation studies have focused on the impact of operational conditions and mixture composition. However, little attention has been given to other important parameters such as the impact of FW composition on VFA yield and VFA profile.

Strazzera et al. (2021) showed that the mono-fermentation of different food waste fractions led to different VFA yield and profile. The highest VFA yields were obtained from the protein-rich fraction followed by the starch-rich fraction and the fruit and vegetables fraction. The VFA yield of the cellulose-rich fraction and lipid-rich fraction was very low for all pH conditions (i.e., uncontrolled pH, 5.5 and 7.0). The highest VFA yields were achieved at pH 7.0 with butyric acid dominating the VFA profile in all fractions. The starch-rich and fruit and vegetable fractions also enriched propionic and acetic acid, whereas the protein-rich fraction also enriched valeric and acetic acid.

The fermentation of proteins has not been studied as thoroughly as sugars and carbohydrates (González-Cabaleiro et al., 2015; Hoelzle et al., 2014; Zhou et al., 2018). Shen et al. (2017) and Bevilacqua et al. (2020) evaluated different types of protein on mono-fermentation performance. Shen et al. (2017), who fermented tofu and egg white at 30 °C and pH 6.0, reported a higher VFA yield for egg white than for tofu and a completely different VFA profile. The tofu VFA profile was dominated by acetic acid, while egg white presented an evenly distributed concentration of acetic, propionic, butyric, and valeric acid. Bevilacqua et al. (2020), who fermented casein and gelatine at 25 °C and circumneutral pH, also reported that different protein types result in different VFA yields (higher for casein than for gelatine) and product profile. The dominant VFA in both fermenters was acetic acid; however, casein fermentation enriched butyric and propionic acid while gelatine fermentation enriched propionic and valeric acid.

In two recent publication using synthetic substrates, Bevilacqua et al. (2022) and Wang et al. (2022) showed that the ratio between carbohydrates and protein can be used to drive the fermentation product profile. In both publications, increasing the amount of carbohydrates favoured the accumulation of acetic and n-butyric. Bevilacqua et al. (2022) and Wang et al. (2022) provided a fundamental understanding of the impact of feedstock composition on co-fermentation performance, however, further research is needed to elucidate the interaction when using complex substrates and the relative importance of FW composition on the VFA yield and VFA profile. This knowledge is important to maximise the VFA yield to make better use of the fermentation infrastructure and tune the VFA profile since different biotechnologies require different VFA as platform chemical.

The goal of this study is to understand how FW composition influences the VFA yield and profile of WAS/FW co-fermentation. Firstly, the impact of each FW component was individually assessed. Secondly, the interaction between different FW components (fish/fruit & fish/cellulose) was evaluated under different proportions. Finally, the interaction observed when mixing a protein-rich and a carbohydrate-rich component was validated using different components (meat/vegetables & meat/rice).

2. Materials and methods

2.1. Substrates origin

Thickened waste activated sludge (WAS) was collected in a municipal WWTP located in the Barcelona Metropolitan Area (ca. 300,000 population equivalent) (Catalonia, NE Spain). After collection, it was stored in a fridge at 4 °C until use (maximum storage time of 3 days). FW was formulated by mixing vegetables (30 %), fruits (30 %), carbohydrates (pasta and rice) (20 %), meat (10 %), and fish (10 %) on wet basis as in Vidal-Antich et al. (2021). Synthetic FW was used to ensure FW reproducibility throughout the experiments as well as to better assess the individual impact of each component. The different components tested were: fruit (apple and banana), vegetable (Veg) (potato and onion), pasta (plain boiled pasta), rice (round-grain boiled rice), meat (canned ham), fish (surimi sticks), and microcrystalline cellulose (Cel). Microcrystalline cellulose was not present in the synthetic FW formulation, but it was used as a reference substrate for carbohydrates.

All ingredients were individually shredded with a kitchen blender (ca. 3 min) to reduce the particle size, where the minimum amount of deionised water was added. The physico-chemical characterisation of the different components and the macromolecular composition of each product can be found in the supplementary material (Table S1 and Table S2).

2.2. Co-fermentation experiments set-up

Co-fermentation batch experiments were carried out in 250 mL serum glass bottles (operating volume of 150 mL) at mesophilic conditions under anaerobic conditions. All WAS/FW co-fermentation experiments were carried out in triplicate using the same mixture ratio, i.e., 70 % WAS + 30 % FW (on VS basis). This WAS/FW ratio provides high VFA yields while maintaining pH above strongly inhibitory levels without adding external chemicals (Vidal-Antich et al., 2021). The FW composition varied depending on the goal of each experiment. No inoculum was added, hence the fermentation process relied on the native fermentative bacteria. The pH was not adjusted at the beginning or during the experiment. After adding the substrates, each bottle was flushed with N₂ gas for 1 min (ca. 5 L/min) to achieve anaerobic conditions and sealed with a PTFE-butyl septum retained with a screwcap. Finally, the bottles were placed in a temperature-controlled incubator at 35 °C. Bottles were manually mixed by swirling each day and before each sampling event. Each fermentation experiment was run for 13 days and monitored by 8 sampling events. In each sampling event, 4 mL of sample were withdrawn with an 18G hypodermic needle connected to a 5 mL syringe (the total withdrawn volume represented about 20 % of the initial volume). The samples were collected to analyse soluble chemical oxygen demand (SCOD), VFA, pH, and total ammoniacal

nitrogen (TAN). Table S3 in the supplementary material provides the amount of each component in each experiment.

2.2.1. Experiment 1. Assessment of individual FW components on FW and WAS co-fermentation

The goal of the first experiment was to assess the impact of each FW component on co-fermentation performance (i.e., VFA yield, VFA profile). Accordingly, seven co-fermentation mixtures were tested: WAS/Fruit, WAS/Veg, WAS/Pasta, WAS/Rice, WAS/Meat, WAS/Fish and WAS/Cel. Two additional experiments were carried out (i) WAS/FW co-fermentation and (ii) WAS mono-fermentation.

2.2.2. Experiment 2. Assessment of mixing a protein-rich and a carbohydrate-rich component in WAS co-fermentation: Fish with fruit or cellulose

The goal of the second experiment was to explore the impact of FW composition on WAS/FW co-fermentation performance. Based on Experiment 1 results, different mixtures between fish (protein-rich component) and fruit or cellulose (carbohydrate-rich components) were co-fermented with WAS to study the effect of FW composition and the influence of the carbohydrate source. Specifically, WAS (main substrate) was co-fermented mixing with fish and fruit or with fish and cellulose in different proportions. The tests carried out in this second experiment were (the numbers in the test identifiers indicate the proportions on VS basis): WAS/Fish_{70/30}, WAS/Fruit_{70/30}, WAS/Cel_{70/30}, WAS/Fish/Fruit_{70/20/10}, WAS/Fish/Fruit_{70/15/15}, WAS/Fish/Fruit_{70/10/20}, WAS/Fish/Cel_{70/20/10}, WAS/Fish/Cel_{70/15/15}, WAS/Fish/Cel_{70/10/20}, and WAS mono-fermentation.

2.2.3. Experiment 3. Corroborate the effect of mixing a protein-rich and a carbohydrate-rich component in WAS co-fermentation: Meat with vegetables or rice

The goal of the third experiment was to validate the response observed in Experiment 2 using meat as a protein-rich component mixed with either vegetables or rice as carbohydrate-rich components. The tests carried out in this third experiment were (the numbers in the test identifiers indicate the proportions on VS basis): WAS/Meat_{70/30}, WAS/Veg_{70/30}, WAS/Rice_{70/30}, WAS/Meat/Veg_{70/20/10}, WAS/Meat/Veg_{70/15/15}, WAS/Meat/Veg_{70/10/20}, WAS/Meat/Rice_{70/20/10}, WAS/Meat/Rice_{70/15/15}, WAS/Meat/Rice_{70/10/20}, and WAS mono-fermentation.

2.3. Analytical procedures and data analysis

Total solids (TS) and volatile solids (VS) were performed according to the Standard Method procedure 2540G (APHA, 2017). pH was measured with a micro pH probe (PHEL-GB3-001) connected to a multi-meter. For analysis of soluble compounds, the samples were centrifuged at 16,000 × g for 15 min and then filtered through a 0.45 µm nylon syringe filter. Soluble chemical oxygen demand (SCOD) was determined following the 5220D Standard Method procedure (APHA, 2017). Individual VFA concentration (i.e., acetic, propionic, i-butyric, n-butyric, i-valeric, n-valeric, i-caproic, n-caproic and heptanoic acid) were determined using a gas chromatograph (GC 2010 plus, Shimadzu) equipped with a capillary column (Agilent technologies DB-FFAP, 30 m × 0.25 mm × 0.25 µm) and flame ionised detector (FID) following the 5560D Standard Method procedure (APHA, 2017). TAN was measured using an ammonia electrode (Orion 9512, Thermo Scientific) following the Standard Method procedure 4500-NH₃D (APHA, 2017).

Principal component analysis (PCA) was used to elucidate the relationships between FW composition and VFA profile in a reduced ordination space. The PCA was performed using as response variables the percentage of each VFA (on COD basis) during the pseudo-stationary stage of the batch (data from 6th, 8th and 10th day). The response variables were z-score standardised before PCA analysis to compare variables with different magnitudes. PCA analysis was carried out using the function *prcomp()* in RStudio (version 4.0.3).

3. Results and discussion

3.1. Assessment of individual FW components on FW and WAS co-fermentation

Fig. 1 shows the VFA yield, pH and TAN concentration over time of the co-fermentation mixtures and WAS mono-fermentation in Experiment 1. All co-fermentation mixtures reached higher VFA yields than WAS mono-fermentation (307 mgCOD/gVS), indicating the higher biodegradability of the FW components compared to WAS. The highest co-fermentation yields were reached by the protein-rich components, 502 and 442 mgCOD/gVS for WAS/Fish and WAS/Meat, respectively. These results align with those reported by Strazzera et al. (2021) and they can be related to the higher energy density of protein-rich substrates compared to carbohydrate-rich substrates (COD/VS of ~1.5 vs. ~1.0, respectively). WAS/Fish and WAS/Meat mixtures also reached a slightly higher pH (pH ~5.5) than the other co-fermentation tests (pH ~5.0), which can be attributed to the additional buffer capacity provided by TAN from protein ammonification. The TAN concentration at the end of the WAS/Fish and WAS/Meat co-fermentation was 2100 and 1525 mgN/L, respectively. For comparison, the TAN concentration at the end of WAS mono-fermentation was 1160 mgN/L.

WAS/Pasta and WAS/Rice (starch-rich components) also showed a relatively high VFA yield of 394 and 419 mgCOD/gVS, respectively. The pH of these mixtures was around 5.0, slightly lower than the observed in the protein-rich components. The WAS/Veg and WAS/Fruit mixtures yielded 432 and 350 mgCOD/gVS, respectively. The VFA production of WAS/Fruit was very low during the first 6 days of the test, suggesting an inhibition of fermentative bacteria due to low pH. The VFA production from WAS/Fruit increased from day 6 once the pH of the fermentation broth was above 4.5. The mixture WAS/FW yielded 390 mgCOD/gVS which falls within the values obtained from the different FW components (350–502 mgCOD/gVS). The VFA yield of WAS/Cel was 350 mgCOD/gVS.

The PCA shows that each component had a distinct cluster based on their VFA distribution (Fig. 2), indicating that each component had a statistically different VFA profile. These results imply that each component is a potential driver of the VFA profile on WAS/FW co-fermentation. The VFAs concentration of each fermentation condition over time and the VFA profile used in the PCA can be found in the Fig. S1 and Fig. S2 of the supplementary material.

Based on their ordination space, four groups could be distinguished: (i) WAS and WAS/Meat were driven by acetic, i-butyric, i-valeric and i-caproic acid, (ii) WAS/Rice, WAS/Pasta and WAS/Veg were driven by propionic, n-butyric and n-valeric acid, (iii) WAS/Cel, and (iv) WAS/Fish that was equally influenced by all the response variables. The WAS/Fruit cluster was not included in any group because of the high variability of the VFA profile in the period considered. WAS/Fruit results can be related to pH variations. The WAS/FW cluster was located nearby the WAS/Rice and WAS/Pasta clusters, indicating that the VFA profile of the synthetic FW resembles the obtained from the starch-rich co-substrates.

The VFA profile of WAS mono-fermentation on day 8 (maximum VFA yield) was dominated by acetic (29 %), propionic (20 %), n-butyric (19 %) and n-valeric (11 %) acid, which is consistent with the results reported by Appels et al. (2011), Morgan-Sagastume et al. (2011) and Peces et al. (2020). WAS/Meat co-fermentation had a similar VFA profile to WAS, i.e., acetic (27 %), propionic (19 %), n-butyric (22 %) and n-valeric (13 %) acid. WAS/Fish showed a more even distribution of VFA, which VFA profile was dominated by acetic (18 %), propionic (19 %), n-butyric (23 %) and n-valeric (19 %) acid. The comparison between the VFA profile of both protein-rich components shows that WAS/Meat enriched i-caproic acid (3 %) while WAS/Fish enriched n-caproic (6 %) and heptanoic (3 %) acid. The VFA profile of WAS/Meat and WAS/Fish is consistent with previous publications where protein fermentation led to the enrichment of acetic and butyric acid (Alibardi and Cossu, 2016; Bevilacqua et al., 2020). The different VFA profile could be related to the different amino acid composition of the two co-substrates since other operational parameters were similar (Bevilacqua et al., 2020; Shen et al., 2017).

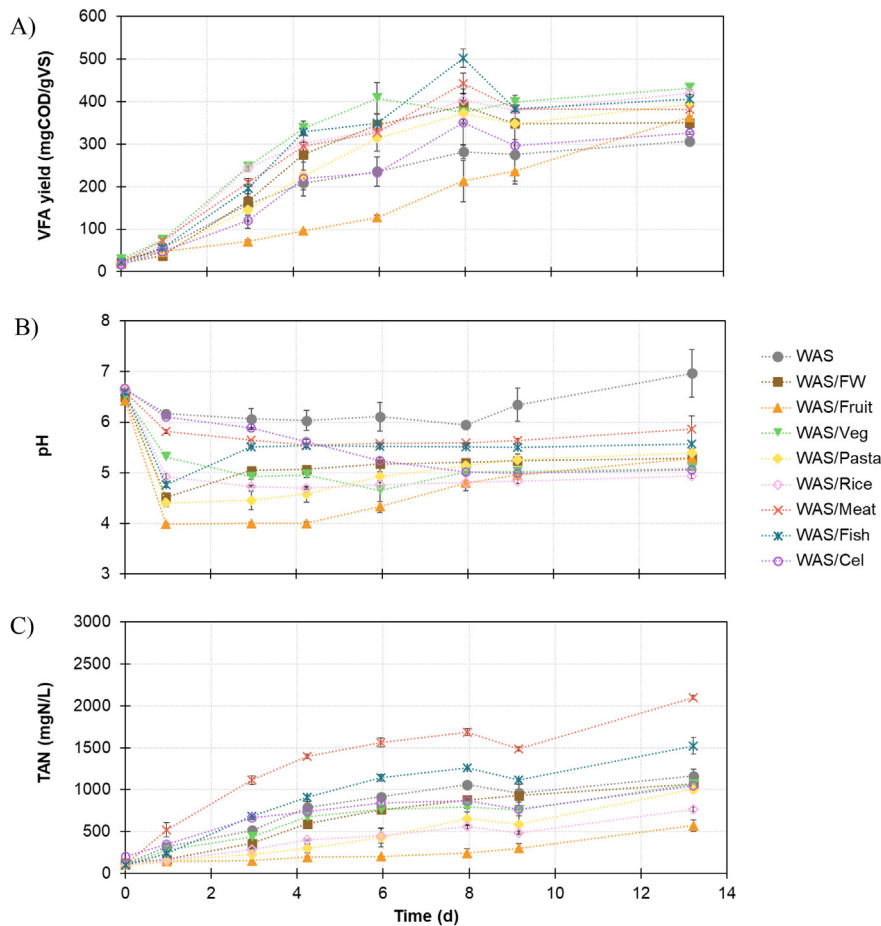


Fig. 1. Evolution of the (top) fermentation yield, (middle) pH and (bottom) TAN concentration in Experiment 1. Error bars indicate the standard deviation.

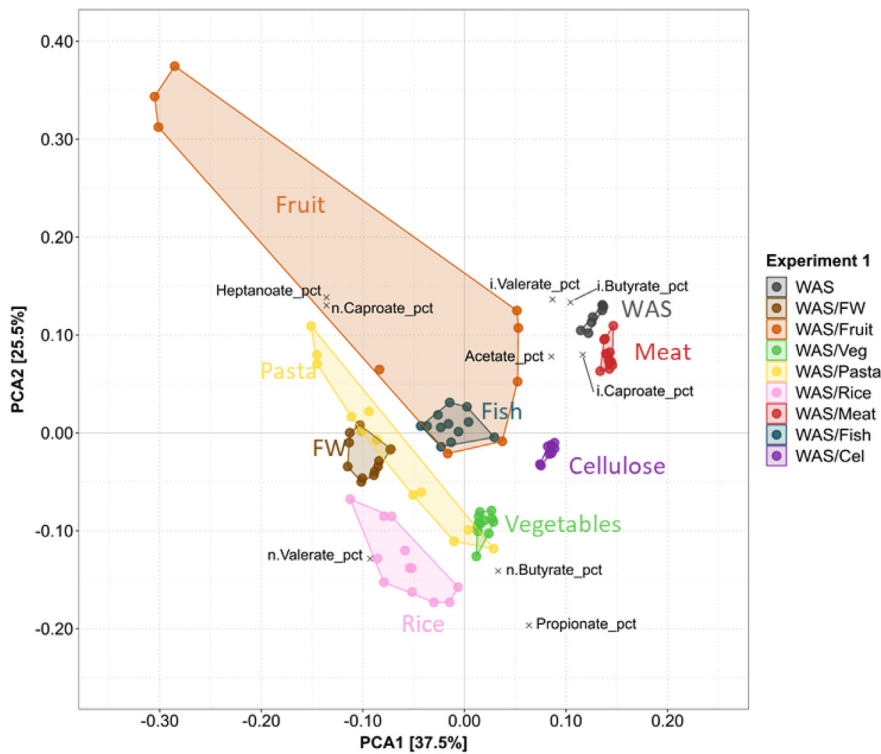


Fig. 2. PCA plot of the VFA profile distribution in Experiment 1.

The VFA profile of WAS/Rice, WAS/Pasta and WAS/Veg were also dominated by acetic (18, 19 and 22 %, respectively), propionic (24, 22 and 27 %, respectively), n-butyric (23, 18 and 22 %, respectively), and n-valeric (23, 22 and 16 %, respectively) acid. In most previous publications, the fermentation of carbohydrates led to a fermentation broth enriched in butyric acid followed by acetic and propionic acid (Alibardi and Cossu, 2016; Greses et al., 2022; Strazzera et al., 2021; Yin et al., 2016). However, Albuquerque et al. (2007), who fermented sugar cane molasses at 30 °C, reported that the concentration of butyric and valeric acid increased to the detriment of acetic and propionic acid when the pH decreased from pH 7.0 to 5.0. Indeed, the most noticeable difference between the protein-rich and carbohydrate-rich components was the higher percentage of n-valeric acid in the latter.

Finally, WAS/Cel co-fermentation was characterised by the accumulation of acetic acid (33 %) followed by propionic (28 %) and n-butyric (19 %) acid. This profile is similar to the reported by Garcia-Aguirre et al. (2017) and Bengtsson et al. (2008) who fermented paper mill wastewater under mesophilic conditions at pH 5.5 and 6.0, respectively.

3.2. Assessment of mixing a protein-rich and a carbohydrate-rich component in WAS co-fermentation: Fish with fruit or cellulose

The WAS mono-fermentation yield in Experiment 2 was much lower than in Experiment 1 (97 vs. 307 mgCOD/gVS). This noticeable difference on VFA yield had a direct impact on the co-fermentation yields (combined effect of WAS and co-substrate fermentation). The WAS/Cel and WAS/Fish VFA yields were lower in Experiment 2 compared to Experiment 1 (276 vs. 350 mgCOD/gVS and 363 vs. 502 mgCOD/gVS, respectively). However, the WAS/Fruit VFA yield in Experiment 2 was much higher than in Experiment 1 (401 vs. 350 mgCOD/gVS, respectively), especially when considering the lower WAS yield in Experiment 2. The higher WAS/Fruit yield in Experiment 2 was related to the higher pH values throughout the

batch (5.0–5.5), likely due to WAS higher alkalinity. The pH during WAS mono-fermentation in Experiment 2 was more stable and higher (6.9–7.3) than in Experiment 1 (5.9–7.0).

Experiment 2 comprised co-fermentation mixtures between WAS and two components, fish as protein source and fruit or cellulose as carbohydrate source (Fig. 3). The maximum VFA yields were reached by the fish/fruit mixtures, i.e., WAS/Fish/Fruit_70/10/20 (508 mgCOD/gVS), WAS/Fish/Fruit_70/20/10 (464 mgCOD/gVS), and WAS/Fish/Fruit_70/15/15 (433 mgCOD/gVS). The VFA yield of the fish/fruit mixtures were substantially higher than the individual co-substrate yields, i.e., WAS/Fish (364 mgCOD/gVS) and WAS/Fruit (401 mgCOD/gVS). These results suggest that there was a positive interaction on VFA yield when mixing different components. A positive interaction on VFA yield when mixing different substrates has also been reported by other publications, including Moscariello et al. (2022), Wang et al. (2022), and Xin et al. (2018).

The co-fermentation tests mixing fish/cellulose also reached higher VFA yields than the co-fermentation tests with one co-substrate, i.e., WAS/Fish (364 mgCOD/gVS) and WAS/Cel (401 mgCOD/gVS). The VFA yield of the three fish/cellulose mixtures was quite similar, i.e., 447, 447 and 433 mgCOD/gVS for WAS/Fish/Cel_70/10/20, WAS/Fish/Cel_70/20/10 and WAS/Fish/Cel_70/15/15, respectively. These results reinforce the idea of a positive interaction between protein and carbohydrates on fermentation efficacy. Indeed, some authors already suggested that carbohydrate-rich substrates enhance the fermentation yield of protein-rich substrates due to a more balanced nutrient composition (Chen et al., 2013; Feng et al., 2009). However, this interaction was not proportional to the amount of co-substrates in the mixtures since in both cases (i.e., fish/fruit, fish/cellulose) the 10/20 and 20/10 mixtures reached higher VFA yields than the 15/15 mixture. However, our data do not allow elucidating the mechanisms behind this improvement.

The PCA shows that each single substrate co-fermentation (i.e., WAS/Fish, WAS/Fruit and WAS/Cel) had a distinct cluster in the ordination

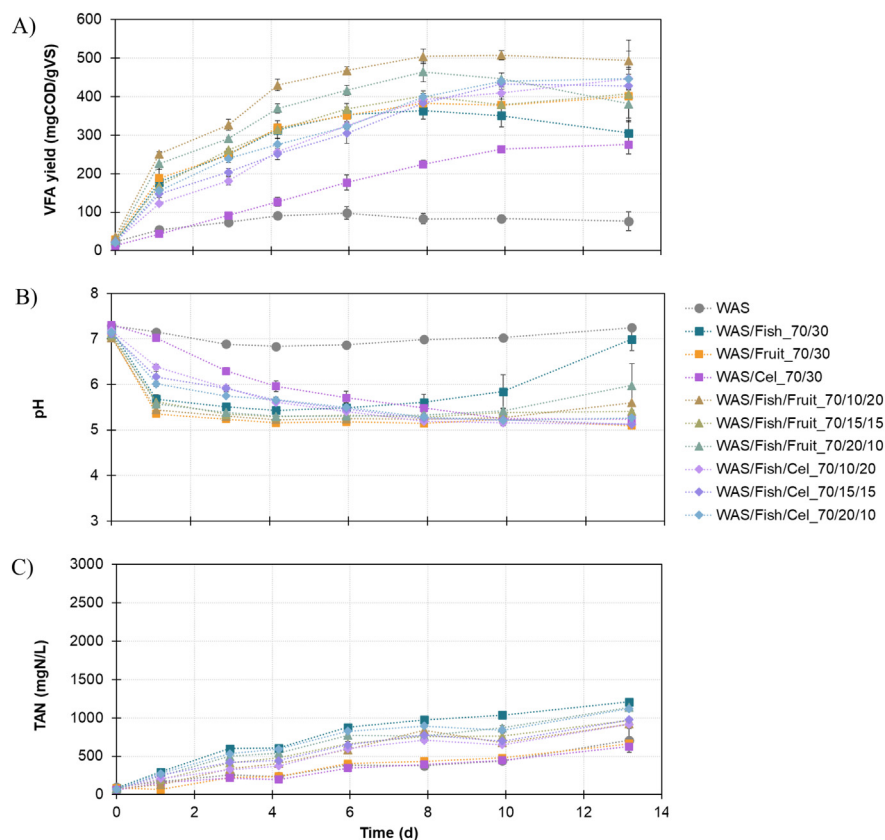


Fig. 3. Evolution of the (top) fermentation yield, (middle) pH and (bottom) TAN concentration in Experiment 2. Error bars indicate the standard deviation.

space (Fig. 4), i.e., each co-substrate had its own VFA profile. The VFA profile of WAS/Cel co-fermentation on day 8 (maximum VFA yield) was dominated by acetic (37 %) and propionic (32 %) acid, while the WAS/Fruit VFA profile was dominated by acetic (24 %) and n-butyric (38 %) acid (see Fig. S4 of the supplementary material). As in Experiment 1, the WAS/Fish cluster was located at the centre of the ordination space (equally influenced by all the response variables) with acetic (31 %), propionic (18 %) and n-butyric (27 %) acid being the main VFA.

The most remarkable result from Experiment 2 was the clear trend observed in the cluster trajectory within the ordination space from fish/fruit and fish/cellulose co-fermentation (see arrows in Fig. 4). The clusters from fish/cellulose co-fermentation were located between the WAS/Fish cluster and the WAS/Cel cluster with the co-fermentation clusters moving from one cluster to the other as the proportion of co-substrate in the mixture increases or decreases. In other words, the WAS/Fish/Cel_{70/20/10} cluster was closer to the WAS/Fish cluster, the WAS/Fish/Cel_{70/10/20} cluster was closer to WAS/Cel cluster, and the WAS/Fish/Cel_{70/15/15} was between them. The same response was observed from the fish/fruit mixtures, which clusters were located between the WAS/Fish cluster and the WAS/Fruit cluster.

The WAS/Fish/Cel mixtures were dominated by acetic (34–36 %), propionic (26–31 %) and, to a lesser extent, n-butyric (14–17 %) acid. The percentage of acetic and propionic acid increased and the percentage of n-butyric and n-valeric acid decreased as the proportion of cellulose in the mixture increased. The WAS/Fish/Fruit mixtures were also dominated by acetic (26–29 %) and n-butyric (36–40 %) acid. For this mixture, the percentage of n-butyric acid increased to the detriment of acetic acid as the proportion of fruit in the mixture increased.

These results indicate that both substrate type (e.g., fish, cellulose, fruit) and macro-composition (e.g., carbohydrates, protein) have a role on VFA profile. These results agree with those reported by Bevilacqua et al. (2022) and Wang et al. (2022), who also observed that adjusting the carbohydrates to protein ratio can be used to drive the fermentation product profile. Therefore, it can be concluded that FW composition can be used to drive the VFA profile to a certain extent.

3.3. Corroborate the effect of mixing a protein-rich and a carbohydrate-rich component in WAS co-fermentation: Meat with vegetables or rice

The maximum VFA yields of the co-fermentation tests carried out with one co-substrate in Experiment 3 were similar to the ones obtained in Experiment 1, despite the lower WAS mono-fermentation yield in Experiment 3 compared to Experiment 1 (162 vs. 307 mgCOD/gVS). In Experiment 3, the VFA yields for WAS/Meat, WAS/Veg and WAS/Rice were 373, 430 and 452 mgCOD/gVS, respectively (Fig. 5).

Experiment 3 co-fermentation tests showed the same positive interaction observed in Experiment 2, i.e., mixtures between protein (meat) and carbohydrates (vegetables or rice) reached higher VFA yields than the co-fermentation tests with one co-substrate (i.e., WAS/Meat, WAS/Veg and WAS/Rice). As in Experiment 2, the interaction between meat and rice was not proportional to the amount of co-substrates in the mixtures since the 10/20 and 20/10 mixtures reached higher VFA yields than the 15/15 mixture. Specifically, the maximum VFA yields were 459, 414 and 529 mgCOD/gVS for WAS/Meat/Rice_{70/10/20}, WAS/Meat/Rice_{70/15/15} and WAS/Meat/Rice_{70/20/10}, respectively. Contrariwise, the maximum VFA yield of the meat/vegetables co-fermentation experiments was linked to the amount of co-substrates in the mixtures, with the VFA yield increasing from 461 (WAS/Meat/Veg_{70/10/20}) to 519 mgCOD/gVS (WAS/Meat/Veg_{70/20/10}) as the meat concentration increased. This trend was unexpected since the VFA yield of WAS/Meat (373 mgCOD/gVS) was lower than WAS/Veg (430 mgCOD/gVS). These results clearly indicate that the prediction of the VFA yield of waste co-fermentation is not straightforward and that further research needs to be carried out to elucidate the mechanisms that control co-fermentation VFA yield.

The PCA shows that rice and vegetables individual co-fermentation had a very similar VFA profile, which on day 6 was dominated by acetic (30 and 33 %, respectively), propionic (17 and 19 %, respectively) and n-butyric (39 and 31 %, respectively) acid (Fig. 6). Meat had a separate cluster driven by its lower percentage of n-butyric acid (17 %) and a higher percentage of i-butyric (7 %) and i-valeric acid (16 %) when compared to rice and

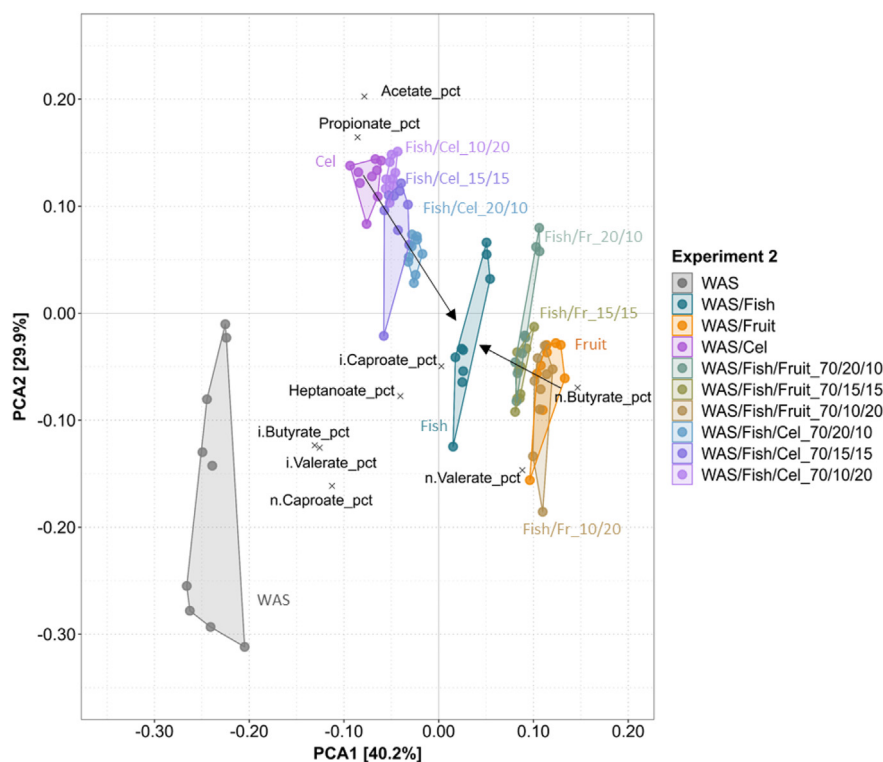


Fig. 4. PCA plot of the VFA profile distribution in Experiment 2. Arrows indicate the trajectory of the clusters from WAS/Cel to WAS/Fish and from WAS/Fruit to WAS/Fish.

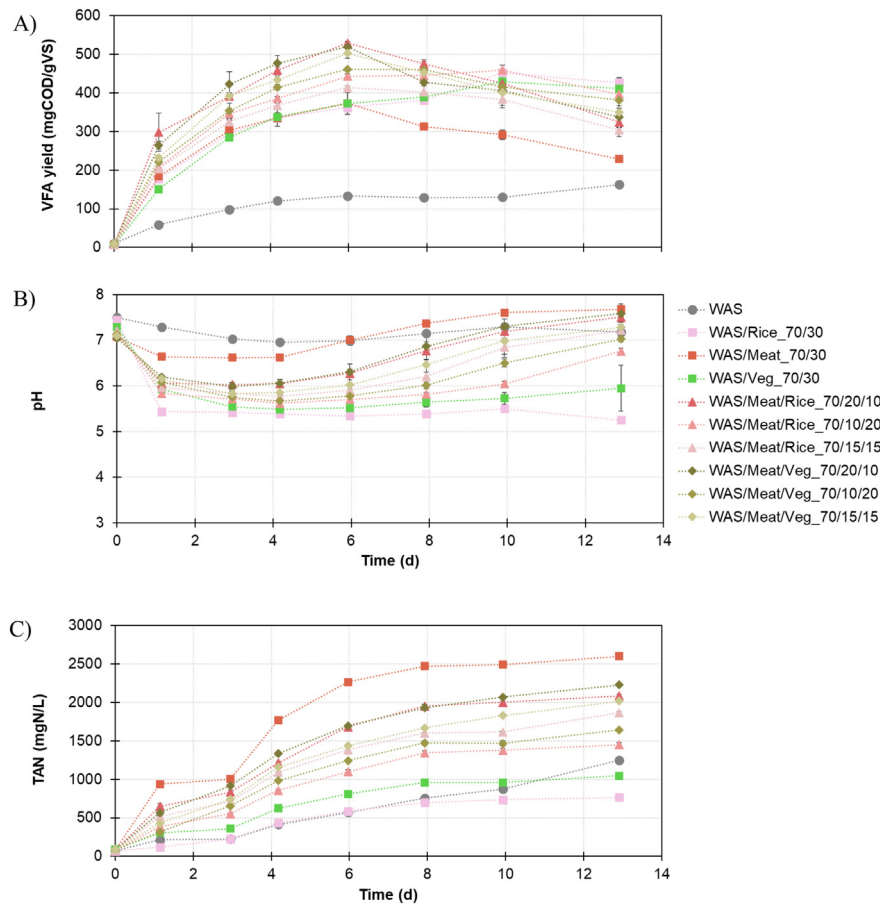


Fig. 5. Evolution of the (top) fermentation yield, (middle) pH and (bottom) TAN concentration in Experiment 3. Error bars indicate the standard deviation.

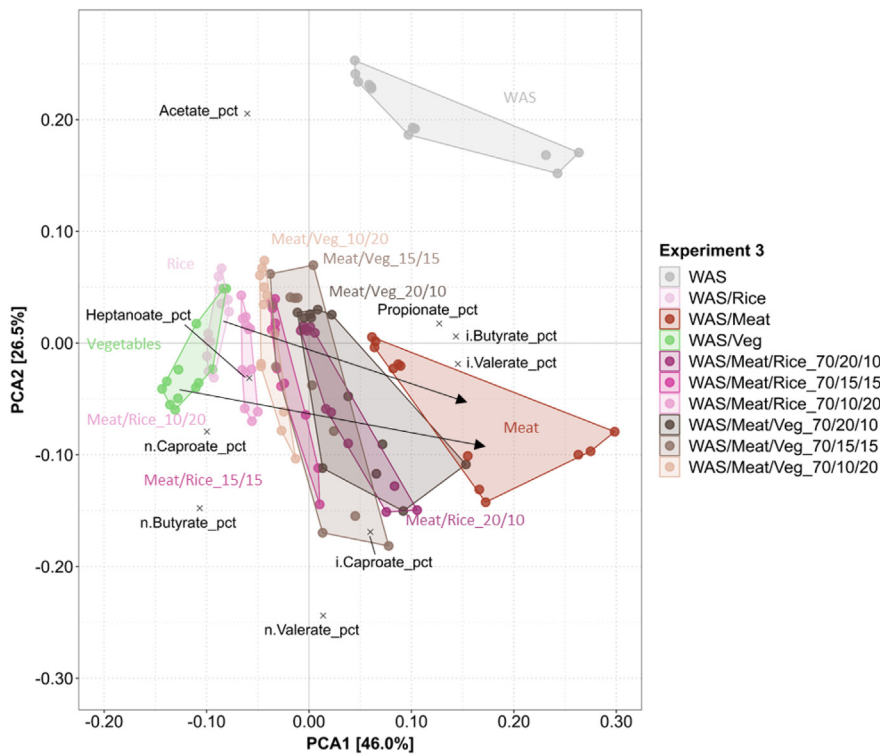


Fig. 6. PCA plot of the VFA profile distribution in Experiment 3. Arrows indicate the trajectory of the clusters from WAS/Rice to WAS/Meat and from WAS/Veg to WAS/Fish.

vegetables. The percentage of acetic and propionic acid in WAS/Meat were 33 % and 20 %, respectively. The most noticeable change in the fermentation broth composition was the decrease of n-butyric acid percentage and the increase of i-valeric acid percentage as the proportion of meat in the mixture increased.

The trajectory of the co-fermentation clusters within the ordination space in Experiment 3 showed the same pattern as Experiment 2, i.e., the clusters of the tests comprising two co-substrates were between the one co-substrate clusters and they move from one cluster to the other as the proportion of co-substrate in the mixture increases or decreases (see arrows in Fig. 6). This reproducible pattern further reinforces the idea that the FW composition can be used to drive the fermentation VFA profile to a certain extent.

3.4. Assessment of the batch effect

Co-fermentation experiments were carried out with three freshly collected WAS from the same WWTP. Experimental results showed that WAS had a noticeable impact on the co-fermentation VFA yield. Indeed, the VFA yield of the WAS mono-fermentation batches was 307, 97 and 162 mgCOD/gVS in Experiment 1, 2 and 3, respectively. Additionally, a PCA analysis showed that each WAS mono-fermentation resulted in a distinct VFA profile (Fig. S7). These results imply that the inherent variability of WAS properties impact fermentation performance. Parameters that could explain this variability include biodegradability, composition, sludge age, seasonality, or microbial community among others. Understanding the mechanisms that control WAS fermentation yield and profile is important for biorefinery applications.

A PCA combining the VFA profile of all the experiments carried out in this research showed a batch effect, i.e., WAS influenced the VFA profile of the co-fermentation mixtures (Fig. S8). For instance, WAS/Meat co-fermentation was located in different regions of the ordination space in Experiment 1 and 3. However, WAS had the same influence over all the mixtures within the same batch. As illustrated in Figs. 4 and 6, the trajectory of the co-fermentation clusters within the ordination space were between the individual co-substrate clusters and they move from one cluster to the other as the proportion of co-substrate in the mixture increases or decreases.

4. Conclusions

The impact of FW composition on WAS/FW acidogenic co-fermentation was investigated through mesophilic batch tests. Experimental results showed that each component had a distinct VFA yield, with protein-rich components reaching the highest VFA yields. Based on their VFA profile, four groups were distinguished: (i) WAS and WAS/Meat driven by acetic, i-butyric, i-valeric and i-caproic acid, (ii) WAS/Rice, WAS/Pasta and WAS/Veg driven by propionic, n-butyric and n-valeric acid, (iii) WAS/Cel, and (iv) WAS/Fish. Co-fermentation experiments mixing a protein-rich co-substrate (i.e., fish or meat) and a carbohydrate-rich co-substrate (i.e., fruit, cellulose, rice, vegetables) showed that VFA yields improve when both components are present in the mixture. However, this positive interaction was not proportional to the amount of co-substrates. Conversely, the VFA profile was driven by both the WAS and the co-substrates proportion in the mixture. For biorefinery applications, these results imply that adding FW is an opportunity to increase WAS fermentation yields and tune the VFA profile. However, these results also indicate that predicting the VFA yield of co-fermentation is not only related to FW composition, but FW composition can be used to drive the VFA profile to a certain extent.

CRediT authorship contribution statement

C. Vidal-Antich: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **M. Peces:** Conceptualization, Formal analysis, Data curation, Writing – review

& editing, Visualization. **N. Perez-Esteban:** Conceptualization, Formal analysis, Writing – review & editing, Visualization. **J. Mata-Alvarez:** Conceptualization, Writing – review & editing, Supervision. **J. Dosta:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **S. Astals:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.157920>.

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