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Research article

Integrated resource recovery from co-fermentation of food waste and sludge: Balancing denitrification potential and biogas recovery

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ABSTRACT

Replacing fossil-based carbon sources with sustainable alternatives for denitrification supports nitrogen compliance, lowers indirect emissions, and mitigates cost increases in WWTPs. This study investigated the pilot-scale fermentation of food waste (FW) and primary sludge (PS) at different ratios to produce volatile fatty acids (VFA) and lactic acid, for use as carbon source in denitrification. Four substrate ratios (FW:PS) were tested (100:0, 50:50, 25:75, and 0:100) under mesophilic conditions (34 ± 0.4 °C) without pH control. Fermentates from the tests produced a wide range of sCOD (6–82 g COD/L) and VFA concentrations (3–56 g VFA_{COD}/L). No statistically significant differences in VFA yields between reactors were detected, whereas a significant difference in COD yields were observed. Higher FW proportion increased VFA concentrations and shifted the VFA composition towards lactic and propionic acid. Despite these differences, denitrification rates remained consistent (7–9 mg NO_3^--N/g VSS•h), suggesting the microbial community's functional flexibility in utilising varying VFA profiles. Methane potential analysis of the solid residues after fermentation and VFA removal indicated that FW-fermentate maintained biogas yields, whilst PS-fermentate showed a 50 % reduction compared to untreated substrates. Estimated carbon source volume demands in large-scale applications varied significantly due to differences in sCOD and nutrient concentrations. Co-fermentation of PS with 25 % and 50 % FW reduced volume requirements by a factor of 8 and 12, respectively, compared to fermenting PS alone. These findings demonstrate the feasibility of fermenting FW and PS to balance denitrification efficiency, biogas recovery, and resource utilisation at WWTPs.

1. Introduction

The new European Union (EU) directive for wastewater treatment prioritises nutrient removal, greenhouse gas emission reduction, and lower energy consumption. Several large European wastewater treatment plants (WWTP) must comply with stricter discharge limits of 8 mg/L of total nitrogen (TN) and 0.7 mg/L of total phosphorus (TP), with certain facilities in Sweden required to meet TN levels as low as 6 mg/L and TP of 0.2 mg/L (Swedish Environmental Protection Agency, 2018; EU Directive, 2024). Biological nitrification/denitrification is generally the preferred method worldwide for nitrogen removal in domestic wastewater treatment (McCarty, 2018). In heterotrophic denitrification, a carbon source is oxidised, and nitrate is used as an electron acceptor, sequentially reduced to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and finally nitrogen gas (N_2) (Tchobanoglous et al., 2014). The readily available chemical oxygen demand (COD) at WWTPs is often insufficient to remove enough nitrate to meet low-nitrogen effluent limits (<6 mg/L). This is predominantly due to low influent biological oxygen demand (BOD) to Total Kjeldahl Nitrogen (TKN) ratios, and decreased microbial activity at lower process temperatures, which decelerates the hydrolysis of particulate organic matter into soluble substrates and limits denitrification, notably in colder climates (Tchobanoglous et al., 2014; Environmental Protection Agency, 2013). Consequently, many WWTPs currently rely on external carbon sources, and demand within the EU is expected to increase soon. In past decades, methanol was the most frequently used carbon source because of its lower prices and accessibility (Environmental Protection Agency, 2013; Foglar and Briški, 2003), however, its fossil-based production has an

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immense impact on CO_2 emissions (Methanol Institute, 2022). Furthermore, the price of methanol has increased by 250 % over the past 10 years (Methanex Corporation, 2025), inevitably affecting the WWTP's operational costs (OPEX).

The results from applying several different alternative carbon sources at WWTPs have been summarised in multiple review papers (Fu et al., 2022; Ahmed et al., 2023; Christensen Henze and Harremoës, 1977). Previously evaluated carbon sources include methanol and ethanol, by-products from industrial processes containing longer-chain sugars or long-chain fatty acids (e.g., glycerol (Alessio et al., 2023), cellulose (Qi et al., 2021a), industrial wastewaters (Fu et al., 2022), etc.), as well as short-chain volatile fatty acids (C2-C5) (VFAs). VFAs are regarded as great carbon sources for denitrification due to their simple structure and ability to efficiently produce energy (Elefsiniotis et al., 2004), which results in higher denitrification rates and less accumulation of intermediate products, such as NO_2 and N_2O, compared to many other carbon sources (Ge et al., 2012; Wei et al., 2022). VFAs can be produced from petrochemical derivatives through thermochemical processes or through biological processes by anaerobic fermentation of organic materials (Agnihotri et al., 2022). VFAs are expensive to produce and in high demand due to their broad applications across multiple industries; therefore, producing VFAs through the fermentation of waste streams (e.g., sewage sludge, food waste) presents an attractive, more sustainable alternative. At WWTP, this approach not only has the potential to improve circularity and reduce emissions; it also enhances self-sufficiency by utilising locally available organic waste as a resource, thereby reducing the reliance on external markets. Despite decades of research on VFA production from waste streams (Liu et al., 2020; Atasoy et al., 2018) and positive results as carbon sources for denitrification (Elefsiniotis et al., 2004; Carranza Muñoz et al., 2024; Lee et al., 2014; Sapmaz et al., 2022), their use on a large scale remains low, and WWTPs continue to choose chemical-based sources such as methanol, ethanol, or glycerol. This is likely due to several factors, such as the ease of operating and handling of traditional carbon sources, the low VFA yields from sewage sludge, or substrate competition with biogas production. There is a trade-off between using sewage sludge and other organic waste materials for VFA or biogas production, and thus it is crucial to consider both the potential GHG reduction and economic viability.

Food waste (FW) has a high content of organic matter, and several studies have used it as a substrate to produce different high-value products, including acetic acid and other VFAs (Sapmaz et al., 2022), lactic acid (Zhou et al., 2018), and hydrogen (Alibardi and Cossu, 2016). Acetic and lactic acids are known to be effective carbon sources for denitrification due to their high solubility, rapid microbial uptake, and their role as electron donors (Tang et al., 2018; Pan et al., 2025). These two acids have been found to produce the same denitrification rates (Tang et al., 2018; Pan et al., 2025), and lactic acid is rapidly converted into acetate and propionate, further supporting denitrification (Zhou et al., 2018). Despite the potential of lactic and acetic acid, their full-scale application for denitrification in wastewater treatment remains marginal. Certain drinking and groundwater treatment plants have used acetic acid as a carbon source since the 1990s (Works and L.A. C.D.o.P., 2013), however, the cost limits its use in WWTPs. Producing lactic acid and other VFAs from food waste could be more economically viable, but collection and logistical constraints may limit availability, even in large metropolitan areas. In addition, food waste is considered a high-value substrate for biogas production (Mirmohamadsadeghi et al., 2019), which may also limit the availability for the production of volatile organic acids. Compared to using solely FW, a more viable approach to both enhance VFA yields and simplify implementation would involve using smaller volumes of FW in co-fermentation with waste-activated sludge (WAS) (Tayou et al., 2022; Vidal-Antich et al., 2021) or primary sludge (PS) (Owusu-Agyeman et al., 2020; Min et al., 2005). The combination of PS and FW is a high-organic-content substrate mix that has been studied for the production of VFAs and caproic acid in both batch and semi-continuous scales (Owusu-Agyeman et al., 2020) and at pilot scale with a 30:70 ratio (Owusu-Agyeman et al., 2023). Semi-continuous trials with HRT of 7–10 days showed promising results for producing longer-chain acids, including valeric and caproic acids, and as a carbon source for denitrification in batch tests. The mechanisms influencing acid production during co-fermentation of PS and FW, including changes in VFA composition and lactic acid formation, are not yet fully described, particularly in relation to their implications for denitrification performance, biogas yield, and the minimum FW proportion needed to sustain high production of short-chain VFAs. In addition, potential biogas losses in large-scale WWTPs caused by diverting sludge for VFA production, which is an important factor in decision-making, have not been extensively investigated.

The aim of this study was to investigate the co-fermentation products (VFAs, lactic acid, and other soluble compounds) of food waste and primary sludge at different proportions, and to compare denitrification rates with the resulting fermentates, to evaluate them as carbon sources. The fermentation process was assessed through analyses of macromolecule composition, sCOD and VFA yields, and microbial composition. Moreover, the biogas potential of the remaining solid fraction after VFA extraction was determined, and the reduction in biogas potential when shifting from conventional anaerobic digestion (AD) to anaerobic fermentation for carbon source production + AD was calculated. The fermentation was performed in two pilot-scale reactors operated under mesophilic conditions, with uncontrolled pH and different FW:PS (% v/v) ratios: 100:0, 50:50, 25:75, and 0:100. This study provides a comprehensive assessment of alternative denitrification carbon sources, contributing to the optimisation of nitrogen removal strategies and advancing circular wastewater treatment approaches.

2. Materials and methods

2.1. Substrates

Primary sludge (PS) was produced in a sedimentation tank with an inflow of municipal wastewater from Henriksdal WWTP (Stockholm, Sweden) at 1.2 m^3/h (hydraulic load: 0.4 m/h). Ferric chloride ($FeCl_3$) was dosed prior to the sedimentation tank with a dose of 8–10 g Fe^{3+}/m^3. Food waste (FW) from Södertörn biogas plant - Biokraft AB (Huddinge, Sweden) was delivered weekly in 1 m^3 batches. The plant processes a mixture of household and industrial FW, which was ground (12 mm) and hygienised at 70 °C for 1 h before delivery. Substrate characteristics are presented in Table 1. Both substrates showed considerable variation in total solids and in carbon, nitrogen, and phosphorus fractions during the experimental period, consistent with earlier reports (Qin et al., 2024; Ossiansson et al., 2023).

2.2. Fermentation experiments

Two fermenters, with a total volume of 2 m^3 and a working volume of 0.9 m^3, were operated under mesophilic conditions (av. temperature 34 ± 0.4 °C, Table 2). The fermenters were used as parallel experimental units and were not biological replicates; each reactor was operated under one substrate ratio at a time. No pH control was used during the experiments, as this would considerably increase costs at full scale and decrease the feasibility. The fermenters were inoculated with sludge from another reactor (Owusu-Agyeman et al., 2023), which was fed with a substrate mix of PS and FW, had low pH (~5) conditions and low methanogenic activity.

Four tests were conducted in two consecutive periods with FW:PS volumetric (v/v) ratios of 100:0 (Test A), 50:50 (Test B), 75:25 (Test C), and 0:100 (Test D) (Table 2). The aim was to evaluate whether mixing low amounts of FW (25 %) with PS could achieve performance comparable to higher FW shares. During days 0–48, Fermenter 1 was operated at a 100:0 ratio (Test A), and Fermenter 2 at a 75:25 ratio (Test C). From day 49, Fermenter 1 was switched to a 50:50 ratio (Test B), and Fermenter 2 to a 0:100 ratio (Test D). All ratios are written in FW to PS

Table 1
Characteristics of substrates, primary sludge (PS) and food waste (FW), used during the fermentation trials.

	TS		VS		Total COD		sCOD		NH4-N		TN - Filtered		PO4-P		tVFA incl. Lactic acid		Proteins		Carbohydrates		Lipids	
	%		% of TS		g/L		g/L		mg/L		g/L		mg/L		gCOD/L		g/kg VS		g/kg VS		g/kg VS	
	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.
Primary Sludge (PS)	2.5	1.3	89.8	2.9	40.2	18.3	1.4	0.9	42.2	12.4	0.1	0.0	20.2	9.5	0.9	0.6	7.1	2.1	17.1	5.5	3.3	0.7
Food Waste (FW)	16.3	1.9	92.5	1.9	151	27.5	87.6	19.7	406	148	1.4	0.5	323	97.6	36.7	9.4	20.2	1.8	75.8	6.0	24.6	11.3

Note: TS: Total solids, VS: Volatile solids, sCOD: soluble chemical oxygen demand, tCOD: total chemical oxygen demand, NH_4^+-N: ammonium nitrogen, $TN_{filtered}$: total nitrogen in filtered samples, PO_4-P: phosphate phosphorus, tVFA: total volatile fatty acids (including lactic acid, expressed as g COD/L), av: average, sd: standard deviation. n = 21 samples for all analyses except for proteins, carbohydrates and lipids, which were for PS, n = 10 and for FW, n = 8.

ratios (FW:PS). Feeding was automatic every 30 min using eccentric screw pumps (Netzsch, Germany) at the predefined ratios, corresponding to a total inflow of around 215 L/d and a hydraulic retention time (HRT) of 4 days in all stages (Table 2). Following each change in substrate ratio, a 3 HRT +2 days (15 days) adaptation period was applied, and data from this period were excluded from the evaluation to ensure steady-state operation under the new feed composition. In continuous fermentation systems, microbial adaptation and stabilisation of metabolite profiles typically occur after approximately 3 HRT following operational changes (Lu et al., 2011; Llamas et al., 2025).

2.3. Sampling and biochemical analyses

The temperature in the fermenters was monitored with thermometers and controlled with electrically heated water jackets. The pilot was equipped with one online pH meter installed in Fermenter 2, and pH was measured in samples from both fermenters twice per week with a handheld pH meter. The substrates (PS and FW) and the resulting fermentates from both reactors were sampled twice per week. Fermentate samples were taken from a sampling point located after the recirculation pump of each fermenter. The samples were centrifuged and filtered through 0.45 μm acetate filters prior to spectrophotometric analysis. Cell tests (MERCK, Germany) were used to determine sCOD and tCOD, total nitrogen (TN) in filtered samples, ammonium nitrogen (NH_4^+-N), phosphate (PO_4-P), and VFA. To quantify VFA species (C1-C6) and lactic acid, the samples were further filtered (0.22 μm), acidified 10 % (v/v) with 37 % H_2SO_4, and analysed using a high-performance liquid chromatograph (HPLC) Agilent 1100 Series with a refractive index detector and an ion exclusion column (Rezex ROA - Organic Acid H^+, 300 × 7.80 mm, Phenomenex). The mobile phase was 5 mM H_2SO_4 with a flow rate of 0.6 mL/min. Lactic acid was included in the total VFA (tVFA) calculations to provide a comprehensive assessment of the available carbon sources. Total solids (TS) and volatile solids (VS) were measured using the standard method 1684 (EPA and U.S.E.P.A., 2001), with values corrected for VFA loss (including lactic acid) following the method described by Vahlberg et al. (2013). Samples for protein, carbohydrate, and lipid content analysis were sent weekly to an external accredited laboratory (Eurofins AB, Sweden). The external laboratory determined NH_4^+-N following a modification of the Standard Methods 1998, 4500; nitrogen Kjeldahl using EN 13342; raw fat using a modified NMKL 160 method; and carbohydrates were calculated with the remaining VS. The same laboratory analysed tCOD in FW and fermentate samples following the ISO/IEC 17025:2017 SWEDAC 10300 method. The total COD content in primary sludge samples was calculated using a tCOD/VS factor of 1.77, as reported by Ahnert et al. (2021).

2.4. Denitrification tests

One FW sample and four fermentate samples of 200 mL (one per test) were collected and immediately stored at −18 °C to limit biological activity and preserve VFA composition, a commonly applied preservation approach shown to minimise VFA degradation and changes in speciation during storage (Wagner et al., 2017). Samples were taken once the processes were considered stable, defined as three retention times plus two days (or 15 days from the start of the test). Sampling was performed on days 37, 30, 42, and 30 for Tests A, B, C, and D, respectively. These samples were used to evaluate the fermentates as carbon sources for denitrification. After thawing, the sludge samples were filtered through a 0.6 mm sieve, with the liquid phase used for denitrification tests and the retained sludge used for biomethane potential (BMP) tests (see description below). The denitrification batch tests were performed in 5 L reactors, following the methodology (test DEN.CHE.1) described by van Loosdrecht et al. (van Loosdrecht et al., 2016). The initial concentrations in the reactors were 30 mg NO_3^--N/L and 150 mg sCOD/L, corresponding to a C/N of 5.4 and a C-to-volatile suspended solids (VSS) ratio of 0.05–0.1. The pH was adjusted to 7 following the

Table 2
Operational conditions of the fermentation tests with different FW:PS ratios.

	Substrate proportion (v/v)		Flow	HRT	Operational Period	Temperature			OLR		
	FW	PS	L/d	days	days	°C			g VS/L•d		
						av.	sd.	*n*	av.	sd.	*n*
Test A (100:0)	100 %	0 %	214	4.0	48	35	1	18	36	7	8
Test B (50:50)	50 %	50 %	213	4.0	50	34	2.3	*15*	19	3.0	*7*
Test C (75:25)	75 %	25 %	224	4.0	48	35	0.8	*18*	16	2.1	*8*
Test D (0:100)	0 %	100 %	212	4.0	50	34	1.4	*15*	3.2	2.1	*7*

Note: Flow: influent flow rate; HRT: hydraulic retention time; OLR: organic loading rate; av: average; sd: standard deviation; n: number of samples.

addition of the carbon sources. Several batches of activated sludge from an MBR pilot described by Andersson et al. (2024), was used as inoculum in the denitrification tests. Characteristics included TS: 2.9 ± 0.9 %, VS: 85 ± 3 % of TS, sCOD: 28.7 m/L. The sludge was not washed and diluted 3 times with tap water to approximately 3 g TS/L. rNOx-N endogenous and rNOx-N exogenous were calculated as the linear regression of the nitrate decreasing slope in mg N/L•min, in the reference reactor and the average of the biological copy reactors, respectively. Anoxic growth yield was calculated as described in formula (a).

$$Y_{OHO} = 1 - 2.86\frac{rNOx_{exo} - rNOx_{endo}}{r_{COD}} \quad \text{(a)}$$

2.5. Biochemical methane potential (BMP) tests

After filtering the fermentate samples (0.6 mm) and extracting the supernatant for the denitrification test, the remaining sludge was used for BMP tests. Four solid fraction samples, one from each fermentation test (A–D), untreated (unfermented and unfiltered) samples of FW, PS, and a mixture of FW and PS (25:75) were used as substrate. Three sets of Automatic Methane Potential Test System (AMPTS) II equipment from BPC Instruments AB (Lund, Sweden) were used; each set consisted of a water bath with a capacity for 15 glass 500 mL bottles with an 80 % active volume. The inoculum-to-substrate ratio was 3:1 (on a VS basis), and the organic load was 3 kg VS/m^3 in all tests. The tests were performed under standard mesophilic conditions (37 °C) for 30 days. The experiments were set up in triplicate, and each BMP set had a control with cellulose and a blank with only inoculum. The inoculum used was digestate from mesophilic anaerobic digester No. 5 at Henriksdal WWTP, with TS: 2.6 %, VS: 63.5 % of TS, NH_4^+-N: 740 mg/L, and sCOD: 362 mg/L. Prior to the BMP tests, the inoculum sludge was degasified at 37 °C for 6 days.

2.6. 16s RNA gene amplicon sequencing and analysis

Samples of substrates and fermenters were collected (see sampling description) for microbial community analysis across the four tests. The sampling occurred on the following days: Test A: on days 16, 27, 43, 44, 45, 47, and 48. Test B: on days 30, 36, 45, 48, 49, and 50. Test C: on days 16, 31, 42, 43, 45, and 48. And Test D: on days 28, 34, 43, 46, 47, and 48. DNA extraction of samples, 16s RNA gene amplicon sequencing, and data analysis were performed following the methodology described by Eliasson et al. (2023). The isolation of total genomic DNA was performed with the FastDNA™ Spin Kit for Soil [MP 45] and FastPrep®–24 instrument [MP 46] and library preparation of 16S rRNA gene V4 region (515F-805R) (Hugerth et al., 2014) was carried out as described by (Müller et al., 2016), followed by paired-end sequencing on Illumina MiSeq with v3 chemistry at the sequencing facility of the SNP&SEQ technology platform in Uppsala (UGC, 2018). The sequencing raw data was carried out with the de-multiplexed fastq reads. Cutadapt (v3.5) (Martin, 2011) was used for the adapter and primer trimming and quality control (Q-score ≥20). On average, 23,774 de-multiplexed fastq paired-end reads per sample were obtained, and 23,449 (98.5 %) were retained after adapter trimming and quality filtering. The quality controlled paired-end reads were merged using VSEARCH (v2.21.1) (Rognes et al., 2016) which resulting in 23,095 merged reads (merging percentage = 98.56 %). Package *dada2* (v1.22.0) (Callahan et al., 2016) was used for the chimeric sequence removal, generation of amplicon sequence variants (ASV), and taxonomic annotations in RStudio (v2022) (RStudio Team, 2020) running R (v4.2.2) (R Core Team, 2022). On average, 14,633 reads per sample were non-chimeric and used to analyse sequence variants which resulted in 1739 ASVs. Taxonomic annotations of ASVs were performed using the 16S rRNA database formatted for DADA2 with Genome Taxonomy Database taxonomies (v207) (Alishum et al., 2021). The abundance table, taxonomy table, sample metadata, and phylogenetic tree were merged into a single object and used for visualisation and statistical analysis using packages *phyloseq* (v1.42.0) (McMurdie and Holmes, 2013), vegan (v2.6.4) (Oksanen et al., 2019), and ggplot2 (v3.4.1) (Wickham, 2016).

2.7. Statistical analysis

For statistical analysis, Welch's ANOVA was applied to the data without assuming homogeneity of variance to evaluate differences in the response variables between reactors ($p < 0.05$). Where applicable, exploratory pairwise comparisons were conducted using Welch's t tests with Holm adjustment for multiple comparisons.

3. Results and discussion

3.1. Solubilisation of organic matter/hydrolysis

Fermentation tests (A–D) were conducted under mesophilic temperatures and HRT of 4 days. This retention time has been shown to promote the production of short-chain VFAs, particularly acetate (Jankowska et al., 2018), which is considered the most effective carbon source for denitrification (Wei et al., 2022). Throughout the experiments, the different batches of FW had average sCOD concentrations of 87.6 ± 19.7 g COD/L and tVFA (including lactic acid) of 36.7 ± 9.4 g VFA_{COD}/L, constituting 53 % and 22 % of the total COD, respectively (Table 1). PS, on the other hand, contained lower concentrations of sCOD (4 % of tCOD) and VFA (2 % of tCOD) compared to FW (Table 1).

These differences in the tCOD and sCOD concentrations between FW and PS resulted in variations among the ingoing substrate mixtures in the tests, with sCOD concentrations decreasing as the proportion of PS increased. During the fermentation tests, the degree of solubilisation exhibited an opposite trend, with the lowest sCOD yield observed in the test fed only with FW (test A) and the highest in the test with solely PS (test D). Test A resulted in no significant increase in the soluble carbon concentration compared to untreated FW and showed an average net carbon solubilisation of only 11 ± 152 g sCOD/kg VS_{in} (VS fed into the reactor) (Fig. 1b). Increasing the proportion of PS to 50 % in Test B and 75 % in Test C resulted in a higher level of solubilisation, reaching 299 ± 217 ($p > 0.05$) and 243 ± 55 g sCOD/kg VS_{in} ($p = 0.02$), respectively (Fig. 1b). Despite the low organic load in test D, solubilisation of sCOD was 450 g sCOD/kg VS_{in}, reaching a sCOD concentration of 5.87 ± 3.0 g sCOD/L ($p < 0.001$), which was an increase from 1.44 ± 0.9 g sCOD/L in untreated PS. Although the solubilisation yield increased with

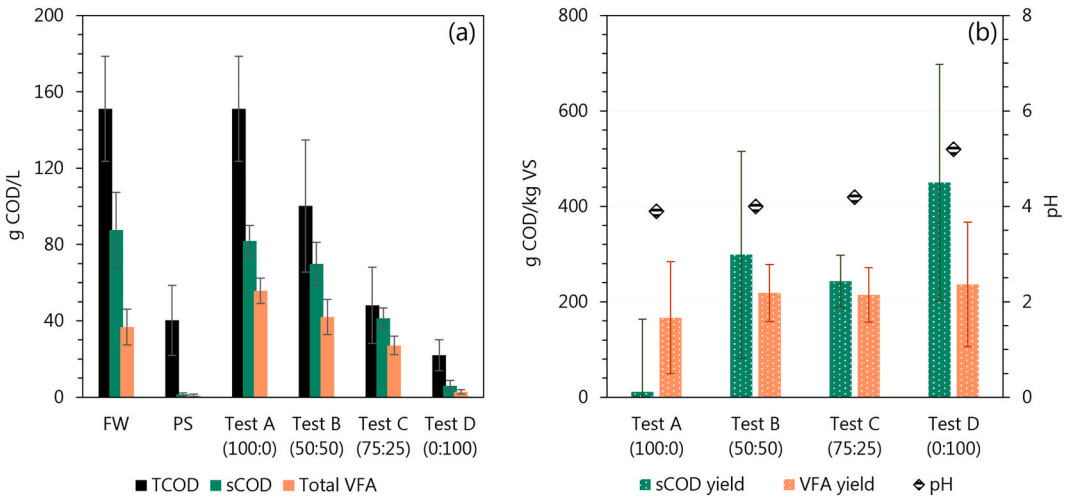

Fig. 1. Characteristics of substrates and fermentates in different tests (A–D), with corresponding yields and pH values **(a)** Concentrations of total COD, soluble COD, and total VFA in substrates, FW (food waste) and PS (primary sludge), and fermentates produced from different mixes of the substrates. Substrate proportions are given as FW:PS (% v/v). **(b)** COD solubilisation and VFA yields. Values in (a) and (b) represent averages from the stable period. Substrate values in (a) are averaged over the 98-day experiment. Error bars were calculated based on: (a) FW and PS: n = 26; A and C: n = 8; B: n = 7; D: n = 8; (b) A, B, and D: n = 6; C: n = 7.

increasing PS proportion ($p = 0.009$), sCOD concentrations in the fermentates significantly decreased ($p = 0.02$), with Test A reaching 82 ± 8 g sCOD/L, 14 times higher than Test D, containing 5.9 ± 3 g sCOD/L. Tests B and C had intermediate sCOD concentrations of 70 g sCOD/L and 42 g sCOD/L, respectively. Although a significant difference was found only between the pairs Tests A-C and A-D, Welch's ANOVA indicated a significant effect on COD yield ($p = 0.009$) and a second-order polynomial relationship ($R^2 = 0.99$) was observed between the share of substrate and sCOD concentrations in all tests. The proportions of sCOD relative to tCOD in the fermentates were 37, 42, 56, and 13 % in tests A, B, C, and D, respectively (Fig. 1a).

The solubilisation of organic matter is known to be influenced by both substrate composition and pH, with neutral pH being the most optimal (Shi et al., 2022a). However, the conversion of different macromolecules has different optima in this regard, and values between 5.5 and 6.5 are ideal for carbohydrate hydrolysis (Zoetemeyer et al., 1982) whereas protein hydrolysis generally performs better at pH 7 (Breure and van Andel, 1984), but can start improving at pH > 5 (Deng et al., 2023). For lipid degradation, the optimal pH of microbial lipase is typically neutral, but varies between species (Yao et al., 2021). Many enzymes have been shown to exhibit reduced functionality at low pH (~3) (Zhou et al., 2018), with reduced activity for both carbon solubilisation and VFA production. Moreover, high levels of VFAs combined with low pH values can cause inhibition. VFAs are weak acids (pKa ~4.7) and primarily exist in their undissociated form at pH < pKa (~4.9). This causes them to diffuse into bacterial cells, dissociate, and lower cytoplasmic pH, which disrupts metabolism and energy balance and ultimately leads to growth inhibition or cell death (Russell et al., 1997). In the present study, the pH in all fermentation tests was low, reaching values between 3.9 ± 0.1 and 5.2 ± 0.1 (Fig. 1b), with the lowest values in the tests with higher FW shares. The FW initially contained high concentrations of sCOD and tVFA, likely due to the hygienisation process (70 °C for 1 h). The combination of initially high levels of lactic acid and VFAs, together with the high organic load, resulted in decreased pH in the fermenters. The low pH and high VFA likely explain the low level of solubilisation observed (Fig. 1b). In line with this, Moestedt et al. (2019) also reported a low degree of solubilisation (~7 %) during hydrolysis/fermentation of FW at pH 3.8. The importance of pH was also presented in a study by Tang et al. (2016), who observed an increase in the hydrolysis rate of FW from 15 % to 28 % after raising the pH from 3.5 to 6. However, Feng et al. (2018) reported a higher degree of solubilisation (~30 %) for FW, compared to our results (0 %) at pH 3.2, suggesting that other factors, such as substrate composition or microbial adaptation, may also influence solubilisation under extreme acidogenic conditions. Test D with only sludge showed the comparably highest degree of solubilisation, which may be connected to the comparably higher pH (5.2).

The observed variability in sCOD and VFA concentrations of food waste during the experimental period highlights the inherent compositional variability associated with changing dietary inputs (Qin et al., 2024). This variability also affected macromolecular composition, resulting in the high standard deviations observed (Table 3). Carbohydrates were the most abundant macromolecule in both substrates and fermentates across all tests (Tables 1 and 3), accounting for 63 % of the VS in PS samples, which is higher than the typical range of chemically enhanced PS (Abdelrahman et al., 2023; Wilson and Novak, 2009), and 65 % in FW, which is consistent with previous studies (Owusu-Agyeman et al., 2023; Strazzera et al., 2018). Carbohydrate degradation was higher in reactors receiving PS, reaching 41 %, 57 %, and 51 % in Tests B, C, and D, respectively (Fig. 2), in which the pH was closer to the ideal range for carbohydrate degradation. In contrast, degradation was lower in Test A (13 %) at pH 3.9. For proteins, the conversion was lower than for carbohydrates in Tests A, B, and C, reaching 4 %, 25 %, and 8 %, respectively, whilst Test D showed a higher value of 58 % (Fig. 2). The difference in protein solubilisation between the different tests was also likely related to the pH. Indeed, protein degradation has been shown to occur 5 to 25 times slower in acidic conditions compared to neutral pH (Duong et al., 2019). FW generally contained a higher proportion and concentration of lipids than PS (Table 1). Hydrolysis of lipids produces glycerol and long-chain fatty acids (LCFAs), which are further degraded via β-oxidation (often rate-limiting) (Zheng et al., 2024; Law et al., 2023). Nevertheless, lipids showed greater degradation than both proteins and carbohydrates in tests A and B (Fig. 2b), with reactors having a higher fraction of FW in the substrate and a higher initial lipid content.

In summary, pH and substrate composition were important in organic matter solubilisation and macromolecule degradation. Although low pH (3.9–4.2) limited enzymatic hydrolysis and protein degradation, it favoured carbohydrate hydrolysis. Higher PS proportions (Test D, pH 5.2) led to improved overall solubilisation, particularly of proteins. Aside from pH, the pattern in this study suggested microbial adaptation: protein-rich substrates promoted protein degradation, whereas substrates with higher lipid content enhanced lipid breakdown. Carbohydrates were highly degraded in all tests, whilst lipid degradation was the most degraded macromolecule in FW-rich conditions.

Table 3
Characteristics of fermentates after reaching stable conditions considered after 15 days (3 HRT + 2 days).

		Reactor 1 (FW:PS -% v/v)						Reactor 2 (FW:PS -% v/v)					
		Test A (100:0)			Test B (50:50)			Test C (75:25)			Test D (0:100)		
		av.	**sd.**	***n***	**av.**	**sd.**	***n***	**av.**	**sd.**	***n***	**av.**	**sd.**	***n***
TS	**%**	15	3.2	*7*	11	3.3	*9*	8.7	1.6	*7*	1.3	0.7	*9*
VS	**% of TS**	91	2.8	*7*	91	1.8	*9*	92	1.0	*7*	84	5.1	*9*
Total COD	**g/L**	151	28	*4*	100	35	*3*	48		*2*	22	8.1	*3*
sCOD	**g/L**	82	8.1	*7*	70	11	*8*	41	5.2	*7*	5.9	3.0	*8*
NH_4-N	**mg/L**	588	188	*7*	452	75	*8*	313	80	*7*	183	86	*8*
TN - Filtered	**mg/L**	1639	442	*7*	1671	279	*8*	916	177	*7*	442	470	*8*
PO_4-P	**mg/L**	345	61	*7*	314	83	*8*	178	43	*7*	39	17	*8*
TVFA $_{+ \text{la}}$	**gCOD/L**	56	6.7	*7*	42	9.2	*8*	27	4.8	*7*	2.8	1.1	*8*
Proteins	**g/kg VS**	21	2.1	*3*	11	3.5	*5*	12	1.7	*3*	2.2	1.9	*5*
Carbohydrates	**g/kg VS**	58	9.0	*3*	33	12	*5*	22	13	*3*	8.0	7.2	*5*
Lipids	**g/kg VS**	33	15	*3*	13	12	*5*	13	2.7	*3*	1.7	0.9	*5*

Note: TS: Total solids, VS: Volatile solids, sCOD: soluble chemical oxygen demand, tCOD: total chemical oxygen demand, NH_4^+-N: ammonium nitrogen, $TN_{filtered}$: total nitrogen in filtered samples, PO_4-P: phosphate phosphorus, tVFA: total volatile fatty acids (including lactic acid, expressed as g COD/L). av: average, sd: standard deviation, n: number of samples.

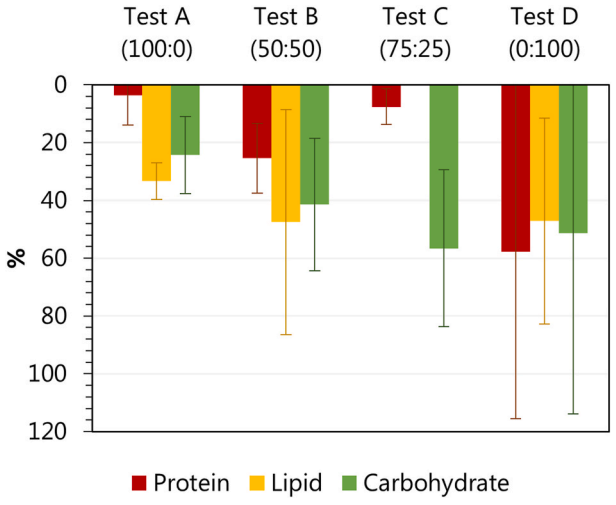

Fig. 2. Macromolecule degradation in % (proteins (red), carbohydrates (green), and lipids (yellow)) during fermentation Tests A–D. FW: Food waste, PS: Primary Sludge. Substrate proportions are given as FW:PS (% v/v).

3.2. VFA species and lactic acid yields

Lactic acid was included in the tVFA calculations to provide a comprehensive assessment of the available carbon sources. The tVFA profiles of the substrates emphasised their differences, with food waste exhibiting a tVFA concentration of 36.7 ± 9.4 g VFA_{COD}/L, with lactic acid (51 %) as the dominant acid, followed by propionic (19 %), butyric (16 %), and acetic acid (14 %) (Fig. 3a and b). By comparison, PS had lower tVFA concentrations (0.9 ± 0.6 g VFA_{COD}/L), with acetic (45 %) and propionic acid (44 %) as the dominant VFAs, and lower concentrations of butyric and valeric acids (Fig. 3a). The tVFA concentration of fermented FW (Test A) reached 55.7 ± 7 g VFA_{COD}/L (Fig. 1a), corresponding to a 34 % increase compared to FW ($p < 0.001$), despite the low solubilisation of carbon. The VFA to sCOD ratio also increased from 42 % to 68 %, and the VFA to total carbon ratio increased by 51 % (from 22 % to 34 %) (Figs. 1 and 3). The VFA production yields (VFA_{COD}/kg VS_{in}) were not significantly different between reactors (Welch's ANOVA, $p > 0.05$). In Test A the yield was 167 ± 117 g VFA_{COD}/kg VS_{in} (Fig. 1b), indicating an active fermentation process where soluble compounds were converted to VFA even at pH 3.9. The results were consistent with those reported by Jiang et al. (2013), who obtained 32 g VFA_{COD}/kg VS_{in} at pH 3 and 137 g VFA_{COD}/kg VS_{in} at pH 5 under mesophilic conditions. Increasing the proportion of PS in the feed increased the average VFA yield, reaching 215–236 g VFA_{COD}/kg VS_{in} in Tests B-D (Fig. 1b). Overall, the VFA yields of the reactors with higher PS proportion, in Tests C and D, were slightly lower than those reported in the literature, likely due to the lower pH. For instance, Owusu-Agyeman et al. (2023) reported a yield of 315 g VFA_{COD}/kg VS_{in} with anaerobic fermentation of 30 % FW and 70 % PS at pH 5, and Yang et al. (2022) and Ucisik and Henze (2008) reported yields of 250–310 g VFA_{COD}/kg VS_{in} and 168–270 g VFA_{COD}/kg VS_{in} with PS, at pH 5-7, respectively. Lactic acid was not measured in any of these studies.

The final tVFA concentrations in the fermentates decreased with an increasing proportion of PS (Figs. 1 and 3), likely because of the lower incoming organic load and VFAs with the substrate. For fermented FW (Test A) the concentration reached 55.7 ± 7 g VFA_{COD}/L (Fig. 1a), corresponding to a 34 % increase compared to FW ($p < 0.001$), despite low solubilisation of carbon. The VFA to sCOD ratio. The tVFA concentration increased compared to untreated FW, but with a decreased level of lactic acid by approximately 4 g COD/L, reducing its share of the tVFA to 27 %. Meanwhile, the share of acetic and propionic acid increased by 40 % and 21 %, respectively. Small amounts of iso-valeric and valeric acids were also formed during fermentation, whilst no butyric acid was produced (Fig. 3b and S1). This pattern in reactors fed with higher FW is consistent with the consumption of influent lactate and its conversion to propionate and acetate. This conversion can occur via established metabolic routes, such as the acrylate pathway or the lactate pathway, which also produces acetate and 1,2-PDO (Gonzalez-Garcia et al., 2017; Moestedt et al., 2020; Tao et al., 2021).

Fermentation of PS (0:100, Test D) increased tVFA concentration to 2.8 ± 1 g VFA_{COD}/L ($p < 0.001$), mainly through the production of propionic (49 %) and acetic (34 %) acid (Fig. 1). These results align with previous studies, which have reported acetate and propionate as the main fermentation products of PS, with acetate typically being the most abundant (Tchobanoglous et al., 2014; Owusu-Agyeman et al., 2020; Ucisik and Henze, 2008; Diaz et al., 2023). The co-fermentation of FW and PS in Tests B and C showed similar VFA profiles to Test A, but with slightly higher lactic acid fractions that primarily originated from the FW, which accounted for 35 % and 30 % of the tVFA in Tests C and D, respectively (Fig. 3). These fermentates also contained propionic (34 and 37 %), acetic (19 and 21 %), and butyric acid (11 and 10 %). The VFA profile in Test C resulted in slightly different results compared to a previous study, where a similar substrate share was used (FW:PS 70:30; HRT 7d), and acetic acid was the dominant VFA, followed by propionic acid (Owusu-Agyeman et al., 2023). Lactic acid was not measured in that study, and unlike our results, longer-chain acids, such as caproic acid, were highly abundant.

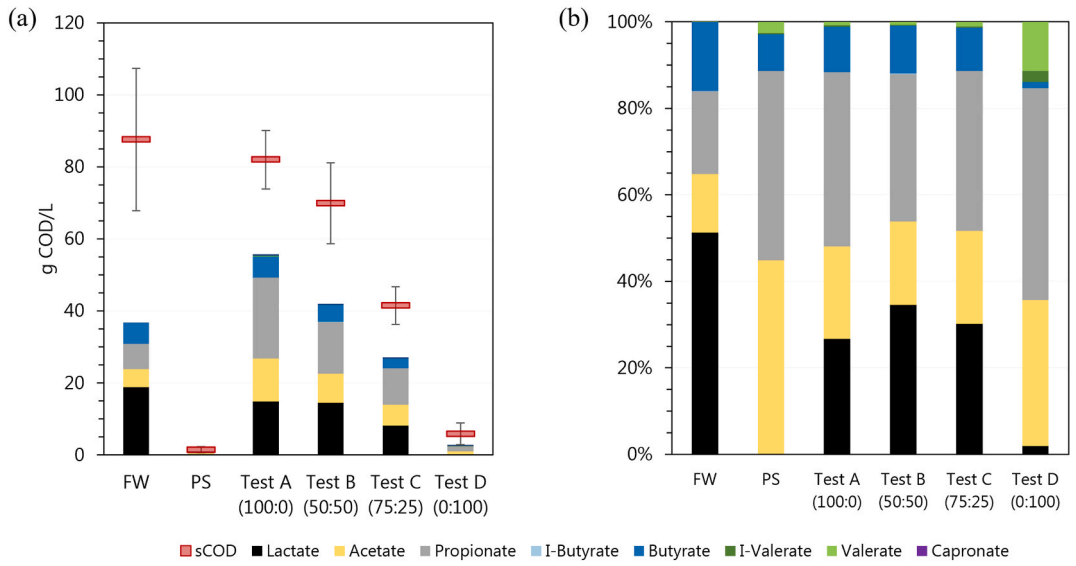

Fig. 3. Concentrations and proportions of volatile fatty acids (VFAs) and lactic acid in the substrates (PS: Primary sludge and FW: Food waste) and fermentates under four test conditions (A, B, C, and D). Substrate proportions are given as FW:PS (% v/v). **a)** Concentrations of VFAs, lactic acid, and soluble chemical oxygen demand (sCOD), presented in g COD/L. Error bars: n = 9. **b)** Relative proportions of VFAs and lactic acid as a percentage of the total VFAs.

During the tests, the production of lactic acid and VFA was determined by the substrate and was also likely affected by the prevailing pH. FW contained high concentrations of VFAs, mainly lactic acid, which primarily degraded into propionic and acetic acid at low pH in Tests A and B. Overall, excluding lactic acid, propionate was the dominant VFA in all tests, which contrasts with the literature that typically reports acetate as dominant at low pH for both FW (Strazzera et al., 2021) and PS (Tchobanoglous et al., 2014) fermentation. Propionic acid

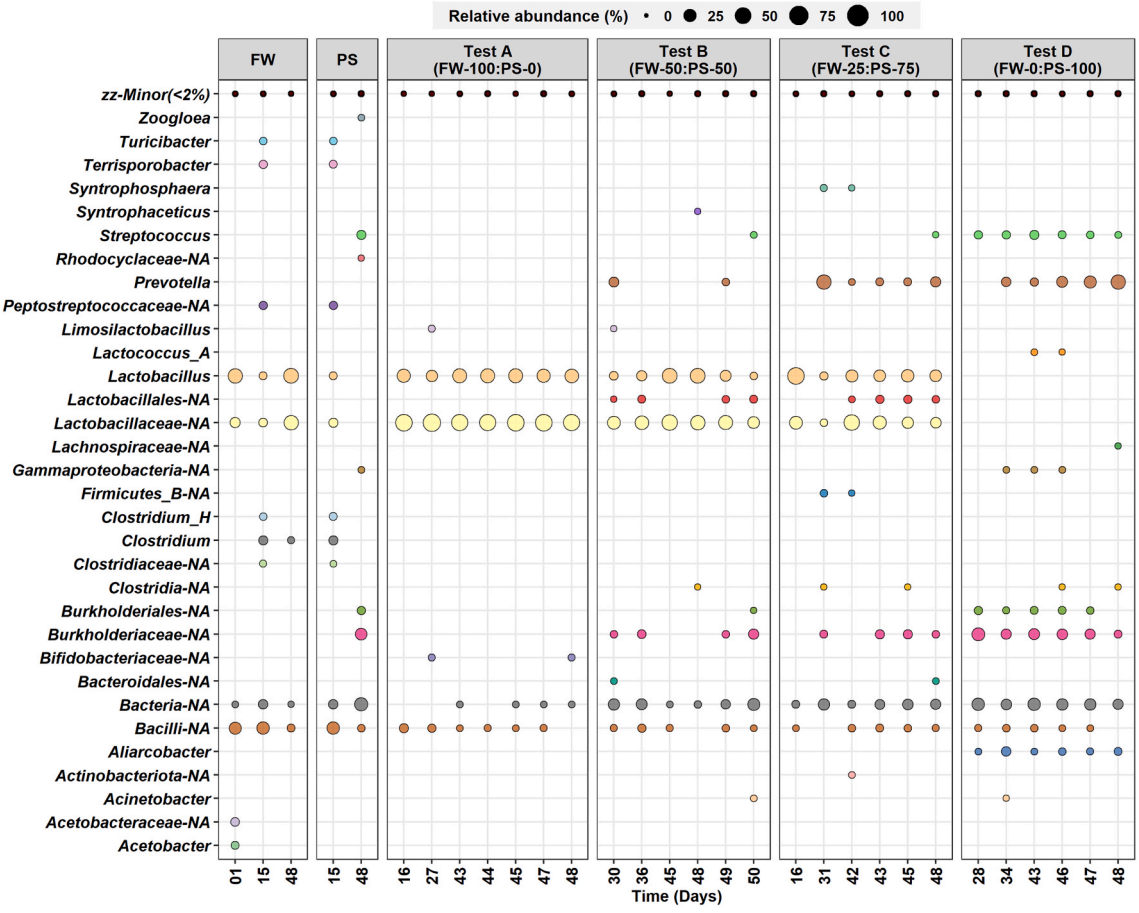

Fig. 4. Genus-level relative abundance (>2 %) of microbial communities in FW, PS, and fermentation tests (A–D) over time. Substrate proportions are given as FW: PS (% v/v).

dominance has instead been reported under alkaline conditions (Atasoy and Cetecioglu, 2022).

3.3. Microbial community composition

Microorganisms from the phylum Firmicutes dominated the substrate FW, whereas PS exhibited a higher abundance of *Proteobacteria* and *Bacteroidota*. This is in line with previous studies investigating the fermentation of FW (Moestedt et al., 2019) and sludge (Iglesias-Iglesias et al., 2019). During fermentation the microbial composition was similar as the substrate and shifted from FW-rich (Test A) to PS-rich (Test D); i.e. the relative abundance of *Proteobacteria* and *Bacteroidota* increased, whilst *Firmicutes* declined. This propose a microbial shift in response to substrate composition (Fig. 4), evidenced by the Non-metric Multi-Dimensional Scaling (NMDS) analysis with Bray-Curtis dissimilarity index at the family level ($p < 0.05$, stress = 0.0983) (Fig. S2). The assembly of the microbial community during mixed culture fermentation of FW and PS depends on various parameters, such as the chemical and microbial composition of the substrate (Shi et al., 2022b), as well as the microbial composition of the selected inoculum (Moestedt et al., 2019), and the prevailing pH (Atasoy and Cetecioglu, 2022).

Reactors fed primarily with FW in Tests A, B, and even C were, similar to the FW, dominated by members within the family *Lactobacillaceae,* with two dominant species identified: one belonging to the genus *Lactobacillus* and the other from an unidentified genus. The dominance of *Lactobacillaceae* during the fermentation of FW has been reported in several previous studies (Pan et al., 2025; Tang et al., 2016; Feng et al., 2018). The ASV related to the genus *Lactobacillus* exhibited 100 % similarity (NCBI blast) with *Lactobacillus acetotolerans*, a species with a high tolerance to acetic acid (Entani et al., 1986), which can explain its prevalence in FW and during fermentation. Species within the family *Lactobacillaceae* utilise a range of carbohydrates whilst producing lactic acid. Lactic acid production can proceed via two primary metabolic pathways: the homofermentative and the heterofermentative pathways, depending on the bacterial species and enzymatic capabilities. The homofermentative pathway results in only lactic acid, whereas the heterofermentative pathway produces lactic acid along with ethanol, acetic acid, and CO_2 as by-products (Song et al., 2022). *L. acetotolerans* uses the homofermentative pathway; however, the observed decrease in lactic acid and increase in the proportion of acetic acid suggest that the heterofermentative pathway may have also been active. Lactic and acetic acid can also be produced via the bifidum pathway (Song et al., 2022), although *Bifidobacteriaceae,* which employ this pathway, were only detected at very low relative abundance in Test A.

Reactors fed with PS demonstrated a decreasing relative abundance of *Lactobacillaceae* and were instead dominated by genera *Prevotella, Streptococcus,* and unclassified genera annotated as *Burkholderiaceae_NA* and *Burkholderiales_NA* to indicate taxa known only to the family and order levels, respectively. Genus *Aliarcobacter* and unclassified *Gammaproteobacteria_NA* were exclusive to the fermentation of PS in Test D, but only at a very low relative abundance (4.6 % and 2 %, respectively) (Fig. 4). *Prevotella* are known to contribute to polysaccharide degradation whilst producing organic acids such as formate, acetate, succinate, and propionate (Zhang et al., 2023), which were the dominating VFA in the reactor fed with PS only. Members within the genus *Streptococcus* also ferment various carbohydrates, whilst producing mainly lactic acid (Toit et al., 2014). Representatives in the family *Burkholderiaceae* utilise numerous organic compounds as carbon and energy sources (sugars, fatty acids, dicarboxylic acids, alcohols, glycols, aromatic compounds, amino acids, etc.) (Coenye et al., 2014). It is notable that certain members within this family, such as the genus *Acidovorax,* have been observed to act as S-oxidising denitrifiers when using acetate. A BLAST search (NCBI) identified *Acidovorax* as the closest relative to the observed ASV within the family *Burkholderiaceae* (Huang et al., 2019). A possible source of S could have been H_2S, which was present at high levels in the gas phase in tests receiving PS (B-D) (>465 ppm).

The carbohydrate-consuming microbial community was evident in all tests and dominated by species from the families *Lactobacillaceae* or *Prevotellaceae*, and the higher protein conversion in Test D was likely linked to the relatively higher abundance of *Burkholderiales-NA*. Clarifying the community responsible for lipid hydrolysis was more challenging and not as obvious. However, representatives within *Burkholderiales* have been proposed to express lipases (Bharathi and Rajalakshmi, 2019) and all processes showed a relatively high abundance of an unknown taxa that were potentially involved in lipid degradation and VFA production via beta-oxidation.

3.4. Denitrification rates – carbon sources

Following fermentation, one sample per test (A-D) and one FW sample were filtered (0.6 mm sieve), using the liquid phase for the denitrification tests. The highest denitrification rate (9.31 g NO_3^--N/L/kg VSS•h) was observed with the fermented FW from Test A (Table 4), which also obtained the highest VFA/sCOD ratio. The higher rate was likely linked to the high concentration of acetic acid (11.9 g VFA/L) and propionic acid (22.4 g VFA/L) (Fig. 3), supporting the results of Elefsiniotis and Wareham (2007) who demonstrated that heterotrophic denitrifiers prefer low molecular weight compounds compared to other VFA species (lactic acid was not included in their study). Fermented FW has demonstrated denitrification rates comparable to the results obtained in this study. For example, Zhang et al. (2016), Kim et al. (2016), and Qi et al. (2021b) reported rates of around 12 mg NOx/g VSS•h, and Tang et al. (2018) reported 5.5 mg NOx-N/g VSS•h, all of which are similar to the rates obtained with either acetic or lactic acid. Furthermore, unfermented FW also displayed good denitrification rates, just slightly lower than Test A. This could be attributed to the high content of lactic acid and potentially other available soluble carbon compounds.

The second highest denitrification rate was observed in Test C, with a value of 8.50 g NO_3^--N/g VSS•h, followed by the unfermented FW, Tests B and D. The denitrification rate of fermented PS in Test D was comparable to 7.1 mg NO_3-N/g VSS•h observed before with alkaline pretreated PS fermentation liquid (Liu et al., 2016) and to 9 mg NOx/g MLSS•h with PS fermentation liquid (Hatziconstantinou et al. (1996). Although a correlation between the VFA/sCOD ratio and the denitrification rates across the tests (A-D) was identified ($R^2 = 0.77$), there was no significant difference between the denitrification rates (for all pair comparisons). This lack of statistical significance indicates that all tested fermentates were similarly effective as carbon sources, thus demonstrating the flexibility and robustness of the denitrification process with different carbon sources.

The sCOD/NO_3^--$N_{removed}$ is an important parameter for determining the efficiency of a carbon source. Based on electron balances, the theoretical consumption of COD per gram of nitrate removed, if no growth occurs, would be 2.86 g O_2/g NO_3^--N and 1.71 g O_2/g NO_2^--N for nitrite (Tchobanoglous et al., 2014). However, actual COD consumption also supports cell growth and maintenance of all types of bacteria (Zhang et al., 2016), and therefore, a ratio of 3.5–4.5 gCOD/g$N_{removed}$ is more accurate. Our tests resulted in higher ratios: 5.7–5.9 for Tests B, C, and D; 6.5 for Test A; and 7.1 for FW. A lower g sCOD/g NO_3-$N_{removed}$ ratio could indicate better denitrification efficiency but may also suggest slower carbon uptake rates. Tests D and B had low g COD/g $N_{removed}$ ratios, as well as low biomass yields (0.33 and 0.53 g COD/g COD, respectively), and lower denitrification rates, indicating that the substrate may not be as readily available. In contrast, the biomass yield in Tests A, C, and FW was within common ranges (0.61, 0.68, and 0.63, respectively), which is consistent with Güven (2009) who reported 0.62–0.66 g COD/g COD for hydrolysis products. These findings suggest that although Tests B and D showed lower carbon consumption rates and potentially higher denitrification efficiency per unit of COD, their overall denitrification performance was limited, likely because of slower carbon uptake.

Table 4
Denitrification performance of liquid fractions (carbon source) and methane potential (BMP) of solid and unseparated fractions of various substrates. Denitrification parameters include specific denitrification rate, growth yield (Y_{OHO}), total nitrogen removal efficiency, and COD/NO_3-N ratio. The BMP value for the sample Mix 50:50 (% v/v) marked with * was calculated theoretically from BMP values for PS and FW.

Sample	Sample fraction	Denitrification rate	Growth yield (Y_{OHO})	N Removal efficiency	Carbon consumption ratio	Sample fraction	BMP	
(FW:PS) % v/v		mg NO_3^--N/g VSS.h	g COD/g COD	% TN	g sCOD/g NO_3^--N		N L CH_4/kg VS	
		av. (n = 2)	av. (n = 2)	av. (n = 2)	av. (n = 2)		av. (n = 3)	sd. (n = 3)
Test A (100:0)	Liquid	9.31	0.61	84.78	6.51	Solid	382.0	6.4
Test B (50:50)	Liquid	7.45	0.53	87.24	5.90	Solid	349.7	16.4
Test C (75:25)	Liquid	8.50	0.68	83.14	5.81	Solid	246.4	6.9
Test D (0:100)	Liquid	7.11	0.33	71.51	5.70	Solid	163.4	0.6
Food Waste	Liquid	8.24	0.63	87.20	7.11	Unseparated	366.2	6.8
Primary Sludge	–	–	–	–	–	Unseparated	330.8	19.4
Mix PS:FW (50:50)*	–	–	–	–	–	Calculated	348.5*	–
Mix PS:FW (75:25)	–	–	–	–	–	Unseparated	325.6	7.8

Total nitrogen (TN) removal efficiency was the lowest in Test D, at 71 %, compared to 85 %, 87 %, and 83 % in Tests A, B, and C, respectively. The low sCOD to ammonium ratio in Test D (Fig. 5) likely contributed to the low TN removal, as ammonium was introduced along with the carbon source. Fermented FW from Test A proved more suitable, achieving a high denitrification rate (9.31 mg NO_3^--N/g VSS•h), 100 % nitrate removal efficiency, 84.8 % TN removal efficiency, and a g sCOD/g NO_3^--$N_{removed}$ ratio of 6.5, which aligns with common values of FW fermentates (Zhang et al., 2016). One factor that should be considered in long-term trials with food waste is that oil and fat can strongly inhibit denitrification by limiting mass transfer (Chen et al., 2021). However, longer trials are needed to evaluate the extent of this potential inhibition.

3.5. NH_4^+-N and PO_4-P in the carbon sources

Fermentates from organic streams contain relatively high concentrations of nitrogen and phosphorus compared to other sources because these nutrients are both present in the substrates and released during fermentation. Ammonium is released from the degradation of amino acids (Wei et al., 2021), and phosphate has been shown to be released from bonds with fibre components (Metzler et al., 2009), which impacts the nutrient concentration in the final carbon source (Ali et al., 2021; Soares et al., 2010). In this study, the differences between FW and PS were evident in both the substrates and the fermentates. FW and fermentates from Tests A, B, and C exhibited sCOD/NH_4^+-N ratios above 130, but were below 50 (Fig. 5) in PS and fermented PS (Test D). The sCOD/PO_4-P ratio was 71 in PS and above 150 in all fermentates (Tests A-D) and FW. The acceptability of nutrient content in the carbon source depends on each specific wastewater treatment system. For example, Soares et al. (2010) reported sCOD/NH_4^+-N and sCOD/PO_4-P ratios of 17 and 117, respectively, which were 47 % and 22 % lower than those observed in fermented PS (Test D), yet they are still considered to be acceptable in a biological nutrient removal (BNR) system. Similarly, a sCOD/NH_4^+-N ratio as low as 16 and 11 has been previously obtained with fermented mixed sludge and with pre-treated fermented digestate (Carranza Muñoz et al., 2024), which could still be applied under certain operational conditions. Low ratios undermine the goal of nutrient removal by introducing additional N and P into the water line, especially in post-denitrification zones. Very low carbon-to-nutrient (N and P) ratios may require additional treatment steps and nutrient recovery processes that preserve soluble carbon content could offer a viable solution.

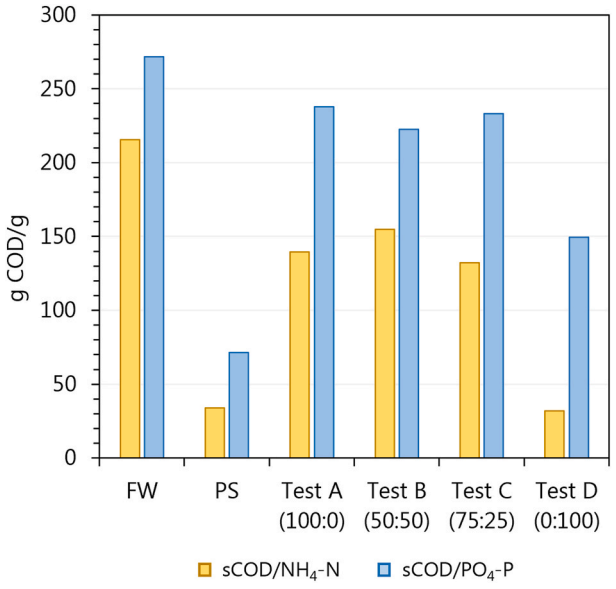

Fig. 5. Ratios of soluble COD to ammonium (NH_4^+-N) and soluble COD to phosphate (PO_4-P) of substrates FW and PS and the four fermentates from tests A-D. Substrate proportions are given as FW:PS (% v/v).

The results indicated that phosphate release, which has been previously shown to be facilitated under acidic conditions (Shi et al., 2022a), did not seem problematic in any fermentate, regardless of the substrate used (Fig. 5). The balance of soluble carbon and these nutrients is important when defining the potential of a carbon source. Substrates such as food waste typically have a better balance between sCOD and nutrients (N and P) than other substrates, such as primary or waste-activated sludge, but the macromolecule composition of the substrates and the dynamics during fermentation define the carbon source characteristics.

3.6. Biochemical methane potential tests

A separate pre-fermentation step has been shown to enhance biogas production of FW and sewage sludge compared to one-stage digestion, due to improved hydrolysis and fermentation (Feng et al., 2020; Nabaterega et al., 2021; Moestedt et al., 2016). However, filtering leads to reduced soluble carbon in the remaining solid fraction, including VFAs and lactic acid, which could reduce the methane potential. Our results indicated that the macromolecular composition of the substrates and fermentates was likely the most influential parameter for the biogas potential of the solid fraction. FW contained higher concentrations of

carbohydrates and lipids, which limited degradation during fermentation due to low pH. This left more particulate carbon available for methane production during AD. Lipids generally yield more biogas than proteins or carbohydrates (Magdalena et al., 2018; Cirne et al., 2007), which can explain the observed higher methane yield from the solid fraction of FW-rich fermentates compared to fermentates with more PS (Table 4, Fig. S3). The solid fraction from Test A yielded the highest methane production (382 ± 6 N m^3 CH_4/ton VS), slightly surpassing unfermented FW ($p = 0.009$). Subsequently, methane yields followed a decreasing trend as the proportion of FW in the substrate mix decreased. Test B resulted in 350 ± 16 N m^3 CH_4/ton VS, whilst methane yields in PS-rich fermentates were significantly lower. The solid fraction from Test D exhibited the lowest methane yield (163.4 ± 0.6 N m^3 CH_4/ton VS), with a 51 % decrease compared to unfermented PS ($p = 0.03$). This suggests that although a pre-fermentation step could enhance BMP and the extraction of VFAs reduces it, the macromolecular composition also plays an important role. In this case, fermentates with a higher FW proportion resulted in higher biogas yields due to a low level of hydrolysis and fermentation of lipid and carbohydrate in previous steps; contrary to fermentates with a higher share of PS (Fig. 2).

3.7. Nutrient removal and biogas production at WWTP

The transition from fossil-based carbon sources at large-scale WWTPs depends on how alternative solutions affect the overall plant operation. This study evaluated two key factors: the carbon source quality, including its production and denitrification efficiency, and the potential reduction in biogas production. In this study, fermentation resulted in no significant differences in tVFA yields among the tests, and differences were only significant between tests A-C and A-D. However, sCOD and tVFA concentrations showed significant differences in all trials with changing VFA profiles and were mainly dominated by propionic and lactic acid. pH and substrate composition had the most significant influence on hydrolysis, fermentation, and microbial community dynamics. Denitrification rates in all tests were statistically identical, suggesting that the microbial community could utilise different carbon compositions efficiently. This points to microbial versatility in the activated sludge, with no strong preference for specific VFA profiles.

An additional factor that is important to consider is the volumes of carbon source required for denitrification, which are influenced by several factors such as the WWTP process, sCOD and VFA concentrations, denitrification rate, denitrification potential, and the nutrient content in the carbon sources. Although statistical significance among the fermentation trials and the denitrification rates was limited, the fermentate volumes needed to reach the target nitrate removal varied significantly depending on the carbon source characteristics (sCOD, tVFAs, and nutrients). In a theoretical WWTP with an activated sludge system, removing **100 kg NO_3-N** per day in the post-denitrification would require an estimated **0.42 m^3/d** of methanol, assuming common methanol parameters (1200 g COD/L, 5 gCOD/gNO_3^--N, and 0.5 g $COD_{biomass}$/g COD_{MeOH}). In contrast, based on measured fermentate characteristics and test results (Tables 3 and 4), the required fermentate volumes would be **7.9 m^3, 8.4 m^3, 11.7 m^3, and 97 m^3** for Tests A, B, C, and D, respectively. These results highlight the importance of substrate composition during fermentation and the resulting nutrient content in determining the efficiency and operational feasibility of carbon source production. Co-fermenting PS with FW significantly reduced the required volume. Additions of 25 % and 50 % FW decreased the needed fermentate volume by a factor of 8 and 12, respectively, compared to PS alone. These results demonstrate the benefit of including high organic content substrates such as food waste, even in small proportions.

Biogas production was the second key factor assessed. Specific methane production was higher in the solid fraction of fermentates produced with more FW (A–B), whereas fermentates from higher substrate shares of PS showed a reduction of biogas potential (C–D). Extraction of the soluble fraction after FW fermentation (Test A) did not affect biogas yield, indicating that VFA recovery can be compatible with energy recovery. However, applying the same process to PS reduced methane production by approximately 50 % in Test D. Upscale calculations were performed using sludge production estimates (based on different Y_{OHO}), BMP values for different fermentates before and after VFA production, and assuming that all the solid residue was recirculated back to the digester for biogas production. In the scenario where the WWTP did not previously handle FW, and is introduced as a new substrate, the impact on biogas production was estimated as an increase of 19 %, 13 %, and 5 % in Tests A, B and C, respectively, and a 35 % decrease in Test D. On the other hand, if the plant had already received FW and decided to divert it for VFA production, the projected reduction in biogas production would be approximately 6 % (Test A).

4. Conclusion

Fermented FW proved to be an effective carbon source for denitrification, offering better $sCOD/NH_4^+$-N and $sCOD/PO_4$-P ratios than fermented PS, while maintaining similar biogas yield compared to untreated FW. However, FW has practical constraints, such as its limited on-site availability at WWTPs and high transport costs for large volumes, both of which can lead to higher costs and increased handling complexity. Additionally, pronounced seasonal and temporal variations in food waste composition affected sCOD and VFA production, indicating that substrate variability should be considered when interpreting fermentation performance and extrapolating results beyond the experimental period. Food waste is already utilised within circular systems through biogas production and nutrient recovery, and in the approach proposed here, it also contributes through the recovery of soluble carbon for use as an internal carbon source that can substitute fossil methanol; however, agricultural nutrient recycling may be restricted when FW is mixed with sewage sludge. This raised the question of how co-fermentation of PS with FW can improve carbon source production without causing excessive biogas losses at WWTPs. Addressing this question by evaluating FW additions of 25 % and 50 % was a central aim of the present study. Our results demonstrate that co-fermenting PS with a low proportion of FW (25 %) may offer a suitable balance between carbon source production, energy yield, and operational feasibility.

From a process perspective, pH and substrate composition were important parameters influencing the fermentation outcomes and microbial community dynamics. Different VFA profiles resulted from the fermentation of different substrate combinations, changing from lactic acid-dominated in FW trials to propionic acid-dominated in PS trials. Nevertheless, denitrification rates remained stable across all tests, indicating a high degree of microbial functional flexibility and a limited dependence on specific VFA compositions. This robustness may improve operational stability, but it also raises the question of whether attempting to direct fermentation toward specific VFA profiles is necessary for full-scale applications. Furthermore, the high standard deviations observed in all trials highlight the inherent variability when using substrates such as FW and PS. Additional studies using more stable and consistent substrates are recommended to confirm these results and improve predictability for full-scale implementation. For WWTPs considering a carbon recovery approach, understanding the trade-offs between carbon redirection, energy recovery, and treatment stability will be essential for a successful full-scale implementation. From a scaling-up perspective, the methodology is technically feasible, as demonstrated by mass balance and design calculations at large scale; however, practical constraints related to solid–liquid separation and handling of variable substrates must be addressed. In particular, improved separation systems, such as centrifugation or equivalent technologies, may be required to ensure stable operation and consistent carbon source quality at full scale.

CRediT authorship contribution statement

A. Carranza-Muñoz: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **C. Baresel:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **A. Malovanyy:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **A. Singh:** Writing – review & editing, Visualization, Software, Data curation. **A. Schnürer:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Christian Baresel reports financial support was provided by Stockholm Water and Waste, Käppalaförbundet, and Syvab AB. Other 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.jenvman.2025.128470.

Data availability

Data will be made available on request.

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