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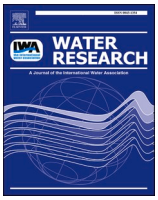

Impact of thermal hydrolysis on VFA-based carbon source production from fermentation of sludge and digestate for denitrification: experimentation and upscaling implications

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ABSTRACT

Stricter nutrient discharge limits at wastewater treatment plants (WWTPs) are increasing the demand for external carbon sources for denitrification, especially at cold temperatures. Production of carbon sources at WWTP by fermentation of sewage sludge often results in low yields of soluble carbon and volatile fatty acids (VFA) and high biogas losses, limiting its feasibility for full-scale application. This study investigated the overall impact of thermal hydrolysis pre-treatment (THP) on the production of VFA for denitrification through the fermentation of municipal sludge and digestate. Fermentation products and yields, denitrification efficiency and potential impacts on methane yield in the downstream process after carbon source separation were evaluated. Fermentation of THP substrates resulted in 37–70 % higher soluble chemical oxygen demand (sCOD) concentrations than fermentation of untreated substrates but did not significantly affect VFA yield after fermentation. Nevertheless, THP had a positive impact on the denitrification rates and on the methane yields of the residual solid fraction in all experiments. Among the different carbon sources tested, the one produced from the fermentation of THP-digestate showed an overall better potential as a carbon source than other substrates (e.g. sludge). It obtained a relatively high carbon solubilisation degree (39 %) and higher concentrations of sCOD (19 g sCOD/L) and VFA (9.8 g VFA_{COD}/L), which resulted in a higher denitrification rate (8.77 mg NOx-N/g VSS•h). After the separation of the carbon source, the solid phase from this sample produced a methane yield of 101 mL CH_4/g VS. Furthermore, fermentation of a 50:50 mixture of THP-substrate and raw sludge produced also resulted in a high VFA yield (283 g VFA_{COD}/kg VS_{in}) and denitrification rate of 8.74 mg NOx-N/g VSS•h, indicating a potential for reduced treatment volumes. Calculations based on a full-scale WWTP (Käppala, Stockholm) demonstrated that the carbon sources produced could replace fossil-based methanol and meet the nitrogen effluent limit (6 mg/L) despite their ammonium content. Fermentation of 50–63 % of the available sludge at Käppala WWTP in 2028 could produce enough carbon source to replace methanol, with only an 8–20 % reduction in methane production, depending on the production process. Additionally, digestate production would be sufficient to generate 81 % of the required carbon source while also increasing methane production by 5 % if a portion of the solid residues were recirculated to the digester.

1. Introduction

Stricter nutrient discharge limits, such as those recently proposed in the new EU legislation for urban wastewater treatment (European Parliamentary Research Service, 2023) or those already approved by the Swedish government, will increase the reliance on external carbon sources at wastewater treatment plants. The Swedish mitigation plan for improving the status of the Baltic Sea, which is affected by eutrophication, already applies stringent effluent requirements on large WWTPs (>100,000 person-equivalent (pe)) (Naturvårdsverket, 2016).

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Consequently, redesign and optimisation of WWTP processes are underway in Sweden. Most of them include dosing external carbon sources in denitrification zones, demand for which is estimated to increase with increasing population. Fossil-based methanol (MeOH) is the preferred carbon source for denitrification because of its high sCOD content and relatively low cost (~EUR 375/ton in Q3 2023) (Methanex Corporation, 2023), but it raises environmental concerns due to the high CO_2 emissions associated with its production and transportation (Methanol Institute, 2022). The methanol consumption for the three WWTPs serving ~2.8 Mpe in the Stockholm area is estimated to reach 6000 tons annually by 2040, significantly influencing operating costs and CO_2 emissions.

The extensive scientific literature on alternative carbon sources for denitrification has been reviewed in several recent publications (Fu et al., 2022; H. Wang et al., 2021). The focus in most previous studies has been on exploring potential carbon sources such as glycerol, methane, ethanol, different pure volatile fatty acids (VFAs), by-products from different industries (Ahmed et al., 2023; Fass et al., 1994; Lee and Welander, 1996) and fermentate liquids produced by sludge fermentation under different conditions (Elefsiniotis et al., 2004; Luo et al., 2019). The results evidence that acetic acid is the most effective carbon source, giving higher denitrification rates than methanol, glycerol or glucose (Pan et al., 2023). This is attributable to its molecular structure, which allows for more efficient production and transfer of electrons and energy, thereby promoting denitrification (Wei et al., 2022). When using a fermentation liquid with a mixture of organic compounds as a carbon source, acetic acid is depleted first, after which the denitrification bacteria use the remaining VFAs (Elefsiniotis et al., 2004), followed by soluble proteins and finally other organic compounds (Guo et al., 2017). Despite this preference of denitrifiers for short-chain carboxylic acids (e.g. acetic acid), denitrification performance remains similar when the carbon source is a mixture of soluble organic compounds. This has led to growing interest in producing carbon sources through fermentation or co-fermentation of organic waste streams at WWTPs to decrease costs and emissions. Studies to date have investigated the fermentation process under different parameters, e.g. temperature (Elefsiniotis and Li, 2006; Ossiansson et al., 2023), hydraulic retention time (HRT) (Khan et al., 2019) and substrates, e.g. food waste and other organic streams as a sole substrate or mixed with sludge (Atasoy et al., 2018; Zhou et al., 2018). Furthermore, pre-treatments can improve carbon solubilisation and VFA yields in sludge fermentation. Thermal hydrolysis pre-treatment (THP), a commonly used method for enhancing anaerobic digestion (AD), uses high temperatures and changes in pressure to destroy floc structures and microbial cell walls (Barber, 2016). THP is currently used on an industrial scale to increase biogas yield and sludge dewaterability, while at a pilot scale, the concentrate produced by THP has been evaluated as a carbon source for denitrification (Barlindhaug and Ødegaard, 1996; Chen et al., 2024; Guo et al., 2017), and as an enhancer for anaerobic fermentation. In terms of fermentation, some studies have observed a positive effect of THP on VFA production from sludge (Hosseini Koupaie et al., 2021; Morgan--Sagastume et al., 2011; Xiang et al., 2023; Zhang et al., 2019), whereas others have found that THP does not enhance biodegradability or VFA production due to the presence of refractory by-products from the Maillard reaction (Castro-Fernandez et al., 2023).

While in-depth investigation has focused on the production of internal carbon sources for denitrification, it has so far only been evaluated using primary (PS) and waste-activated sludge (WAS) and not digested sludge. Using digestate as substrate could enable the production of a carbon source without biogas reduction and with significant reductions in sludge handling volumes, resulting in cost savings. Previous studies have examined the effects of THP (at different temperatures) on digestate for improving dewaterability and enhancing biogas yield (Cai et al., 2021; Nordell et al., 2022; Svensson et al., 2018), but not for further fermentation. The Norwegian company Cambi now offers the option of post-THP (SolidStream) as a pre-treatment for improved dewatering, although the performance of this application has not been fully documented.

Additionally, little attention has been given to the overall implementation of the process in full-scale WWTP or its impact on biogas production and sludge management, which directly impacts WWTP operating costs. Most WWTPs in Sweden produce biogas through AD of sludge, contributing 31 % of the national biomethane production (Klackenberg, 2023). Using sewage sludge for other purposes, e.g. producing a carbon source for denitrification, could theoretically reduce biogas production. This would affect the production of renewable energy in Sweden and would represent a significant revenue loss for WWTPs (of ~EUR 1/Nm^3 CH_4).

In the present study, we compared internal carbon sources for denitrification produced from fermentation of raw and THP-treated sewage sludge and digestate. The aims were to determine the effect of THP on fermentation, denitrification and biogas loss after VFA extraction and to evaluate the potential of digestate as a substrate for VFA production compared with sludge. Additionally, we investigated the potential benefits of feeding a mix of THP sludge and raw sludge to the fermentation. A pilot-scale THP Cambi-pilot plant, two bench-scale fermenters, denitrification test reactors and biomethane potential (BMP) tests were used in the study. The results obtained were used in process calculations and preliminary cost estimates for Sweden's third-largest WWTP, Käppala (Stockholm), in 2028. Käppala WWTP is currently undergoing construction to increase capacity, which will increase the external carbon source requirement, so identifying the most beneficial scenario for producing an internal carbon source for denitrification is of great interest.

2. Materials and methods

2.1. Experimental design

Four different experiments were conducted, as shown in Fig. 1. Each experiment consisted of two fermenters (F1, F2). Fermenter F1 was used as a control and was fed with either raw digestate or a mixture of PS and WAS, while Fermenter F2 was fed with the corresponding thermally hydrolysed substrate [Cambi-Pilot]. After fermentation, the fermentates were centrifuged, resulting in a solid fraction and reject water containing sCOD and VFA (referred to hereafter as carbon source). The carbon source was used in denitrification activity tests, and the solid fraction was used in BMP tests. Thermally hydrolysed sludge (THP-sludge) and thermally hydrolysed digestate (THP-digestate) without fermentation were also centrifuged and evaluated as carbon sources.

In experiments 1 and 2, the fermenters were fed with a mix of PS+WAS (35:65 on a total solids basis; PS_{65}/WAS_{35}) with a final TS of 3.5 %. The same substrate mix was used in experiment 3 but with a higher final TS content (5 %), while digestate was used as the substrate in experiment 4. In experiments 1, 3, and 4, fermenter F2 was fed with 100 % THP-substrate, whereas in experiment 2, F2 was fed with a mixture of 50 % raw sludge and 50 % THP-sludge (volume-based) (Table 1). To validate the results from Cambi-Pilot, an additional experiment was performed with full-scale THP-sludge taken from a thermal hydrolysis Cambi process (HIAS, Norway) (results included in the supplementary material).

2.2. Substrates and inoculum

The PS, WAS and digestate used as substrates were collected weekly from Käppala WWTP. The anaerobic reactors used for sludge treatment and biogas production at Käppala WWTP, from which the digestate was taken, operate under mesophilic conditions (37 °C) and an average retention time of 20 days. This digestate was used as substrate in experiment 4 and as inoculum for all fermentation experiments and BMP tests. Activated sludge from the post-denitrification zone of Käppala WWTP and carriers from the end of the pre-denitrification MBBR zone at

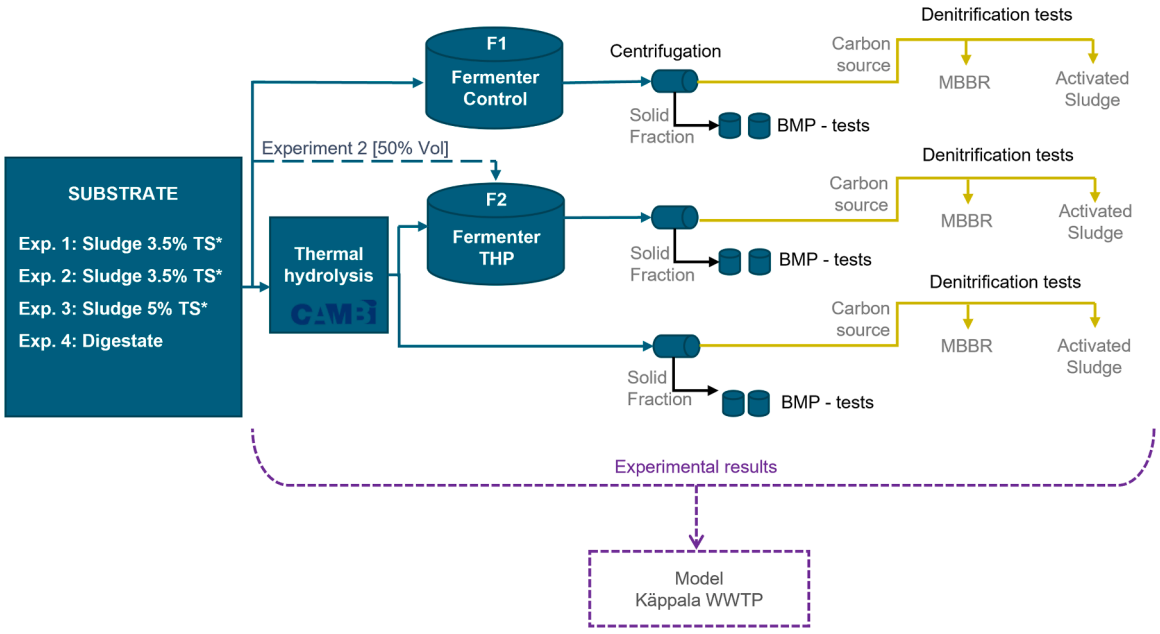

Fig. 1. Experimental scheme: Experiments 1–4. The process included thermal hydrolysis, fermentation, denitrification tests on the fermentation liquid and biomethane potential (BMP) tests on the solid fraction. It also included a control fermenter with no thermal hydrolysis. MBBR: moving bed biofilm reactor. * Mix 65 % PS:35 %WAS (TS based).

Margretelund WWTP (Åkersberga, Sweden) were used as inocula in the denitrification tests (the MBBR lines at Käppala are currently under construction and no samples were available). The characteristics of the substrates and inocula are summarised in Table 1.

2.3. THP pilot plant

THP mini-pilot plant (model 2 L/5 L; Cambi, Norway- Figure S1) was operated in batch mode at 165 °C and 6 bar for 30 mins (effective contact time). The pilot was preheated before each run and then fed with different substrates once the desired temperature was reached. It was operated once a week. The thermally hydrolysed samples were stored in the fridge at 4 °C until fed into the fermenters. In the last run of each experiment, additional samples were stored in a freezer at −18 °C to be used as a carbon source for denitrification and for the BMP tests.

2.4. Fermentation

The fermentation trials were performed in a bench-scale dolly reactor system (Belach Bioteknik AB) (Figure S1). Two twin reactors, each with a total volume of 8 L and an active volume of 6.15 L, were fed semi-continuously at 1.75 L per day, six days a week, reaching an HRT of 4 days. A 5 L gas-tight sample bag (SKC, USA) was used during the feeding of the reactors to prevent oxygen leakage and avoid impacting the gas flow meters. Gas samples were collected and sent for GC analysis to determine gas composition on days 2, 7, 10, 15, 17 and 18 of each experiment. Both were operated in mesophilic conditions at 37 °C. The inoculum was sourced from the AD at Käppala WWTP (Table 1). At the beginning of each trial, the fermenters were inoculated, and methanogen activity was inhibited by adding 2-bromoethanoesulfonic acid sodium salt to a final concentration of 30 mM, following the procedure described by Gong et al. (2021). The fermenters' organic loading rate (OLR) ranged between 7.3 and 11.0 g VS/L•d in all trials (Table 1). Each experiment operated for four HRTs plus an additional two days (18 days) to ensure a steady state. During the last three days of each trial, all chemical analyses were performed, and one additional sample per day was collected and frozen for subsequent denitrification and BMP tests (figure S2-monitoring plan).

In order to investigate the influence of pH, experiments 2 and 3 were each extended by an additional four days, and the pH was increased to 6 in all fermenters, using NaOH in experiment 2 and Na_2CO_3 in experiment 3. Only VFA and sCOD were measured during this additional period.

2.5. Denitrification tests

After the fermentation trials were completed, six samples were selected as the most suitable carbon sources for denitrification. The selected samples were centrifuged in the laboratory in a centrifuge (Megafuge 40, Thermo Fisher Scientific) at 4100 rpm for 5 min, and the reject water obtained was used as a carbon source. Batch denitrification activity tests were performed with activated sludge or MBBR carrier AnoxKaldnes k5 (800 m^2/m^3) as inoculum (Table 1). Tests with activated sludge were performed in 5-L reactors, following the methodology (test DEN.CHE.1) described by van Loosdrecht et al. (2016). Starting reactor concentrations were 25 mg NO_3–N/L and 165 mg sCOD/L, corresponding to a C/N ratio of 6.6 and a C/VSS ratio of 0.05–0.1 g COD/VSS. The sludge was not washed. For the tests performed with MBBR biofilm media, a carrier filling ratio of 45 % (same design in Käppala WWTP) was used in 5-L reactors, and initial concentrations were the same as those for activated sludge. The temperature was controlled at 20ºC, and the pH was adjusted to 7 after adding the carbon source in all experiments. The mixing was done with mechanical metal stirrers.

2.6. Biomethane potential tests

After centrifugation of the selected samples and removal of the liquid phase/carbon source, the solid fraction was evaluated in BMP tests (Fig. 1). Three sets of Automatic Methane Potential Test System II (AMPTS II) from BPC Instruments AB (Lund, Sweden) were used, each consisting of 15 glass bottles (500 mL) with 80 % active volume. The inoculum-to-substrate ratio was set to 3:1 (3.6 g $VS_{inoc.}$/1.2 g $VS_{subs.}$), and the organic load was 3 kg VS/m^3 in all tests. The volume was adjusted by the addition of distilled water. The tests were performed under standard mesophilic conditions (37 °C) for 30 days. The inoculum

Table 1
Characteristics of substrates and inocula for fermentation (experiments 1–4) and denitrification, as well as organic loading rates and inflows for fermentation. PS: Primary sludge, WAS: waste-activated sludge, TS: total solids, VS: volatile solids, TCOD: total chemical oxygen demand, sCOD: soluble COD, VFA: volatile fatty acids, TN: total nitrogen, NH4-N: Ammonium nitrogen, PO4-P: phosphate phosphorous, av: average and sd: standard deviation ($n = 9$ for all samples; except protein, lipids and carbohydrates where 2 samples of 1 batch were sent to the external laboratory).

		Raw substrate								Thermally hydrolysed substrate								Inoculum Fermentation [Digester]				Inoculum Denitrification [Activated sludge]	
Experiment		1		2		3		4		1		2		3		4							
		PS + WAS 3.5%TS		PS + WAS 3.5%TS		PS + WAS 5%TS		Digestate		TH 3.5%TS		50% TH Sludge 50% raw sludge 3.5%TS		TH 5%TS		TH Digestate							
Parameter	unit	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd			av.	sd
TS	[%]	3.6	0.5	3.7	0.1	5.4	0.4	5.8	0.4	3.6	0.4	3.8	0.3	5.8	0.0	5.8	0.2	3.1	0.0			0.22	0.02
VS	[% of TS]	83.3	1.0	83.2	0.7	82.2	0.8	67.2	0.7	83.1	1.1	82.3	0.9	83.6	0.1	68.2	1.7	64.6	1.2			0.18	0.01
TCOD	[g/L]	42.4	7.0	46.5	0.5	70.2	3.5	57.9	6.1	46.9	7.7	48.8	2.9	74.7	4.2	64.3	3.0	36.0	0.0			–	–
sCOD	[g/L]	1.5	0.2	1.3	0.2	2.5	0.9	0.0	0.1	10.7	0.5	4.6	0.3	12.3	1.3	24.1	0.6	0.9	0.2			0.1	0.0
VFA	[g/L]	0.9	0.1	0.8	0.1	1.4	0.4	0.1	0.0	1.5	0.1	1.1	0.1	1.5	0.5	3.4	0.6	0.6	0.1			–	–
TN (filtered)	[mg/L]	73	24	75	5	107	15	1	57	845	56	414	43	929	281	2 006	105	761	507			6.8	2.2
NH4-N	[mg/L]	52	16	45	2	71	49	802	73	127	26	73	7	109	13	395	18	748	283			0.2	0.5
PO4-P	[mg/L]	23	6	36	8	42	4	37	8	38	28	47	6	53	3	104	11	–	–			0.9	0.5
Protein	[mg g^{-1}VS]	295	–	316	–	365	–	454	–	322	–	308	–	320	–	484	–	–	–			–	–
Lipids	[mg g^{-1}VS]	120	–	135	–	146	–	62	–	138	–	127	–	141	–	60	–	–	–			–	–
Carbohydrates	[mg g^{-1}VS]	585	–	549	–	489	–	484	–	540	–	564	–	540	–	455	–	–	–			–	–

(Table 1) was degasified for seven days prior to the start of the test. Six samples from the fermentation step and one sample of untreated sludge [PS_{65}/WAS_{35}, TS-basis] were analysed. All samples were analysed in triplicate, and each BMP set had triplicate control samples (cellulose) and triplicate blank samples (inoculum).

2.7. Chemical analyses and analytical methods

Soluble and total COD, total nitrogen (TN), ammonium-nitrogen (NH_4-N), phosphate (PO_4-P) and VFA were analysed in triplicates using spectrophotometric cuvette tests from Hach (Germany). Before the cuvette tests were used, the samples were prepared by centrifugation and filtration through 0.45-μm acetate filters. In addition, to quantify VFA species (C1-C6) and lactic acid, the samples were further filtered (0.22 μm) and acidified 10 % 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). Protein (determined by organic nitrogen using the single factor: 6.25) and lipid content were analysed on sludge samples by an external laboratory (Agrilab AB, Sweden) where the following methods were used: TS determination - Standard Methods: 2540 B, total nitrogen modified method from SS-ISO 13 878, ammonium-nitrogen modified method AN 5226 based on ISO 11,732. Finally, carbohydrates were calculated using the remaining VS.

For statistical analysis, Welch's ANOVA was applied to the data, not assuming homogeneity of variance, to determine the significance of the response variables between scenarios ($p < 0.05$). Tukey's test was used to quantify differences between specific pairs of samples.

2.8. Käppala WWTP – case study

Käppala WWTP currently serves a population of 615 000 pe but is under reconstruction to increase the treatment capacity and meet new discharge nutrient requirements (TN <6 mg/L; total $P < 0.2$ mg/L). By 2028, the plant will serve an estimated 641,000 pe. The new design involves eleven separate treatment lines, nine with conventional activated sludge treating 68 % of incoming water and two with MBBR treating 32 %. All process reactors will have pre- and post-denitrification zones to achieve the nitrogen removal goal, requiring an external carbon source according to design calculations. Estimations of incoming water characteristics and seasonal variations were based on eight years of historical data, as well as design documentation provided by the consultant (Ramboll, Sweden). System boundaries for the calculations in this study included the primary sedimentation tanks, biology reactors (including nitrification and pre-post denitrification in both activated sludge and MBBR systems), secondary clarifiers and anaerobic digesters. Calculations and mass balances for assessing different carbon sources, effluent concentrations, sludge production and biogas production were based on design dimensions (Ramboll, Sweden), as well as specific activity rates, microbial performance, and other parameters from Tchobanoglous et al. (2014). The results obtained from the experimental phase of this study (THP, fermentation, denitrification, and BMP) were used to simulate scenarios using internal carbon sources, with methanol (design carbon source) as a benchmark for comparison. The Supplementary Material provides specific values and assumptions used in calculations and economic analysis and a description of Käppala WWTP.

3. Results and discussion

3.1. Thermal hydrolysis of mixed sludge and digestate

Three different substrates were subjected to THP in the Cambi-Pilot: one digestate sample and two mixed sludge samples with lower (3.5 % TS) and higher (5 % TS) total solids (Fig. 1). The highest sCOD

concentration after THP was obtained with the digestate (24 g sCOD/L), while corresponding values for the lower and higher TS sludge were 10.7 ± 0.5 and 12.2 ± 1.3 g sCOD/L, respectively. These values represented a carbon solubilisation degree of 23, 15 and 41 % for low and high TS sludges and the digestate, respectively (Table 1).

The sCOD increase after THP of sludge was similar to that observed in a previous study using full-scale THP treatment of mixed PS+WAS, where the solubilisation degree was 25 % (Zhang et al., 2019). However, Castro-Fernandez et al. (2023) found lower values (8 % solubilisation degree) also for mixed PS+WAS. The difference between the studies could be linked to different proportions of WAS in the substrate mixture. THP of WAS alone generally results in a higher solubilisation degree than when WAS is mixed with PS, resulting in a range of 19–49 % (Morgan-Sagastume et al., 2011; Zhou et al., 2021). A study by Yang et al. (2019) investigated a THP process with digestate and found 40 % carbon solubilisation and a final sCOD concentration of 15 g sCOD/L, which aligns with our results.

The higher carbon solubilisation post-THP, usually observed for digestate and WAS, may be attributable to differences in macromolecule composition. THP is known to have a more significant effect on proteins and carbohydrates, with lipids remaining unaffected (Li et al., 2022; Wilson and Novak, 2009). WAS is known to have higher protein and lower fat content than PS (Xiao and Zhou, 2020). In our digestate sample, proteins and carbohydrates accounted for 94 % of the VS, while in sludge samples, they accounted for 85–88 % (Table 1). This likely explains the higher solubilisation observed for digestate, as also reported by Svensson et al. (2018). Further, decomposition of amino acids to VFA, which is typically promoted at temperatures above 190 °C (Chen

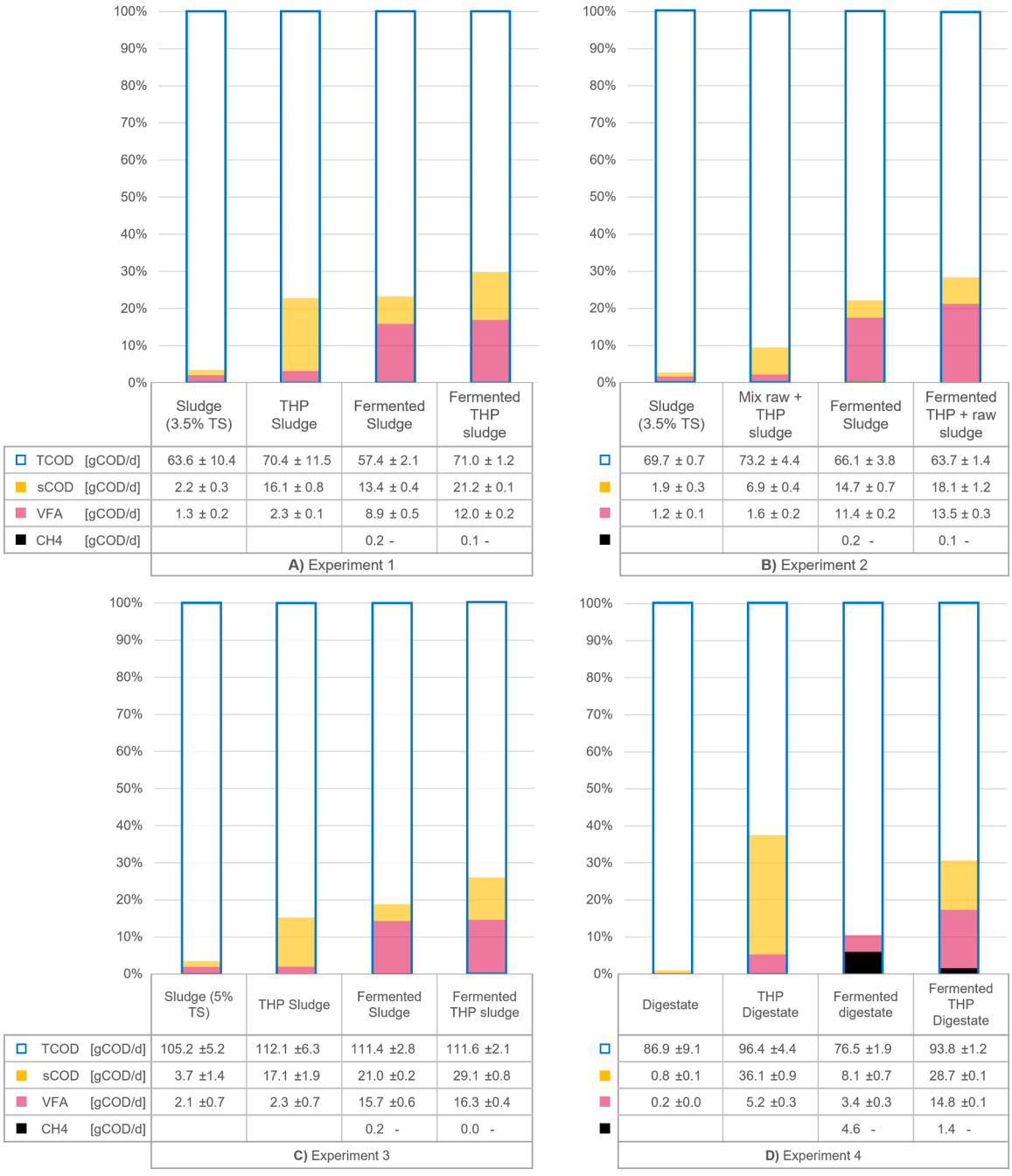

Fig. 2. Carbon characteristics presented as load [gCOD/d], including total (T-) and soluble (s-) chemical oxygen demand (COD), volatile fatty acids (VFA) and methane (CH_4) in a) experiment 1, b) experiment 2, c) experiment 3 and d) experiment 4. $n = 9$ for SD.

et al., 2019; Körner, 2021), seemed to have occurred at 165 °C, as indicated in this study by the increase in VFA concentration in post-THP samples (before fermentation). The VFA concentration in THP-digestate, which had the highest protein content of the substrates tested, was twice as high (3.4 g VFA_{COD}/L after THP) as in the THP-sludge samples (1.1–1.5 g VFA_{COD}/L) (Table 1).

3.2. Influence of THP on acidogenic fermentation of sludge and digestate

3.2.1. Solubilisation – effect on carbon

The degree of carbon solubilisation during fermentation was generally low relative to that following THP alone (Fig. 2, Table 2). Higher carbon solubilisation was observed after fermentation of the untreated substrates (controls) compared with the THP-substrates, which was expected and supported previous findings (Zhang et al., 2019). However, acidogenic fermentation of THP-sludge and THP-digestate yielded higher total concentrations of soluble carbon and VFA compared with the controls (no THP) in all experiments (Fig. 2). Total carbon solubilisation degree, including THP plus fermentation of sludge, ranged from 24 to 31%, whereas in the controls (fermentation of untreated sludge), it was 16–18 % (Table 2). Fermentation of THP-digestate resulted in the highest total solubilisation degree (39.1 ± 0.1%) despite methane formation (Fig. 2) and a decrease in sCOD concentration during fermentation (from 24.1 ± 0.6 to 19.2 ± 0.1 g sCOD/L). Methane was also formed in the control fermenters in experiments 1–3 but at a lower level than in experiment 4 with digestate (Fig. 2). The formation of methane and pH are discussed later in Section 3.2.3.

During fermentation, significantly greater carbohydrate degradation was observed in the fermenters fed with THP-sludge (experiments 1–3) compared with the controls fed with untreated sludge (Fig. 4a). In contrast, experiment 4 (THP-digestate) showed low to no degradation of carbohydrates, with protein being the only macromolecule degraded (Fig. 4a). This differs from a previous study on the fermentation of THP-WAS, which, in spite of high protein content, showed high carbohydrate degradation (Shana et al., 2013). Lipids (<13 % of VS in all substrates) showed the lowest degree of degradation during fermentation. Lipid degradation is usually slow and requires longer retention times than carbohydrates and proteins (Law et al., 2023), but the fact that lipids were not reduced in the fermentation step is beneficial to the downstream biogas production (Section 3.4).

In experiment 2, fermenter F2 was fed with a 50:50 mixture of raw sludge and THP-sludge, which was expected to benefit the fermentation process. The results indicated that soluble carbon concentration and carbon solubilisation degree were only slightly lower in this fermenter (12.1 ± 0.1 g sCOD/L and 24 %, respectively) than in the fermenter fed with 100 % THP-sludge in experiment 3 (14. 1 ± 0.8 g sCOD/L and 31 %, respectively) (Table 2). These results show that reducing THP reactor volume by 50 % would decrease carbon solubilisation by only 25 % during fermentation. The possible influence of refractory products on fermentation is discussed in Section 3.2.2.

3.2.2. VFA yield

Despite the increase in the total degree of carbon solubilisation in all fermenters fed with THP-substrate compared with their controls, the VFA yield of the fermenters fed with THP-sludge in experiments 1 and 3 was not significantly higher than in their control fermenters ($p = 0.60$ and $p = 0.56$, respectively), indicating low conversion efficiency of solubilised organic matter to VFA (Fig. 3). However, in experiment 4, fermentation of THP-digestate resulted in a significantly ($p < 0.001$) higher yield (235 ± 2 g VFA_{COD}/kg VS_{in}) than the corresponding control, leading to a concentration 9.9 ± 0.9 g VFA_{COD}/L. The use of the mixed 50 % raw and 50 % THP sludge in experiment 2 was also beneficial for the fermentation process, yielding a significantly ($p = 0.01$) higher VFA (283 ± 7 g VFA_{COD}/kg VS_{in}) than the control (248 ± 4 g VFA_{COD}/kg VS_{in}).

The low VFA yield in experiments 1 and 3 contradicts the findings by Morgan-Sagastume et al. (2011) and Zhang et al. (2016), who reported that THP of sludge increases VFA yield during fermentation compared with untreated sludge. However, the pH in those studies was between 6.0 - 6.5, compared with 5.1 and 5.4 in our sludge fermenters, which

Table 2
Characteristics during the stable period of fermenters fed with untreated (F ctrl) and thermal hydrolysis pre-treated (THP) substrates in experiments 1–4. PS: Primary sludge, WAS: waste-activated sludge. ($n = 9$ for all samples; except protein, lipids and carbohydrates where $n = 3$ – samples taken in three different days.).

		Fermented Raw substrate (control)								Fermented THP substrate							
Experiment		1		2		3		4		1		2		3		4	
	Substrate	PS+WAS 3.5%TS		PS+WAS 3.5%TS		PS+WAS 5% TS		Digestate		TH 3.5%TS		50% TH Sludge 50% raw sludge 3.5%TS		TH 5%TS		TH Digestate	
Parameter	unit	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.	av.	sd.
TS	[%]	2.7	0.1	3.1	0.1	5.2	0.2	4.9	0.1	3.3	0.0	3.0	0.0	5.5	0.4	5.4	0.1
VS	[% of TS]	81.9	0.4	83.0	1.7	81.9	0.3	66.5	0.2	82.3	0.5	80.0	0.4	81.0	1.4	68.4	0.3
TCOD	[g/L]	38.2	1.4	44.0	2.5	74.3	1.9	51.0	1.3	47.3	0.8	42.5	0.9	74.4	1.4	62.5	0.8
sCOD	[g/L]	8.9	0.3	9.8	0.5	14.0	0.1	5.4	0.5	14.1	0.1	12.1	0.8	19.4	0.5	19.2	0.1
VFA	[g/L]	5.9	0.3	7.6	0.1	10.5	0.4	2.2	0.2	8.0	0.1	9.0	0.2	11.0	0.3	9.9	0.1
TN (filtered)	[mg/L]	500	43	618	58	1 038	127	1 711	28	915	10	800	69	1 330	106	2 102	113
NH4-N	[mg/L]	430	21	447	20	871	143	1 301	206	582	8	604	11	856	103	1 642	72
PO4-P	[mg/L]	178	4	197	11	203	17	118	5	217	1	187	5	268	21	208	6
Protein	[mg g^{-1}VS]	333	13	311	13	289	2	410	8	313	14	334	8	316	8	373	7
Lipids	[mg g^{-1}VS]	134	16	141	8	158	2	63	0	160	3	143	12	162	4	70	1
Carbohydrates	[mg g^{-1}VS]	533	3	548	21	553	4	527	8	527	17	522	21	522	6	557	6
OLR fermenter	[kgVS/d]	7.3	0.9	7.4	0.2	11.0	0.7	9.4	0.7	7.4	1.0	7.3	0.0	11.8	0.1	9.6	0.5
Inflow fermenter	[L/d]	1.5	0.0	1.5	0.0	1.5	0.0	1.5	0.0	1.5	0.0	1.5	0.0	1.5	0.0	1.5	0.0
Solubilisation degree Thermal hydrolysis	[% - sCOD/ TCOD in]	–	–	–	–	–	–	–	–	22.6	–	22.6	–	14.5	–	41.0	–
Solubilisation degree Fermenter	[% - sCOD/ TCOD in]	18.2	0.7	18.72	1.1	16.5	0.3	10.3	1.0	12.9	0.2	17.4	1.8	12.1	2.7	0 (−15.6)	0.2
Solubilisation degree TH + Fermenter	[% - sCOD/ TCOD in]	18.2	0.7	18.72	1.1	16.5	0.3	10.3	1.0	31.0	0.2	23.8	1.8	24.4	1.1	39.1	0.1

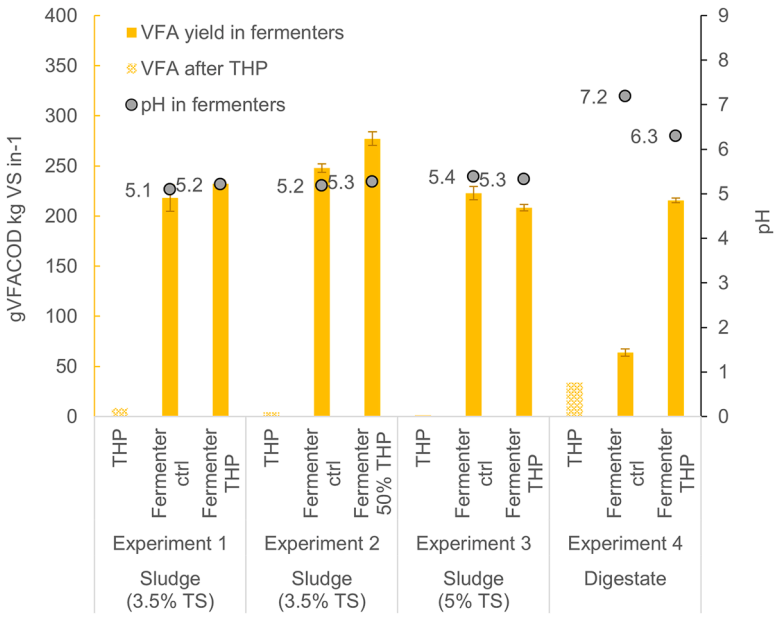

Fig. 3. Volatile fatty acid (VFA) yield and pH in the fermenters run in experiments 1–4, which were fed with different sludges (1–3) and digestate (4), un-treated (ctrl) or pre-treated with thermal hydrolysis (THP).

possibly affected the overall sCOD conversion efficiency (Fig. 3). The importance of pH is discussed in Section 3.2.3.

Another potential explanation for the low VFA yield could be attributed to the Maillard reaction, which has been suggested as a cause for the ineffectiveness of THP for enhanced fermentation and VFA production (Castro-Fernandez et al., 2023; Yan et al., 2022). Maillard reaction products (MRPs), such as melanoidins and other refractory dissolved organic compounds, are formed from the reaction of reducing sugars with amines triggered by increasing temperatures above 140 °C (Ngo et al., 2021; Wilson and Novak, 2009). MRPs can be toxic to biochemical processes, including anaerobic digestion (Q. Wang et al., 2021). In Experiment 2, the highest VFA yield was observed while the fermenter received only half of the substrate from THP, likely diluting the inhibitory compounds. Similary, in Experiment 4, the fermenter fed with THP-digestate resulted in a significantly higher VFA yield than the control, even after accounting for methane production. This outcome suggests that the limited degradation of carbohydrates (as seen in Fig. 4a) may have reduced the likelihood of the Maillard reaction occurring. Additionally, Experiments 1 and 3, which involved sludge with higher carbohydrate content, showed no significant difference in VFA yield compared to their controls, further supporting the hypothesis that inhibition may be linked to the presence of certain compounds generated during the THP.

The dominant carboxylic acids observed were acetic and propionic acids, with lower levels of longer carbon-chain acids in all experiments (Fig. 4a). In contrast, Xiang et al. (2023) observed a pattern of increase in acetate and a simultaneous decrease in propionate during the fermentation of THP-sludge, compared to untreated sludge. Specific

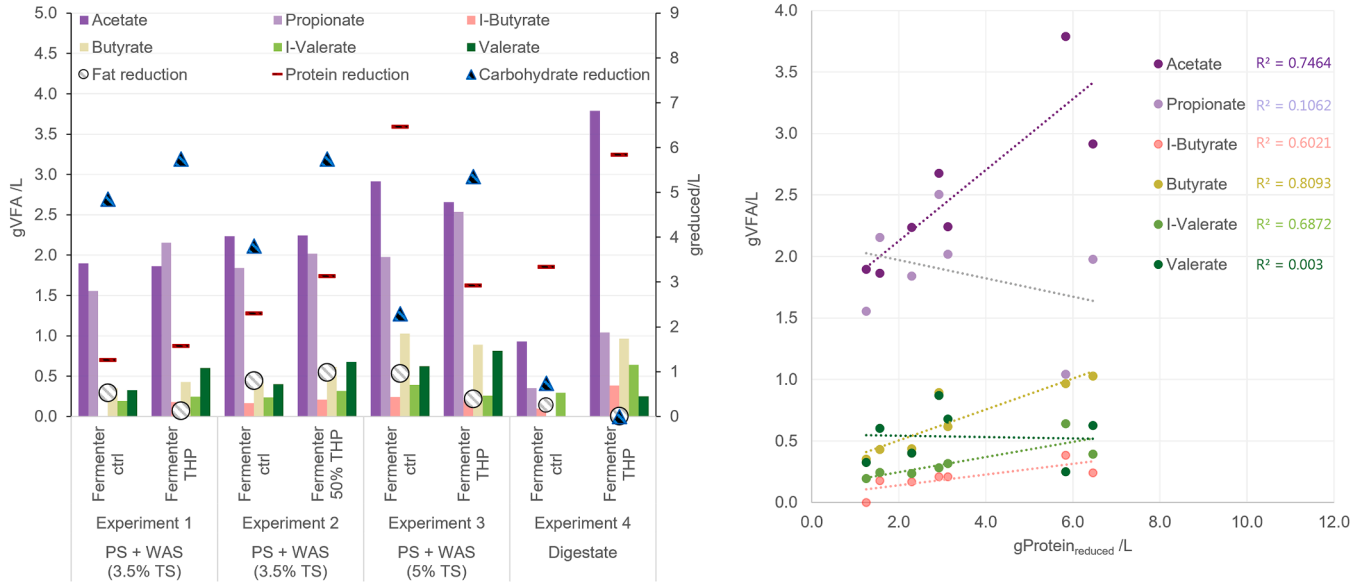

Fig. 4. a) Carboxylic acids (C2-C5), protein reduction (red dashes), carbohydrate reduction (blue triangles) and lipid reduction (grey circles) in fermenters fed with different sludges (experiments 1–3) and digestate (experiment 4), un-treated (ctrl) or pre-treated with thermal hydrolysis (THP). b) Yield (g/L) of volatile fatty acids (VFA) versus reduction in proteins, using results from all fermentation experiments.

VFA production in the fermentation of mixed waste streams is a complex process involving a dynamic community of microorganisms with somehow unpredicted behaviour (Regueira, Lema, et al., 2020). Generally, the VFA species formed during fermentation seem to be determined mainly by the composition of the substrate (Regueira, Bevilacqua, et al., 2020), degradation of macromolecules and operating conditions, and not by the THP per se (Liu et al., 2022; Morgan-Sagastume et al., 2011; Zhang et al., 2019).

Our results suggest that acetate production is mainly linked to protein degradation. This is supported by the observation that acetate concentrations were highest in fermenters with the greatest extent of protein degradation. Additionally, a linear positive correlation was observed between levels of acetic and butyric acid and protein reduction (R^2 >0.75) (Fig. 4b). While protein degradation does not follow fixed stoichiometry, acetate has been identified as the main degradation product. The yield ratio of acetate to protein (g Ac/g Prot) tends to increase with higher pH (Regueira, Lema, et al., 2020). This trend was also evident in our results (Fig. 4b). No other correlations were seen between the reduction in macromolecules and the production of specific acids. However, carbohydrate degradation appeared to follow propionate and valerate formation in experiments 1–3 (Fig. 4a), similar to findings by Rafay et al. (2022).

3.2.3. Influence of pH

The fermentation inoculum (digestate from Käppala WWTP) had an initial pH of 7.1 ± 0.2 (Table 1). During the first 5–10 days of fermentation, the pH decreased and stabilised between 5.1 and 5.4 in experiments 1–3 (Fig. 3). Detailed pH, VFA and sCOD profiles throughout the experiments are included in the supplementary material (Figure S3). pH plays a crucial role in the acidogenic fermentation process because carboxylic acids, including VFA, are weak acids (pKa 4.75–4.90). At lower pH, a higher proportion of VFA exists in their undissociated form, negatively affecting microbial growth (Infantes et al., 2012). A pH between 5.5 and 7.0 is beneficial for higher VFA yields during fermentation (Agnihotri et al., 2022).

To determine if the low VFA yields in experiments 2 and 3 resulted from low pH rather than poor substrate biodegradability (i.e. MRPs), the pH was adjusted to 6 after the fermentation trials ended on day 19. The fermentation was continued for an additional 4 days. An immediate increase in sCOD and VFA concentrations was seen in both experiments. Specifically, in experiment 2, the sCOD concentration increased by 35 % [F1-control] and 23 % [$F2_{50\ \%}$raw and $_{50\ \%}$THP]. In experiment 3, the increases were 28 % [F1-control] and 2 % [F2 THP-sludge]. The increase in VFA yield was greater in the fermenters fed with raw sludge (45 % and 19 % in experiments 2 and 3, respectively) than in those fed with THP-substrate in those experiments (31 % and 19 %, respectively). The results suggest that increasing pH had an immediate positive effect on the solubilisation of carbon and VFA production, especially in the fermenters with untreated sludge. However, the process was only operated for one retention time, and longer-term experiments are needed to confirm these indications.

In experiment 4, the pH stabilised at 6.3 in the fermenter fed with THP-digestate (without pH control), which led to increased methanogen activity while still allowing the accumulation of VFAs (Fig. 3). In contrast, the pH in the control fermenter (untreated digestate) was higher (7.2), favouring methanogenesis and converting most VFA to methane (Fig. 2d, Fig. 3). The observed methanogen activity (215 mL CH_4/kg VSin) could also have been influenced by the feed of untreated digestate, which likely contained active methanogens.

3.3. Performance of carbon sources in denitrification

Based on the results obtained during THP and fermentation, six samples were chosen for evaluation as carbon sources in denitrification tests (Fig. 1). The selection criteria were the levels of VFA and soluble COD. One sample from experiment 2 (fermented $THP_{50\ \%}$), all three samples from experiment 3 and two samples from experiment 4 (THP-digestate with and without fermentation) were selected. Comparing denitrification rates between studies is difficult due to variations in microbial communities and operating conditions, so reference substances are commonly used to contextualise results (Elefsiniotis et al., 2004; Grana et al., 2024). In this study, acetic acid was used as the reference, which resulted in a denitrification rate of 8.1 mg NOx-N/g VSS•h in activated sludge (Fig. 5) and 0.5 g NOx-N/m^2•d in MBBR. The denitrification profiles can be found in the supplementary material (S4-S10).

The results obtained for the six carbon sources tested (Fig. 6) suggested a correlation between the VFA/sCOD ratio and denitrification rate (R^2= 0.702). This is consistent with the notion that carbon sources with simpler molecular structures, e.g. VFAs, are preferred by heterotrophic denitrifiers due to their ability to produce electrons and energy more efficiently, thereby promoting the denitrification process (Elefsiniotis et al., 2004; Wei et al., 2022).

The lowest denitrification rates (<5 mg NOx-N/g VSS•h) were observed with carbon sources produced by non-fermented THP substrates (both sludge and digestate) (Fig. 5). Despite the high soluble carbon content, these had low VFA concentrations and hence low VFA/sCOD ratio. This suggests that the sCOD was composed mainly of longer chain products, including soluble protein, reduced sugars and humic acid substances, that were not converted to VFA, and cannot be used as ready biodegradable material (Chen et al., 2024). Moreover, despite a decrease in nitrate concentrations, no reduction in sCOD was seen during these denitrification tests (Figure S6 and Figure S9 – Supplementary material), indicating simultaneous production and consumption of organic matter, as reported previously for similar carbon sources (Guo et al., 2017).

The fermentation liquid from the control fermenter in experiment 3 displayed a significantly *($p = 0.004$)* higher rate (6.8 mg NOx-N/g VSS•h) than the hydrolysis liquid from unfermented THP-sludge (4.6 mg NOx-N/g VSS•h). This result aligns with previous research indicating that thermal hydrolysis liquids perform less effectively than fermentation liquids as carbon sources for denitrification (Guo et al., 2017; Sun et al., 2016). The carbon sources from fermented sludge and unfermented THP-substrates resulted in significantly ($p = 0.01$) lower rates compared to the control acetic acid. However, contrary to our results, some studies have reported higher denitrification rates with fermentation liquid (Liu et al., 2016) and THP liquid (Barlindhaug and Ødegaard, 1996) than with methanol, acetic acid and propionic acid.

The carbon sources produced by fermentation of THP-substrates (F2 THP) achieved the highest denitrification rates in all experiments. Specifically, fermented THP-digestate and fermented 50:50 mixed raw-THP sludge resulted in 8.8 and 8.7 ± 0.3 mg NOx-N/g VSS•h, respectively, followed by fermented THP sludge (7.6 ± 0.8 mg NOx-N/g VSS•h) (Fig. 5). No significant difference was found between these rates and the acetic acid reference ($p = 0.51$). These results indicate that the addition of THP prior to fermentation improved the quality of the carbon source produced. Additionally, in experiment 3, the denitrification rate using fermented THP-sludge was 13 % higher than that of the control fermented untreated sludge and 66 % higher than for THP-sludge without fermentation. Despite the lack of impact of THP on VFA yield (Fig. 3), it positively influenced the denitrification rates obtained.

Interestingly, THP-fermented digestate, despite its low VFA/sCOD ratio (<0.5), achieved one of the highest denitrification rates (Fig. 5). To our knowledge, no previous study has investigated fermented THP-treated digestate as a carbon source for denitrification. One contributing factor to the high rate of this substrate could have been the higher acetate and butyrate concentrations in the sample. Previous studies have shown that acetic and butyric acid led to higher denitrification rates than other acids (Elefsiniotis and Wareham, 2007; Grana et al., 2024). However, the preference for single VFAs seems to be dependent on the microbial community and not on electron transfer efficiency alone (Li

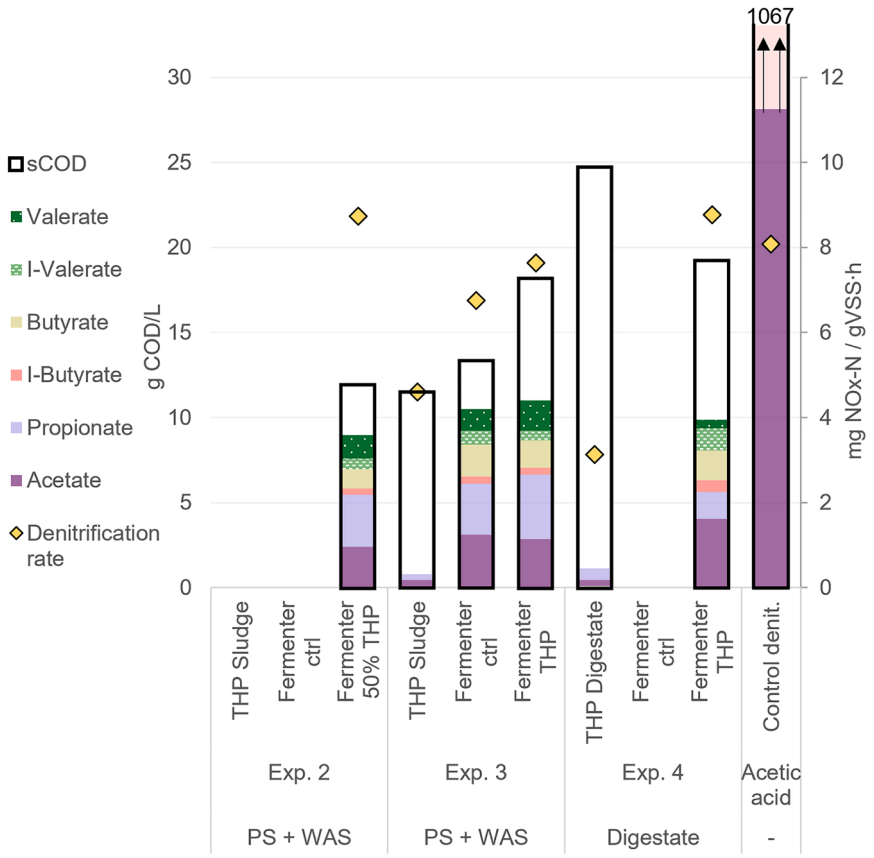

Fig. 5. Carboxylic acids (C2-C6; g COD/L), soluble chemical oxygen demand (sCOD, black rectangles, g/L) and denitrification rates (yellow diamonds, mg NOx-N/g VSS•h) for carbon sources produced during fermentation of sludges and digestate, untreated (Ctrl) and THP. Experiments 2–4. Acetic acid sCOD=1067 g/L (reference).

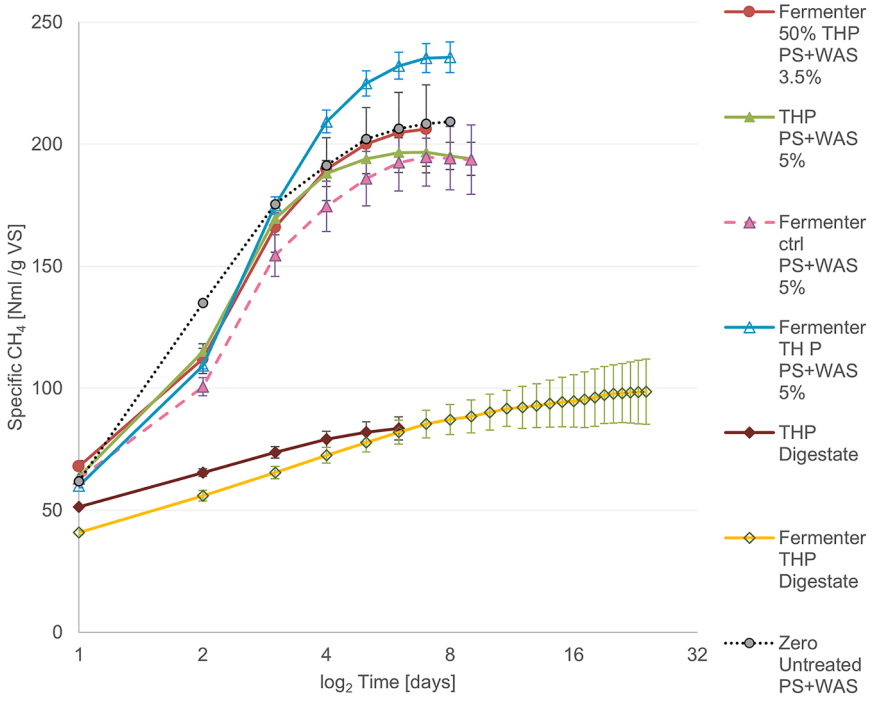

Fig. 6. Biomethane potential (BMP) from sludge solid phase samples recovered after centrifugation of fermentates from the 4 experimental trials performed in the present study (Fig. 1) and THP-treated sludge and digestate.

et al., 2023).

Protein degradation during fermentation was correlated with the denitrification rate (R^2=0.7), likely due to the higher acetate and butyrate production resulting from this degradation. Another contributing factor to the high rate of fermented THP-digestate could be the relatively low biomass growth yield obtained ($Y_{OHO\ gCOD/gCOD}$), which leaves more carbon source available for denitrification (Zhang et al., 2016).

The nitrate removal efficiency exceeded 96 % with fermented THP-substrates, while the unfermented THP-based carbon sources achieved ~70 %. However, the presence of other nitrogen fractions (e.g. NH_4-N and organic nitrogen) in the carbon sources reduced total nitrogen removal efficiency to 75–88 % with fermented THP-substrates and to below 50 % with unfermented THP substrates (Table 2). The release of NH_4-N and PO_4-P during the fermentation of proteins and carbohydrates is a significant concern when using carbon sources from sludge fermentation for denitrification. Nevertheless, process calculations in this study (see Section 3.4) showed that the effluent limits were met with all carbon sources.

Finally, two denitrification tests with MBBR as inoculum were performed with acetic acid and with the carbon source from fermented THP-digestate. They achieved denitrification rates of 0.4 and 0.5 gNOx-N/m^2•d, respectively ($p = 0.003$), which despite being in the low range (Owusu-Agyeman et al., 2023; Sapmaz et al., 2022), indicated comparable performance to that obtained with AS. The results reveal that digestate-based carbon sources had similar or higher efficacy than acetic acid in both inocula.

The high rate obtained using the fermented THP-digestate is a novel finding and holds promise for the integration of a process to produce carbon sources at WWTP. Additionally, the fermentate from 50:50 mixed raw and THP sludge showed a high denitrification rate (Fig. 6), illustrating the potential for reducing energy consumption and investment costs by requiring 50 % of the THP system volume compared to the other scenarios (Fig. 1).

3.4. Downstream biogas production

In the full-scale installation, the solid phase obtained after centrifugation and removal of the VFA-rich carbon source was assumed to be used for biogas production. To simulate this process and evaluate biogas loss by removal of VFA, the methane potential of the solid phase samples was determined in BMP tests (Figure S9). In total, one sample of mixed raw sludge and the solid phase of the six samples from which the liquid phase was used in the denitrification tests (Fig. 1) were evaluated. The methane produced by the non-centrifuged mixed raw sludge represented the zero scenario when calculating biogas loss.

All samples reached their maximum methane potential within 10 days after initiation of the BMP test, except for the fermented THP-digestate, which continued to produce methane until day 25 (Fig. 6). The slower methane production rate in that sample suggested the presence of more complex organic matter. Digestate is known to contain high residual protein coupled with recalcitrant material, such as collagen, which is known to be difficult to degrade (Ekstrand et al., 2022). The mixed untreated raw sludge (zero) sample achieved a BMP of 209 NmL CH_4/g VS, which is within the normal range for mixed PS and WAS (160–350 NmL CH_4/g VS) (Schnürer and Jarvis, 2010). No significant difference was found between the BMP from the zero scenario and that from the samples originating from fermented $THP_{50\ \%}$, THP-sludge and untreated fermented sludge ($p = 0.9$) (Table 3). In other words, methane yield (Nm^3/kg VS) was not lower for any of the mixed sludge samples where the VFA-rich liquid phase had been extracted compared with the zero scenario, regardless of the method of VFA production. The fermented THP-sludge sample exhibited a higher BMP value (236±6 NmL CH_4/g VS) than the zero scenario. While the difference was not significant ($p = 0.06$), it indicated that when mixed sludge was subjected to both THP and fermentation, the methane yield per g VS could increase, even after extracting VFA. One influencing factor for the results was that the lipid share of VS was higher in the fermented sludge and the fermented THP-sludge (with the highest BMP) than in the other samples. This was indicated by a positive correlation (R^2=0.9421) between the lipid content in mg g^{-1} VS in the fermentates (Table 2) and their methane potential (Table 3). Lipids have the highest energy yield of all macromolecules (1.014 L CH_4/g VS), compared with 0.496 L CH_4/g VS for proteins and 0.415 L CH_4/g VS for carbohydrates (Cirne et al., 2007; Magdalena et al., 2018).

The sample from the fermented THP-digestate, which exhibited the highest denitrification rate, had a yield of 101 mL CH_4/g VS, while the sample from unfermented THP-digestate had a yield of 84 mL CH_4/g VS. Similarly, Svensson et al. (2018) obtained BMP of around 50 mL CH_4/g TS (VS 68 %) for dewatered digestate cake exposed to THP (165 °C). These results demonstrate the potential of utilising the digestate for carbon source production without reducing biogas production, as the substrate is taken after AD. Additionally, recirculating part of the solid fraction could even increase biogas production.

3.5. Evaluation of carbon sources

The experimental results were used as a base for scaling-up calculations to determine potentially feasible scenarios at Käppala WWTP. Evaluation criteria included carbon solubilisation, VFA yield, denitrification rate, sCOD/TN ratio, sCOD/TP ratio and BMP (Table 3). Among the scenarios considered, the best overall results were obtained for: fermented 50:50 mixed raw and THP sludge in experiment 2; fermented sludge and fermented THP-sludge in experiment 3; and fermented THP-digestate in experiment 4 (marked * in Table 3). These scenarios were further assessed in process calculations and full-scale implementation

Table 3
Summary of results of the different scenarios, including Carbon solubilisation, VFA yield, Denitrification rate, C/N ratio, TP ratio and BMP. TN: total nitrogen, TP: total phosphorous. The red numbers in the upper right corner represent the ranking of scenarios used to select the most suitable. $n = 9$.

	Carbon Solubilisation		VFA yield		Denitrification rate		sCOD/ TN	sCOD/ TP	BMP	
	[g sCOD/TCOD in]		[g VFACOD/kg VS in]		[mg NOx-N/g VSS.h]		–	–	[mL CH_4 /g VS]	
Fermented * 50 % THP PS+WAS Exp. 2	**3**		**5**		**5**		**3**	**4**	**4**	
	23.8	± 1.8	279	± 7	8.7	± 0.3	15.1	61.8	206	± 18
THP PS+WAS Exp. 3		**1**		**1**		**2**	**2**	**4**		**4**
	14.7		1.1		4.6	± 0.02	10.0	72.8	197	± 6
Fermented * ctrl PS+WAS Exp. 3		**2**		**3**		**3**	**3**	**4**		**4**
	15.5	± 0.3	223	± 6	6.8	± 0.1	12.9	63.6	195	± 12
Fermented * THP PS+WAS Exp. 3		**3**		**3**		**3**	**3**	**4**		**5**
	22.3	± 1.1	208	± 3	7.6	± 0.8	13.8	69.6	236	± 6
THP Digestate Exp. 4		**5**		**2**		**1**	**2**	**5**		**5**
	41.0		34.1		3.1	± 0.3	11.4	112.8	84	± 5
Fermented * THP Digestate Exp. 4		**5**		**4**		**5**	**1**	**4**		**5**
	39.1	± 0.1	235	± 2	8.8	± 0.4	9.1	87.9	101	± 7

for Käppala WWTP in 2028.

3.6. Upscaling results - Käppala WWTP 2028

Käppala WWTP has a set yearly average discharge limit of 6 mg TN/L. In this study, projected data for Käppala 2028 were used for assessment, including an average inflow of 1.97 m^3/s, with 32 % entering the MBBR reactors and 68 % the activated sludge reactors. The inflow characteristics were: BOD_5 60 g/p.d, Tot-N 12.8 g/p.d, Tot-P 1.6 g/p.d, SS 90 g/p.d and alkalinity 88 g/p.d, and monthly variations were calculated using historical data from 2013 to 2020. All input data are presented in Supplementary Material. A summary of the main results is presented in Table 4.

In the base scenario with methanol, the estimated early average nitrate removal requirement in the post-denitrification at Käppala was 629 kg NO_3/d, consuming on average 3 m^3 MeOH/d with a dose of 18 gCOD/m^3 incoming water. This consumption corresponded to a cost of EUR 0.3–1 million per year, depending on methanol prices.

To estimate the amount of internal carbon source needed for nitrate reduction, the concentrations of sCOD and VFA, the NH_4-N load accompanying the carbon source, and the denitrification rates were considered. The nitrate load to be removed increased in all scenarios compared with the base (methanol) scenario due to higher NH_4 (Table 2). The volume of carbon source required to reach 6 mg TN/L ranged between 382 and 708 m^3/d and was ~200 times higher than that of methanol (Table 4). The scenario with fermented THP-digestate had an estimated requirement of 517 m^3/d, which was relatively high due to the high ammonium content in the carbon source. Furthermore, the dose (g COD/m^3 incoming water) was higher in all internal carbon sources scenarios than in the base scenario, meaning that more carbon was used overall. One explanation is the extra nitrogen in the system, but it should also be noted that anoxic growth yield (Y_{OHO}) is lower in methanol than in VFA or other carbon sources (Tchobanoglous et al., 2014).

The amount of substrate required to produce each carbon source was estimated to evaluate the feasibility of internal production at Käppala WWTP. The estimates were based on VFA yield (g VFA/kg VS_{in}) and any VS losses during THP and fermentation, as obtained in the bench-scale fermenter trials. The activated sludge produced with different carbon sources was based on bacterial growth yield (Y_H) and carbon source addition (Table 4). The WAS level increased in all scenarios by between 0.5 and 1.9-tonVS/d (3 % and 11 %), compared with the base scenario. Furthermore, with a fermenter operating with HRT of 4 d, the sludge requirement in scenarios using substrates from experiments 2 and 3 was 46–60 % of total sludge production at Käppala, which can be considered feasible (Table 4). On the other hand, the volume of digestate seemed to be insufficient to replace all the carbon source required, as calculations showed a need for 120 % digestate. However, using digestate would considerably reduce the costs of sludge handling, which for Käppala WWTP accounted for EUR 2.3 M in 2022. Since transportation of centrifuged digestate accounts for a large part of the operating costs (OPEX) of WWTPs, a reduction in the amount could have a great impact on the general costs.

Loss of biogas was calculated using the BMP of the different carbon sources obtained in the tests, and the loss of VS due to THP, fermentation and separation of VFA-based carbon source (Table 3). The estimated total biogas production in Käppala 2028 with methanol (not using any sludge for VFA production) was an average of 13,209 Nm^3 CH_4/d. The use of sludge for the production of internal carbon sources led to a reduction in biogas production: 15 % with fermented 50:50 mixed raw and THP-sludge, 20 % with fermented untreated sludge, and 9 % with fermented THP-sludge. If the digestate were used, the reduction of biogas would be 0 %, and there could even be an increase in biogas production, e.g. by recirculating back 50 % of the residue and producing an extra 1351 Nm^3 CH_4/d (Table 4).

Energy consumption in a thermal hydrolysis system usually varies between 17 and 34 kWh/m^3, but in most cases, it has a positive impact on the overall energy balance of a WWTP and could even be energetically self-sufficient (Ferrentino et al., 2023). Due to the complexity of the current energy flows at Käppala, we did not include THP energy calculations in this study. In terms of costs, without including capital costs, using fermented THP-digestate would be beneficial, even compared with the base scenario. The extra biogas produced (+0.4 M €/year - Table 4), in addition to the reduction in methanol costs (−0.3 M €/year), shifted the balance towards this scenario (Table 4). However, as mentioned, the nitrogen content is a concern, and longer-term trials should be performed to evaluate this scenario fully. Finally, a more extensive economic assessment, including capital costs, will facilitate the decision-making.

Analysis of an approach for producing internal carbon sources to replace methanol in Käppala WWTP by 2028 indicated the benefits of including THP before acidogenic fermentation without exponentially increasing the OPEX. The sludge scenarios analysed could be

Table 4
Results from process calculations and costs associated with scaling-up calculations for the 4 selected scenarios. The red values represent an optional recirculation of digestate. Methanol price=444€/m^3.

	Unit	Methanol	Fermented 50 % THP PS+WAS Exp. 2	Fermented ctrl PS+WAS Exp. 3	Fermented THP PS+WAS Exp. 3	Fermented THP Digestate Exp. 4
Nitrate requirement	Ton NO_3–N /d	0.6	1.1	1.0	1.0	1.5
CS requirement	m^3/d	3	708	491	382	517
Dose	gCOD/m^3 incoming water	18	55	47	46	57
PS production	Ton VS/d	33	33	33	33	33
WAS production	Ton VS/d	17	19	17	17	18
PS+WAS production	Ton VS/d	50	52	51	50	51
Digestate production	Ton VS/d	25	26	25	25	25
Sludge requirement for CS production	Ton VS/d	–	31	32	24	–
Digestate requirement for CS production	Ton VS/d	–	–	–	–	31
	VS% av TS					
	Ton TS/d		39	40	31	46
% of total produced	%	0 %	59 %	63 %	48 %	123 %
Volume fermenter	m^3	240	5 107	2 989	2 185	3 349
Methane production	Nm^3 CH_4/d	13 209	11 262	10 588	12 046	13,209 + 1351
Cost methanol	M euros/ year	−0.3	0	0	0	0
Biogas revenue	M euros/ year	4.3	3.6	3.4	3.9	4.3 + 0.4
Cost of sludge handling	M euros/year	−3.6	−3.7	−3.7	−3.6	−3.7
Total	M euros/year	0.3	−0.1	−0.2	0.3	0.6 + 0.4

implemented in practice to internally produce enough carbon source for denitrification. The digestate could be sufficient to replace 80 % of the carbon source on a yearly average basis, although there is great variation between winter and summer months due to seasonal temperature variation (Figure S4).

4. Conclusions

- The inclusion of THP before acidogenic fermentation had an overall positive effect on the production and quality of the carbon source used for denitrification and further generation of biogas.
- Fermentation of THP-digestate resulted in a better carbon source for denitrification compared with fermented raw and/or THP-mixed sludge. It achieved a higher degree of carbon solubilisation (39 %) and VFA (9.8 g VFA_{COD}/L), which resulted in a higher denitrification rate (8.77 mg NOx-N/g VSS•h).
- Carboxylic acid production during fermentation may be correlated with specific macromolecule degradation. For example, protein degradation led to an increase in acetate and butyrate, while carbohydrate degradation seemed to be related to propionate and valerate production.
- Process calculations based on Käppala WWTP 2028 showed that all carbon sources tested could replace fossil-based methanol and meet the effluent nitrogen limit (6 mg N/L) despite their high nitrogen content. Using THP-digestate could potentially also increase biogas production at Käppala WWTP and decrease the costs of sludge handling.

CRediT authorship contribution statement

Andrea Carranza Muñoz: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jesper Olsson:** Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Andriy Malovanyy:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Christian Baresel:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Nethra Machamada-Devaiah:** Validation, Investigation, Data curation. **Anna Schnürer:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122426.

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