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Thermal hydrolysis on the edge of thermophilic anaerobic digestion: a pilot-scale operation experience

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This study investigated the integration of the thermal hydrolysis process (THP) as a pretreatment with thermophilic anaerobic digestion (TAD) at a pilot scale using sludge from a full-scale wastewater treatment plant. This is the first pilot-scale evaluation of THP–TAD employing thermophilic inoculum adapted to hydrolysed sludge, offering critical insights into the potential of THP (155 °C, 30 minutes) to enhance TAD (55 °C) performance and contribute to sustainable sludge management. This study assessed the effects of THP on process stability at reduced hydraulic retention times (HRTs), biogas production, sludge dewaterability, and antibiotic resistance gene (ARG) reduction. The THP achieved a sludge disintegration degree of 26.8%, enabling a 50% reduction in HRT without compromising the reactor stability or process efficiency. At an HRT of 12 days, the specific biogas production averaged 0.28 Nm^3 kg^{-1} VS_{in}. Additionally, compared with traditional processes with longer HRTs, THP significantly enhanced ARG reduction, achieving a maximum reduction of 3.5 log units, while improving sludge hygienization and maintaining volatile solids reduction (VSR). Despite performance improvements, THP–TAD requires higher energy input, underscoring the need for optimization strategies. This study demonstrated that THP–TAD is a robust and effective approach for intensifying anaerobic digestion, offering notable reductions in capital costs (digester volume) while addressing critical environmental challenges such as ARG mitigation. Further investigations into sludge thickening and energy efficiency optimization are necessary to fully realize the potential of this technology as a cornerstone of sustainable wastewater management.

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Water impact

This pilot-scale study confirms that integrating thermal hydrolysis with thermophilic anaerobic digestion enables stable operation at reduced retention times while reducing antibiotic resistance genes. This approach allows a 40–60% reduction in digester volume, improves sludge dewaterability, and supports energy-efficient operation, offering a scalable solution for wastewater treatment plants facing capacity, cost, and environmental constraints.

Introduction

There is a growing interest in wastewater treatment as well as in the development of sustainable and efficient methods to manage sludge, a by-product of the treatment process. Anaerobic digestion (AD) is one of the most widely used processes in wastewater treatment plants (WWTPs), offering sludge stabilisation, volume reduction, and energy recovery through biogas production.[1] However, conventional mesophilic AD (MAD) is constrained by slow hydrolysis, which limits the breakdown of complex organic matter into simpler substrates required for subsequent metabolic phases.[2]

A promising alternative is thermophilic AD (TAD), which operates at temperatures up to ~55 °C. TAD results in higher degradation rates, shorter hydraulic retention times (HRTs), enhanced volatile solids reduction (VSR), improved hygienization, and greater biogas production.[3] However, the TAD tends to be more sensitive to the process's instability, which is mainly linked to high ammonia concentrations and reactor acidification caused by volatile fatty acid accumulation.[4]

Another strategy to increase the extent and rate of AD is the thermal hydrolysis process (THP). Applying heat and pressure

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results in the breakdown of complex organic compounds, increasing substrate availability for the subsequent AD stages.[5,6] Recently, the optimum THP operating conditions have been intensively studied, identifying temperature as the most important parameter. With respect to biogas production, dewatering, and filtrate quality, the optimum range is 140–160 °C, and the corresponding pressure is approximately 8 bar, with a process duration of approximately 30 minutes.[7,8] In this range, THP is widely applied at the full-scale in combination with mesophilic AD (MAD), leading to 15–60% increases in biogas production, up to a 50% reduction in the HRT, and 3–9% improvement in dewaterability.[9–11]

In addition to these advantages, the main drawback of THPs is their high energy consumption,[12,13] which can be reduced by the optimal energy integration of THPs in WWTPs.[14] Fernández-Polanco and Tatsumi[14] reported that the sludge outlet temperature can decrease THP energy consumption by decreasing the energy demand for cooling. After hydrolysing and depressurising, the sludge is typically cooled by dilution to 40 °C (MAD). By applying TAD at an operating temperature of 55 °C, the heat loss can be reduced.

However, research on combining THP pretreatment with TAD (THP–TAD) is limited. To date, the literature referring to THP–TAD has been conducted at the laboratory scale, with only two studies upscaling it to pilot and full-scale operations. Sørensen, *et al.*[15] used hydrolysed mixed raw sludge (MRS, mixture of primary sludge and waste activated sludge (WAS)) in TAD, which demonstrated greater biogas production and increased organic reduction than did untreated sludge. Ngo *et al.*[16] reported that hydrolysed MRS can reduce ammonia and volatile fatty acid (VFA) release and increase biogas production. In addition to these two studies, which were both at the laboratory scale, the following studies focused on the THP–TAD treatment of WAS, whereas the performance of the combined process in digesting MRS/hydrolysed MRS requires further investigation.

HRT and OLR are among the most critical operational parameters in AD, influencing process stability, biogas production, and VSR. In conventional TAD, HRTs typically range between 10 and 20 days, with OLRs varying from 1.5 to 5 kg VS_{in} $(m^3\ d)^{-1}$, balancing organic degradation efficiency with reactor stability.[2,4] The introduction of THP before TAD is expected to increase substrate degradability, potentially allowing for shorter HRT and higher OLR without compromising performance. Despite this potential, the minimal HRT and optimal OLR required for stable THP–TAD operation remain unknown.

In addition to process operation, the THP–TAD performance may also be influenced by inoculum origin and adaptation. Although microbial adaptation plays a crucial role in process stability, no study has systematically evaluated THP–TAD using a fully adapted thermophilic inoculum sourced from a full-scale TAD reactor. This knowledge gap necessitates further investigation to determine the feasibility of THP–TAD with a fully adapted thermophilic inoculum.

Additionally, antibiotic resistance genes (ARGs) are emerging as an urgent environmental concern because of their role in antimicrobial resistance, which poses global health risks.[17,18] These genes enable bacteria to withstand antimicrobial treatments and can spread readily through horizontal gene transfer; notably, these genes are concentrated from wastewater in sewage sludge and later in AD.[19,20] While THP has been linked to a more efficient reduction of certain ARGs,[21,22] research on ARG dynamics during AD remains inconclusive. Some studies report that AD can substantially lower certain ARG levels,[23] whereas others indicate minimal or even increased ARG abundance during digestion.[24] In TAD, elevated operating temperatures can mitigate ARG proliferation by inactivating certain resistant bacterial strains, diminishing the viability of plasmid-mediated ARGs, and achieving ARG reductions ranging from 50 to 90%.[25] However, the effect of combining THP–TAD on ARG dynamics remains unexplored.

Therefore, the objectives of this study are to I. assess the effects of THP–TAD on MRS treatment, using specific biogas production, VSR, sludge dewaterability and hygienization as key indicators, an approach not previously explored; II. examine THP–TAD process stability at short retention times (≤12 days); III. investigate the possible inhibition by ammonia and VFA from hydrolysed MRS on TAD; IV. examine the effect of acclimated TAD inoculum on THP–TAD process performance; and V. clarify the effects of thermal hydrolysis pretreatment and THP–TAD on the reduction in ARG concentrations.

To the best of our knowledge, this study is the first to systematically investigate THP–TAD *via* an original thermophilic inoculum that has been fully adapted to hydrolysed MRS. The experiment was conducted at a pilot-scale facility directly integrated into a full-scale WWTP with a population equivalent (PE) of 1 million. This setup enables direct bypass of MRS and inoculation with thermophilic sludge from an operational full-scale TAD reactor, ensuring an accurate representation of real-scale conditions. Key parameters, including substrate solubilisation, process stability, biogas production, VSR, ARG removal, and sludge dewaterability, are evaluated under varying HRTs and OLRs. Given the contradictory results from previous studies, this research aims to provide a comprehensive assessment of THP–TAD performance under practical operating conditions.

Materials and methods

Inoculum and substrate

Two inocula (Inoculum 1 and 2) were used for the inoculation of the pilot-scale reactor, both of which were freshly withdrawn from the full-scale TAD (Prague, Czech Republic) before the start of each operational phase (Table 1). The total solids (TS) contents of these inocula were 31.0 and 29.6 g L^{-1}, with corresponding volatile solids (VS) contents of 17.3 and 17.9 g L^{-1}, respectively.

The substrate used for the pilot plant operation was sourced directly from the full-scale sludge line at Prague WWTP. The MRS was composed of primary sludge and mechanically thickened WAS in a 1 : 1 ratio. The untreated MRS1 used in phase 1 had an average TS content of 49.9 g L^{-1} and a VS

Table 1 Average inoculum and substrate compositions during phases 1 and 2 and batch experiments, including standard deviations

	Inoculum 1[a,d]	Inoculum 2[a,d]	Inoculum 3[b,e]	Inoculum 4[b,e]	Inoculum 5[b]	Inoculum 6[b]	MRS1[c]	HMRS1[c]	MRS2[c]
Origin	Thermophilic full-scale (WWTP Prague, CZ)		Thermophilic pilot-plant (this study)		Thermophilic full-scale (WWTP Prague, CZ)	Mesophilic full-scale (medium-sized WWTP, CZ)	—	Hydrolysed MRS1	—
TS [g L^{-1}]	31.0	29.6	21.2 (± 0.0)	31.7 (± 0.7)	30.7 (± 0.0)	14.5 (± 0.4)	52.4 (± 5.1)	35.0 (± 9.3)	49.9 (± 6.8)
VS [g L^{-1}]	17.3	17.9	12.7 (± 0.1)	18.6 (± 0.4)	18.3 (± 0.1)	6.1 (± 0.2)	40.4 (± 3.5)	26.9 (± 6.7)	39.2 (± 5.2)
tCOD [g L^{-1}]	38.0	39.0	21.5 (± 0.8)	28.7 (± 2.3)	26.3 (± 0.7)	14.8 (± 2.1)	84.3 (± 21.7)	50.6 (± 16.6)	79.61 (± 18.0)
sCOD [g L^{-1}]	—	—	2.6 (± 0.0)	2.6 (± 0.2)	2.1 (± 0.0)	0.6 (± 0.1)	2.1 (± 0.4)	13.1 (± 1.9)	2.5 (± 0.7)
DD [%]	—	—	—	—	—	—	—	26.8 (± 10.3)	—
Dilution factor [–]	—	—	—	—	—	—	—	1.6 (± 0.6)	—
$N\text{-}NH_4^+$ [g L^{-1}]	1.28	1.53	1.02	1.03	1.30	—	—	—	—
VS/TS [–]	0.56	0.61	0.60 (± 0.01)	0.59 (± 0.05)	0.59 (± 0.01)	0.42 (± 0.10)	0.77 (± 0.02)	0.78 (± 0.03)	0.79 (± 0.03)
VFA-C2 [g L^{-1}]	0.09	0.22	—	—	—	—	—	—	—

[a] This measurement is based on a single sample; thus, the standard deviation is not applicable. [b] Each measurement was performed in triplicate, and the standard deviation was calculated to account for experimental variability within these replicates. [c] The standard deviation reflects the variability across multiple samples collected throughout the experimental phase, accounting for process fluctuations over time. [d] A single sample from the pilot plant digester was used as inoculum for batch experiments. [e] Average inoculum composition from the pilot plant digester during phase 1 and phase 2.

content of 39.2 g L^{-1}. The hydrolysed MRS1 (HMRS1, after THP) had an average TS content of 35.0 g L^{-1} and a VS content of 26.9 g L^{-1}. The untreated MRS2 used in phase 2 had an average TS content of 49.9 g L^{-1} and a VS content of 39.2 g L^{-1}.

The biodegradability of MRS1 and hydrolysed MRS1 (HMRS1) was tested *via* batch experiments. Additionally, acetic acid (99%) was used as a control substrate to determine the specific methanogenic activity (SMA) of the inocula taken from phases 1 and 2. In the batch experiments, four different inocula were used. The first two, Inoculum 3 and 4, were sampled from the pilot-plant thermophilic digester during phases 1 and 2 (T5) after stable process performance was achieved. The third inoculum (Inoculum 5) was collected from a full-scale thermophilic digester at the Prague WWTP (Czech Republic), whereas the fourth inoculum (Inoculum 6) was obtained from a full-scale mesophilic digester at a medium-sized WWTP (Czech Republic), all from sludge-based digesters.

Pilot setup and operation

Experimental setup of the pilot plant. The pilot plant setup comprised six interconnected tanks (Fig. 1). Two of these, T1 and T2, were open-air stirred tanks designed to store MRS withdrawn directly from the full-scale WWTP sludge line, ensuring stable operation of the THP reactor. The thermal hydrolysis occurred in T3, an autoclave reactor with a working volume of 100 L, where water vapour was dosed to induce high temperatures and high pressures. Directly below T3, T4 functioned as a flash tank, where hydrolysed sludge was depressurised and cooled before being transferred into the digester. T5 was the primary anaerobic digester, constructed as a closed, continuously stirred tank reactor (CSTR) with a working volume adjusted according to the operating conditions, between 3.4 and 6.1 m^3. This digester was equipped to continuously monitor biogas production, temperature, pH, and sludge level to ensure optimal process performance and stability. Finally, T6 served as a storage tank for digested sludge, allowing accumulation prior to dewatering in a small-scale centrifuge (ANDRITZ).

Operation of the pilot plant. As shown in Fig. 1, the pilot-scale experiment was divided into two phases (phase 1 and 2) to compare the performance of the THP–TAD and TAD only. Phase 1 was additionally divided into two predefined HRTs of 8 and 12 days with corresponding OLRs of 3.16 kg VS_{in} m^{-3} and 2.41 kg VS_{in} m^{-3}, respectively. Phase 1 included THP as a sludge pretreatment prior to TAD. To simulate the current operational conditions of the full-scale process at the WWTP of Prague, the MRS was withdrawn daily from the full-scale sludge line and temporarily stored at T1 or T2 for approximately 30 minutes before being pumped into T3 for thermal hydrolysis. THP was conducted in three sequential runs (100 L each), as shown in Fig. S1, to ensure the 300 L required feed volume for T5. The hydrolysis process was carried out at 155 °C (±5 °C) for 30 minutes, after which the treated sludge was released into T4. In T4, after all three

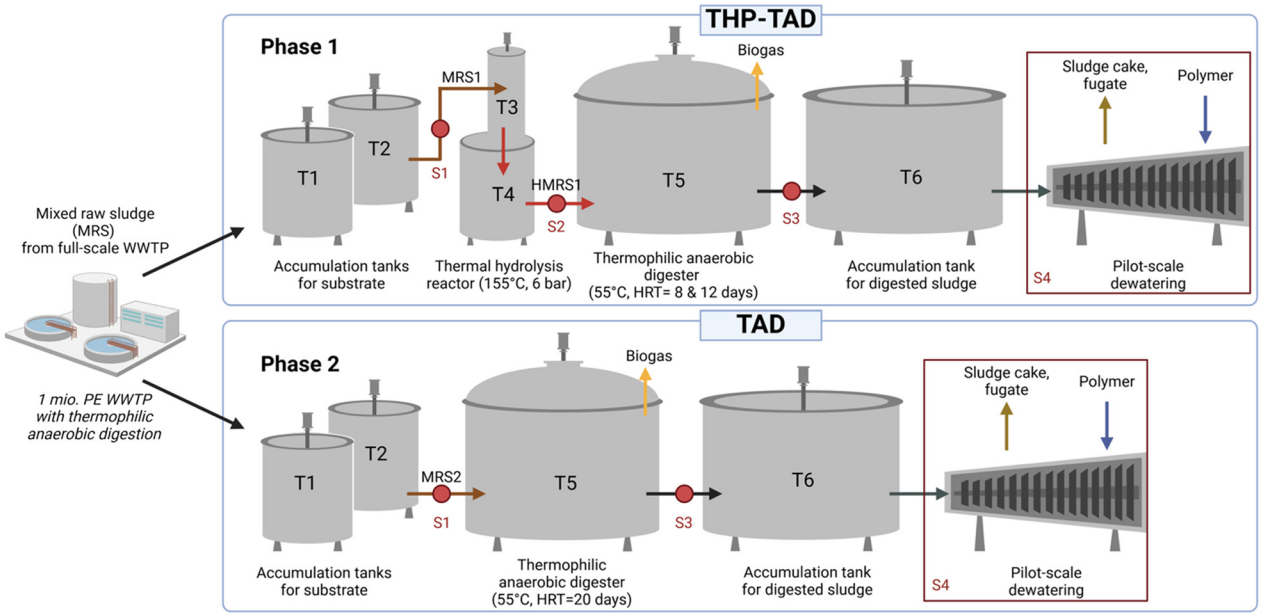

Fig. 1 Schematic of the configurations of the pilot plant with (THP–TAD) and without the thermal hydrolysis process (TAD). Phase 1 (top) included tanks T3–T4 for sludge hydrolysis before digestion in T5 and accumulation of digested sludge in T6. Phase 2 (bottom) does not include THP, sending untreated sludge from T1 or T2 directly to digestion (T5), accumulated digested sludge (T6), and dewatering. The mixed raw sludge (MRS) was removed from a full-scale wastewater treatment plant (WWTP) with one million population equivalents (PEs).

runs, the accumulated sludge naturally cooled to approximately 60 °C before being fed into T5 once per day.

During the second phase (phase 2), THP pretreatment was omitted, and the sludge was directly withdrawn from the full-scale sludge line, stored for less than 5 hours, and then pumped directly into T5 once per day. During phase 2, the HRT was extended to 20 days with a corresponding OLR of 1.97 kg VS_{in} $(m^3\ d)^{-1}$.

Throughout the entire experiment, T5 was maintained at a constant operating temperature of 55 °C (±1 °C) to ensure stable thermophilic conditions. Each HRT change was followed by adaptation, during which the sludge feed was gradually increased until stable process performance was achieved. The stable process performance was determined when the average volatile solids removal (VSR) standard deviation was ≤10%.

To ensure systematic monitoring of process performance, all samples were positioned throughout the pilot plant, typically close to pumps, as marked in Fig. 1. Sampling point S1, located after T1 and T2, was designated for the collection of MRS1 and MRS2. Sampling point S2 was used to collect HMRS1 immediately after thermal hydrolysis in T3 and T4, whereas sampling point S3 was placed at the effluent of the TAD (T5) to collect digested sludge. Throughout the entire experiment, samples were collected daily, ensuring continuous process monitoring. To maintain analytical consistency and data reliability, all samples were directly analysed on the same day of collection, excluding weekends, when they were stored in a refrigerator at 4 °C until the next working day. In addition to the sludge samples, biogas samples were collected from the T5 after the gas meter and transported in 500 mL glass gas tubes (Lenz Laborglasintrumente™ 05680158, Germany). Continuous biogas measurements at T5 were conducted *via* a gas meter (DOG-6, KOBOLD), which was calibrated prior to each operational phase. Furthermore, a combined pH and temperature probe (XB90-02 pH, GRYF) was installed in T5, ensuring real-time pH monitoring and temperature control throughout the digestion process.

Batch experiments

Biodegradability test. The batch assays were conducted in 100 mL Schott bottles (E8R06857, Simax, Czech Republic) equipped with rubber septa to maintain anaerobic conditions. All experiments were performed in triplicate, following the standardised biochemical methane potential (BMP) protocol outlined by Holliger *et al.*[26] A blank sample containing only inoculum was included in determining the baseline methane production from endogenous microbial activity. As detailed in Table 2, the inoculum-to-substrate ratio (ISR) was set at 2 : 1 for all batch assays.

Specific methanogenic activity tests. The SMA tests were conducted with acetic acid (99%) as the sole substrate, following the same batch assay procedure described previously. This approach allowed for a direct assessment of the acetoclastic methanogenic activity of the inoculum after adaptation to hydrolysed or non-hydrolysed substrates.

Analytical methods

Physicochemical analysis. The pH was measured with a Sentro pH meter (SI400, Netherlands) and calibrated with standard solutions before use. The solids contents were

Table 2 The laboratory batch assay setup using inocula and substrates in batch assays, together with conditions such as pH, temperature, and the inoculum-to-substrate ratio (ISR)

Inoculum	Inoculum 3[a]	Inoculum 5[b]	Inoculum 6[c]
Substrate	MRS1	HMRS1	
pH [–]	8.0	8.1	7.9
Temperature [°C]	55	55	40
ISR [VS_{inoc} : $tCOD_{sub}$]	0.5		

[a] Taken from the pilot plant digester (this study) during phase 1. [b] Taken from the full-scale thermophilic digester. [c] Taken from the full-scale mesophilic digester.

determined gravimetrically by drying samples at 105 °C for TS and 550 °C for VS, following the Standard Methods for Examination of Water and Wastewater.[27] Dissolved solids (DS) were analysed *via* the same gravimetric method but with pre-centrifugation of the sample in a SIGMA 3-16P centrifuge at 13 000 rpm for 12 minutes.

The determination of sCOD and tCOD was performed *via* the dichromate oxidation method and titration with diammonium ferrous sulfate, following CSN ISO 6060. Prior to sCOD measurement, the samples were homogenised and centrifuged in a SIGMA 3-16P centrifuge at 13 000 rpm for 12 minutes, after which the supernatant (fugate) was used for analysis. VFAs were analysed *via* a Shimadzu 2010 gas chromatograph (GC) equipped with a flame ionisation detector (FID) and measured at a temperature gradient of 145–230 °C in a Lion LN-FFAP column at a flow of 1 mL min^{-1} and nitrogen as carrier gas. Ammonium nitrogen ($N–NH_4^+$) concentrations were determined volumetrically following CSN ISO 5664 (757449) *via* a Vapodest 500 automatic distillation system (Gerhardt, Germany). The total phosphorus (TP) content was measured *via* a GBC Integra XL spectrometer (RMI, Czech Republic), whereas the phosphate ($P–PO_4^-$) concentration was analysed *via* a SAN++® Classic flow analyser (Skalar, Netherlands) according to EN ISO 15681-2:2018.

Biogas measurement. At the pilot plant, the biogas volume was continuously measured *via* a gas meter (DOG-6, KOBOLD), which was calibrated before each operational phase to ensure accuracy. The methane concentration in the biogas samples was analysed *via* a Hewlett Packard 5890 Series II GC equipped with a thermal conductivity detector (TCD) and a glass column packed with Chromosorb 102. The column and detector temperatures were set at 40 °C and 120 °C, respectively, with argon as the carrier gas at a flow rate of 45 mL min^{-1}.

Prior to biogas volume measurement, all bottles of the batch assays were manually shaken for approximately 30 seconds. The biogas volume was measured *via* the liquid displacement method, following the protocol established by Holliger *et al.*[26] All volumes were normalised to standard temperature and pressure conditions (101.325 kPa, 20 °C) to allow direct comparisons between experimental conditions. The methane concentrations were measured *via* a Shimadzu GC-2014 gas chromatograph equipped with a HayeSep D packed column and a TCD. The column and detector temperatures were maintained at 40 °C and 120 °C, respectively, with argon as the carrier gas flowing at 30 mL min^{-1}.

Cultivation test. To evaluate the process performance of THP–AD, the pathogen removal efficiency, expressed as the degree of hygienization, was also tested. The microbiological risk of hygienised sewage sludge was assessed according to Czech legislation (regulation Nr. 273/2021 Sb.). Thermotolerant coliforms and *Escherichia coli* (TCOLI/ECOLI), intestinal enterococci (ENTERO), and *Salmonella spp.* (SAL) were determined according to CSN 757835, CSN EN ISO 7899-2, and CSN EN ISO 6579, respectively, by culturing methods, and the determination procedure followed the AHEM Methodological Guide Nr. 1/2008. To determine ECOLI and ENTERO, 10 g of MRS, HMRS, and the digested sludge samples were weighed, and 90 mL of phosphate buffer was added. A dilution series was then prepared to calculate the number of colony-forming units (CFUs). For each dilution, two plates were plated on the weighted average. To determine the amount of SAL, 50 g of sludge was weighed, and 450 mL of nonselective multiplication medium was added. Selective multiplication was assessed in 2 liquid media, followed by culture on two solid media for qualitative analysis.

Dewaterability. The pilot-scale dewatering unit was part of the pilot plant installed at the WWTP. The unit comprised a mobile dewatering centrifuge (ANDRITZ) and a flocculation unit (ANDRITZ). As a flocculant powdered polymer, 55 GP (SOKOFLOK) was used. The settings, such as the polymer dosage rotation speed, were adjusted to achieve a sludge cake with the highest dry solids content and fugate with the lowest undissolved solids concentration.

To compare these results, a laboratory centrifugation method was used to exclude the impact of the polymer and adjust the centrifuge settings. This method was closely adapted from Jin *et al.*,[28] and it aims to remove bulk water from sludge in a SIGMA 3-16P centrifuge at 13 000 rpm for 12 minutes.

Antibiotic resistance gene analysis

Sludge samples of MRS, HMRS, and digested sludge were collected in sterile 2 ml Eppendorf tubes during three sampling campaigns to assess the relative abundance of ARGs. Each sample was weighed to fall within 0.25–0.31 g. DNA extraction was performed using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, and the extracted DNA was stored at −20 °C until further analysis.

Quantitative PCR (qPCR) was performed externally by Resistomap (Finland) using a high-throughput SmartChip Real-Time PCR System (Takara Bio, USA). Target ARGs were selected from a panel of 128 genes based on a preliminary screening of wastewater treatment plant (WWTP) samples collected from seven sites in Czechia. The selection criteria were designed to ensure broad and representative coverage of resistance determinants commonly found in wastewater sludge. Specifically, genes were chosen according to their high

abundance and prevalence in WWTPs, their representation of major antibiotic resistance classes—including β-lactams, tetracyclines, macrolides, sulfonamides, aminoglycosides, and multidrug resistance genes—and their relevance to clinically significant or environmentally persistent resistance mechanisms. This ensured inclusion of both widespread and high-risk ARGs, reflecting enzymatic degradation, target modification, and efflux-based resistance mechanisms typical of complex microbial communities in sludge.

All primers employed in the Resistomap array were validated for specificity, amplification efficiency, and taxonomic coverage using the UniPriVal tool,[29] and full primer details are provided in Table S1. Quantification followed established protocols[30,31] with calibration curves prepared from known standards.

Calculations and statistics. The disintegration degree (DD) is commonly calculated to evaluate the efficiency of THP. This parameter helps quantify THP's impact on sludge solubilisation. The VSR, on the other hand, quantifies the efficiency of organic matter degradation during digestion and is one of the critical operational parameters.

During THP, water vapour is injected into the reactor, leading to condensation in the flash tank. This condensation alters the sludge TS concentration, affecting the calculation of DD. Since the exact volume of condensed water was not measured directly, the dilution factor (DF) was calculated to account for this effect. DF enables the correction of COD concentrations to ensure that changes in solubilisation are attributed solely to THP without being skewed by dilution effects.

DF was calculated as the ratio of total solids before and after THP treatment, as shown in eqn (1):

$$\mathrm{DF} = \frac{\mathrm{TS_{in}}}{\mathrm{TS_{out}}} \tag{1}$$

$\mathrm{TS_{in}}$ (g $\mathrm{L^{-1}}$) is the total solids concentration before THP, and $\mathrm{TS_{out}}$ (g $\mathrm{L^{-1}}$) is the total solids concentration after THP.

To eliminate the dilution effect on the sludge disintegration efficiency caused by the THP, the DD was calculated according to Zhang *et al.*[32] incorporating the DF (eqn (2)):

$$\mathrm{DD}(\%) = \frac{\mathrm{sCOD_H} - \frac{\mathrm{sCOD_0}}{\mathrm{DF}}}{\mathrm{tCOD} - \mathrm{sCOD_0}} \times 100\% \tag{2}$$

where $\mathrm{sCOD_H}$ and $\mathrm{sCOD_0}$ (g $\mathrm{L^{-1}}$) represent the sCOD concentrations of HMRS1 and MRS1, respectively, and tCOD (g $\mathrm{L^{-1}}$) represents the total COD of the untreated sample (MRS1).

The VSR was calculated *via* eqn (3):

$$\mathrm{VSR}(\%) = \frac{\mathrm{VS_{in}} - \mathrm{VS_{out}}}{\mathrm{VS_{in}}} \times 100\% \tag{3}$$

$\mathrm{VS_{in}}$ (g $\mathrm{L^{-1}}$) is the volatile solids concentration before digestion, and $\mathrm{VS_{out}}$ (g $\mathrm{L^{-1}}$) is the concentration after digestion. Removed organics ($\mathrm{VS_{removed}}$) in kg per day were derived from VSR following eqn (4).

$$\mathrm{VS_{removed}} = \mathrm{VS_{in}} \times \mathrm{VSR} \tag{4}$$

Statistical analysis was conducted *via* analysis of variance (ANOVA) with a significance threshold of $p \leq 0.05$. For *post hoc* comparisons, the Bonferroni correction was applied to the operational data, whereas the Tukey–Kramer test was used to determine the statistical significance of differences in ARG concentrations between the THP and non-THP samples. The absolute quantification of ARG abundance was conducted following the protocols outlined by Rocha *et al.*[31] and the methodological framework described by Muurinen *et al.*[30] Data visualisation and statistical evaluations were performed *via* RStudio (version 2023.06.2), which employs various software packages, including ggplot2, ggpubr, ggpattern, ggh4x, agricolae, broom, plotly, dplyr, and plyr.

The energy net balance of THP–TAD (phase 1, 12 days) and TAD (phase 2) were calculated based on the average sludge volume fed into THP and AD, as well as on the average TS, VS concentration and average methane yields.

First, the specific energy required for sludge thickening (E_t) was calculated by adapting eqn (5) from DWA-A 216 CZ.[33] For MRS1, the specific energy ($E_{\mathrm{spez.\_thick}}$) required for thickening was set at 0.7 kWh $\mathrm{m^{-3}}$. The sludge volume QRS is the volume prior to thickening.

$$E_t = Q_{\mathrm{RS}} \times E_{\mathrm{spez.\_thick}} \tag{5}$$

Secondly, the energy required for heating ($E_{\mathrm{THP\_heat}}$) the sludge to 160 °C (eqn (6)) was adapted from Barber.[34] Here, the temperature of the sludge prior to THP treatment is the average temperature of aerobic treatment (15 °C), while the THP process temperature is set at 160 °C. The temperature difference, expressed as ΔTs and ΔTw (for sludge and water respectively), is 145 °C (145 K). The mass of sludge (m_s) is the average mass of sludge fed into THP, the mass of water (m_w) was based on average TS concentration. Specific heat capacities of 1.5 kJ $\mathrm{kg^{-1}\ K^{-1}}$ for sludge (C_{ps}) and 4.2 kJ $\mathrm{kg^{-1}\ K^{-1}}$ for water (C_{pw}) were applied. η accounts for heat losses caused by convection and radiation (65%).

$$E_{\mathrm{THP\_heat}} = \frac{\left(m_s \times C_{\mathrm{ps}} \times \Delta T_s + m_w \times C_{\mathrm{pw}} \times \Delta T_w\right)}{\eta} \tag{6}$$

In THP, the heat recovered during flash evaporation (25.40%)[35] was taken into account following eqn (7).

$$E_{\mathrm{THP\_heat\_B}} = E_{\mathrm{THP\_heat}} \times 0.254 \tag{7}$$

For phase 2, the energy required for heating sludge to the temperature of AD (E_{AD}) was assessed following eqn (8). This amount of energy was simplified as the amount of energy required to heat the required mass of sludge (m), temperature difference between the raw sludge (T_{raw}) and digester temperature (T_{AD}) and specific heat capacity of sludge (C_{ps}) including heat losses, estimated to be 10%.

$$E_{\mathrm{AD}} = m \times C_{\mathrm{ps}} \times \left(T_{\mathrm{raw}} - T_{\mathrm{AD}}\right) \times 1.1 \tag{8}$$

Finally, energy produced in the form of biogas (E_{BG}) was calculated following eqn (9). Where average methane yield (Y) in $m^3\ kg^{-1}\ VS_{in}$ as well as OLR in kg $VS_{in}\ m^{-3}$ together with combustion energy of methane (Δc = 36.0 MJ m^{-3}) were used.

$$E_{BG} = Y \times OLR \times \Delta c \quad (9)$$

Results and discussion

General operation performance

Thermal hydrolysis process. The primary objective of the THP reactor is to disintegrate sludge flocs and, hence, solubilise the organic matter in the sludge. The DD, which compares the solubilities of organic matter in treated and untreated sludge, reached an average of 26.8%, consistent with the findings of other studies, such as Kepp *et al.*[36] However, the DD fluctuated significantly by ±10.3%. These fluctuations in TS, VS, tCOD, and sCOD concentrations, indicated in Table 1 by the standard deviation of all measured samples, resulted from two factors: (1) fluctuations in the quality of full-scale sludge and (2) the fluctuating volume of condensed water vapour injected during THP operation. These fluctuations resulted from the simulated full-scale operating conditions, such as sludge bypass, a variable that could not be controlled. The volume of condensed water vapour, expressed as a dilution factor, reached, on average, 1.6 (±0.6), indicating the addition of approximately 33% condensed water vapour to the non-hydrolysed sludge. Nevertheless, the concentration of sCOD, which highlights the disintegration of organic matter in addition to DD, increased with increasing THP by a factor of six. Here, the dilution factor was not further considered, as this sludge was then directly pumped into AD, where it represented easily biologically degradable organics that have the potential for biogas production enhancement.

Volatile solids reduction. In this study, a stable process performance of the anaerobic digester was determined by a VSR of less than 10%. At the onset of phase 1, as shown in Fig. 2B, the organic level at the digester's outlet (VS_{out}) was higher than that at the digester's inlet (VS_{in}) because of the short (<10 days) organics washout. This resulted in a negative VSR and an adaptation period that lasted until day 45. From that day onward, the operation of THP–TAD with an HRT of 8 days (OLR = 3.2 kg VS_{in} $(m^3\ d)^{-1}$) was considered stable, with an average VSR of 54.6% (±9.5%). The transition to an HRT of 12 days (OLR = 2.4 kg VS_{in} $(m^3\ d)^{-1}$) was accompanied by an adaptation period of 23 days, after which the average VSR slightly increased to 56.9% (±8.1%), indicating that HRT prolongation led to an improved reactor stability and an increased removal of organic matter. These findings correlate with those of Gianico *et al.*,[37] who connected the deteriorating VSR of hydrolysed WAS to increasing organic OLR in THP–TAD at an HRT of 8 days. The robustness of the THP–TAD is evident in Fig. 2B at the end of phase 2, where, despite strong VS_{in} fluctuations, VS_{out} remained stable. During phase 2 (non-THP), the increased VS_{in} was attributed to the avoidance of dilution by condensed water vapour in the THP reactor. Nevertheless, phase 2 with an HRT of 20 days (OLR = 2.0 kg VS_{in} $(m^3\ d)^{-1}$) resulted in stable process performance with a VSR of 54.5% (±4.2%), similar to what was achieved during phase 1 with an HRT of 8 days or 12 days. Compared with TAD, THP–TAD achieved the same VSR but with a reduced HRT.

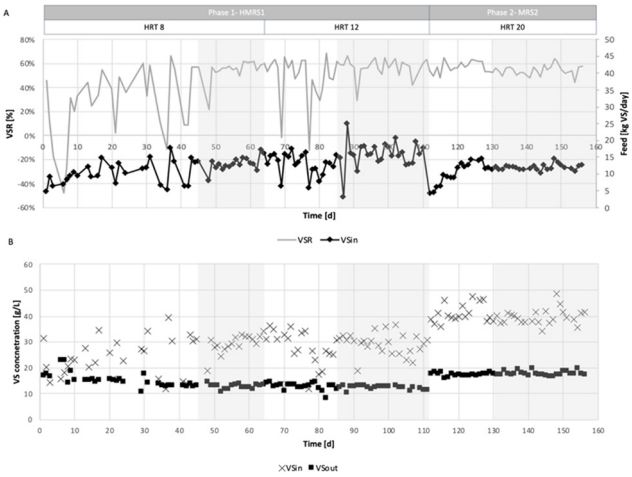

Fig. 2 (A) Shows volatile solids removal (VSR) during thermophilic anaerobic digestion in phase 1 (with THP pretreatment) and phase 2 (without THP pretreatment) on the primary axis as a grey solid line. Phase 1 is further divided into two parts based on the hydraulic retention time (HRT). The grey boxes indicate stable process performance, which is determined by a VSR of less than 10%. The organic's washout caused negative VSR values. On the secondary axis, the daily organic load (black line with squares) is shown in kg VS_{in} per day. (B) Displays volatile solids (VS) concentration at the inlet (VS_{in}), marked with crosses, and at the outlet (VS_{out}) from the thermophilic anaerobic digester, marked with squares.

Volatile fatty acid concentrations. According to Chen *et al.*,[38] the decrease in methane content can be linked to increased VFA concentrations, which were also observed in our study during phase 2. The acetic and propionic acid contents reached averages of 0.51 g L^{-1} and 0.15 g L^{-1}, respectively. In phase 1, acetic acid was present only at low concentrations, below 0.15 g L^{-1}, and the propionic acid concentration was under the detection limit (UDL). The increased propionic acid concentration, which tends toward a slowdown in degradation, points to incomplete digestion. Its further degradation and transformation into methane would be expected if the HRT was prolonged.[39] In this context, THP–TAD outperformed one-stage TAD, where higher VFA concentrations were measured, presumably leading to process instabilities.

Ammonium nitrogen concentration. The degradation of proteins during THP increases the ammonium nitrogen concentration,[40] a known inhibitor of TAD. In this study, the $N\text{–}NH_4^+$ concentrations were, on average, the lowest during phase 1, with an HRT of 8 days, *i.e.*, 1041 mg $N\text{–}NH_4^+\ L^{-1}$. With a prolonged HRT, $N\text{–}NH_4^+$ increased to 1153 mg $N\text{–}NH_4^+\ L^{-1}$. In contrast, the one-stage TAD presented an

average higher $N–NH_4^+$ concentration of 1558 mg $N–NH_4^+$ L^{-1}. During phase 2, ammonia inhibition initially led to the accumulation of VFAs in the reactor, as discussed previously. Based on these results, THP–TAD is beneficial for preventing ammonium accumulation because it enables a reduction in the HRT. Oosterhuis *et al.*[41] reported a potential adaptation of the inoculum to increased $N–NH_4^+$ concentrations, which aligns with the findings of this study, as elevated $N–NH_4^+$ concentrations of 1280 mg $N–NH_4^+$ L^{-1} and 1530 mg $N–NH_4^+$ L^{-1} were observed at the beginning of each phase, respectively. Similarly, Li *et al.*,[42] who conducted a detailed study on ammonia inhibition aimed at setting the boundary for THP–MAD intensification, concluded that the primary limitation during intensification is not the inhibition by free ammonia itself but rather the increased viscosity in AD. In TAD, the $N–NH_4^+$ L^{-1} concentration is higher than that in MAD; however, all other measured parameters do not indicate any ammonia inhibition in THP–TAD. Therefore, in this study and under specific operating conditions, the THP–TAD outperformed the one-stage TAD.

Biogas yield with and without the thermal hydrolysis process

Sludge degradability in anaerobic digestion. The biological degradability of MRS1 and HMRS1 was tested in batch experiments under mesophilic and thermophilic conditions. For thermophilic conditions, two types of inoculum (full-scale and pilot) were used to compare the adaptation of hydrolysed sludge to that of sludge. As depicted in Fig. 3, when mesophilic conditions and inoculum were used, THP enhanced methane production by 14%. This result correlates with the review published by Barber,[5] which exclusively focused on THP–MAD. The author further stressed the importance of the previous exposure of the inoculum to hydrolysed sludge. Under thermophilic conditions, HMRS1 in our study showed a more prolonged lag phase and reduced cumulative methane production. These results were more significant when the inoculum was not adapted to the hydrolysed sludge (Inoculum 6 + HMRS1, Fig. 3). The lag phase was eliminated when the adapted inoculum was used for TAD, and MRS1 and HMRS1 achieved similar results (full-scale and pilot), regardless of whether the sludge was pretreated or not. Furthermore, Bi *et al.*[43] made similar observations for the cumulative methane production of hydrolysed sludge digested by mesophilic inoculum adapted for 55 °C and 41 °C. This finding highlights the importance of thermophilic sludge adaptation, which seems essential for adequately evaluating the THP–TAD system. However, to the best of the authors' knowledge, the literature data published on THP–TAD resulted mainly from mesophilic inocula adapted to thermophilic conditions[37,38,44] or their origin was unclear,[45] and not from an adapted inoculum.

Nevertheless, adapting mesophilic inocula to thermophilic conditions is vital for achieving stable process performance, as the microbial composition requires enough time to transit. For example, Boušková *et al.*[46] achieved stable performance of mesophilic inoculum adapted to thermophilic conditions after an adaptation period of 28 days with an HRT of 20 days within a one-step temperature increase (from 37 °C to 55 °C). However, the adaptation was prolonged to 70 days when the temperature was increased stepwise (3 °C → 42 °C → 47 °C → 51 °C → 55 °C). Another study by Westerholm *et al.*[47] revealed that the microbial community of mesophilic sludge still changed after 117 days of operation when the HRT was 29–35 days, increasing the temperature stepwise by 2 °C per week. Generally, the adaptation period should be at least 2 HRTs, while other digester results, such as biogas production and composition, together with VFA content, can affect process stability. Excluding inoculum adaptation might, therefore, lead to a misinterpretation of the system's results.

During the SMA test, an enhancement of the inoculum's methanogenic activity adapted to hydrolysed MRS was observed during phase 1 toward the end of the test (Fig. S2). This enhancement is significant as it indicates the potential of THP–TAD to improve digestion efficiency.

Daily biogas rate and hydraulic retention time reduction. Fig. 4 shows the daily biogas production, which significantly fluctuated throughout both observed phases. These data were not selected to determine the reactor's stability but rather to compare the operational performance. In correlation with VSR, the biogas production rate remained unaffected by thermal pretreatment (4.49 ± 0.78 and 4.51 ± 0.60 Nm^3 per day, respectively). A slight decrease was observed as the HRT decreased from 12 to 8 days, and the OLR increased from 2.41 to 3.16 kg VS_{in} $(m^3\ d)^{-1}$, decreasing to an average of 3.89 ± 0.56 Nm^3 per day. This phenomenon of a decrease in the biogas production rate with increasing OLR is consistent with the findings of the study conducted by Braguglia *et al.*[44] Additionally, a decrease in the biogas production rate depicted in Fig. 4 occurs at the beginning of each phase. This can be attributed to the adaptation of the inoculum from the two-stage full-scale TAD to the one-stage pilot TAD, which initially had a high specific biogas production of 0.62 m^3 kg^{-1} VS_{in}. Fig. 4 also

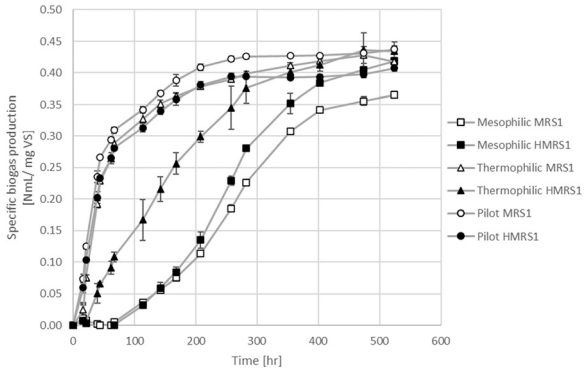

Fig. 3 Specific biogas production of hydrolysed (HMRS1, filled markers) and non-hydrolysed (MRS1, empty markers) mixed raw sludge in anaerobic digestion assays operated under i) mesophilic conditions with mesophilic inocula (squares), ii) thermophilic conditions with thermophilic inoculum from a full-scale (triangles), and iii) thermophilic conditions and thermophilic inoculum from the pilot (circles).

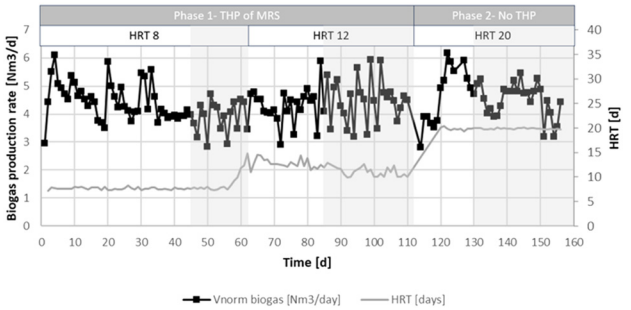

Fig. 4 Comparison of daily biogas production rates between THP–TAD (phase 1) and conventional TAD (phase 2). The secondary axis depicts the real HRT, with shaded areas indicating stabilised operation periods.

presents the main advantage of the THP: a significant reduction in the HRT while maintaining the same biogas production rate. The reduction in the HRT further leads to significant operation and investment savings in annual operating costs, providing apparent savings for plants while considering extending their existing AD capacity.[48] However, these conclusions were drawn mainly for the combination of THP–MAD, whereas for THP–TAD energy efficiency studies, well-organised energy flows at the pilot scale initially led to the development of sustainable processes.[13] Nevertheless, significant shortening of the HRT enables increased sludge flow in AD, further impacting investments and operating costs.[34] By reducing the HRT from 20 days to 12 days, the size of the digester can be reduced to 60%, and if the HRT is reduced to 8 days, to 40% of the original volume while maintaining the same OLR (1.97 kg VS_{in} $(m^3\ d)^{-1}$). Meaning that practically, the sludge line can handle greater sludge volumes with the existing digester, or that the existing capacity can be consumed by adding co-substrate. This significant reduction in digester size underscores the potential cost savings and efficiency of THP–TAD.

Specific biogas yield and biogas quality. After adaptation, phase 1 - HRT 8 achieved an average specific biogas yield of 0.30 (±0.05) $Nm^3\ kg^{-1}\ VS_{in}$ and a CH_4 content of 60.9% (±2.3%). With the HRT prolonged to 12 days, the average specific biogas yield was 0.28 (±0.06) $Nm^3\ kg^{-1}\ VS_{in}$ and 62.2% (±0.7%) CH_4. The specific biogas yield is comparable to the findings of Chen *et al.*,[38] who reported an average specific biogas yield of 0.34 (±0.15) $Nm^3\ kg^{-1}\ VS_{in}$ with 66.3% CH_4 at an HRT of 15 days and a slightly greater OLR of 3.35 (±1.42) kg VS_{in} $(m^3\ d)^{-1}$; and Ferrer *et al.*,[49] who reported an average of 0.22–0.30 $m^3\ kg^{-1}\ VS_{in}$ at an HRT of 10 days with sludge pretreated with low-temperature THP (70 °C). However, the specific biogas production reached in this study is far from the optimal value of 0.6 $m^3\ kg^{-1}\ VS_{in}$ determined by Jenicek *et al.*[50] This optimal value should initially lead to energy self-sufficiency in WWTPs. Moreover, their study did not consider the energy consumed by the THP, which is estimated to be approximately 110% of the initial specific biogas yield. During phase 2, the specific biogas yield reached an average of 0.37 (±0.05) $Nm^3\ kg^{-1}\ VS_{in}$, but had a lower methane content of 56.9% (±4.4) CH_4 than that in phase 1. Although the specific methane yield remained comparable between THP–TAD and TAD (Table 3), the application of THP enabled equivalent methane production at a 40% shorter HRT (12 days). However, these results contradict the findings of Chen *et al.*,[38] who suggested that the HRT should be longer than 20 days for THP-TAD, as an HRT of 15 days led to reactor failure.

Digested sludge quality

Pathogen removal efficiency. In this study, the pathogen removal efficiency was assessed *via* a cultivation test of coliform bacteria, *Enterococci*, and *Salmonella* in samples before and after THP after TAD (phase 1) and before and after TAD (phase 2). As presented in Table S2, in phase 1, THP ensured complete hygienization of the samples after THP (MRS1 and HMRS1), so no pathogens were found. These findings, supported by those of Wang *et al.*,[51] show that the THP treatment temperature and time (160 °C, 30 min) lead to complete pathogen reduction.

However, TAD alone achieved the same results with longer retention times, which aligns with the findings of other studies.[41,52] Therefore, THP–TAD does not outperform TAD in terms of hygienization.

Sludge dewaterability. The dewaterability of digested sludge is an essential parameter in further sludge handling and processing, and it significantly contributes to the operational costs of the wastewater treatment process. As shown in Fig. 5, the THP pretreatment of sludge positively impacted the resulting dewaterability of 22%, 24%, and 16%, respectively, when tested *via* a laboratory centrifuge without adding polymer.

However, when the polymer was added on pilot-scale, this improvement in sludge dewaterability was suppressed, resulting in an average TS contents of 25%, 24%, and 24%, which are lower than those reported by Han *et al.*[53]

The dewaterability depicted in Fig. 5 was the highest during phase 1 – HRT 8, possibly because the dosage of the polymer was 0.88 kg t^{-1} of sludge TS, in contrast to the phase 2 – HRT 12, which achieved similar results, with 0.54 and 0.49 kg t^{-1} of sludge, respectively.

Table 3 Summary of the operating parameters for phase 1 and phase 2, detailing the HRT, organic loading rate, total duration, specific methane yield, and average methane concentration

	HRT [d]	OLR [kg VS_{in} $(m^3\ d)^{-1}$]	Total operation time [d]	Specific methane yield [$Nm^3\ kg^{-1}\ VS_{in}$]	Average CH_4 concentration [%]
Phase 1	8	3.2	61	0.20 (± 0.05)	60.85 (± 2.31)
	12	2.4	48	0.21 (± 0.13)	62.20 (± 0.66)
Phase 2	20	2.0	45	0.21 (± 0.04)	56.89 (± 4.40)

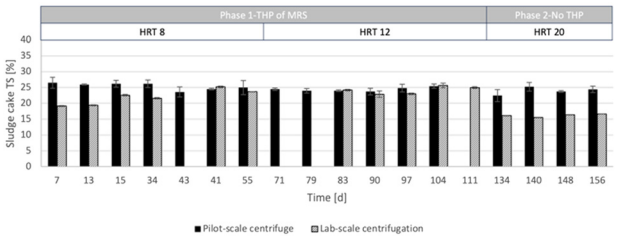

Fig. 5 Dewaterability of sludge expressed as TS in [%] of sludge cake—results from laboratory-scale (grey) and pilot-scale centrifuge (black).

Overall, the results show only a slight improvement in dewaterability when THP–TAD is applied. This could be partially explained by high VS content in digested sludge, which was earlier linked to reduced sludge dewaterability.[54] Yet, limited improvement of digested sludge dewaterability also contradicts the findings of Hamze *et al.*,[55] who indicated two-fold dewaterability improvement (from 8% TS to 16% TS in cake) with polymer addition, therefore further research is recommended. From the operational experience obtained from the pilot-scale dewatering unit, the resulting dewaterability of sludge greatly depends on the optimisation of this unit, the type of polymer used, and its dosage. Therefore, if comparing sludge dewaterability is crucial, the polymer dosage should be excluded from testing if possible. Focusing on the fugate quality, phase 1 with HRTs of 8 and 12 days outperformed phase 2 with an HRT of 20 days in all observed parameters (TS, VS, DS, tCOD, N–NH4+, and TP), which, on the one hand, points to better dewaterability performance and, on the other hand, confirms that the digestion performance was sufficient even at short HRTs.

Reduction in antibiotic resistance genes. An analysis of the alterations in ARG concentrations from sludge samples before THP (MRS1) and after THP (HMRS1), after pilot AD (phase 1), and after full-scale two-stage AD (phase 2) provides a comprehensive understanding of the efficacy of these treatment processes and the potential persistence of ARGs. The reduction in ARG concentrations from MRS1 to HMRS1 suggests the effectiveness of THP in reducing ARG levels shown in Fig. 6, and hence the efficacy of THP in degrading antibiotic-resistant bacteria and ARGs in wastewater sludge.

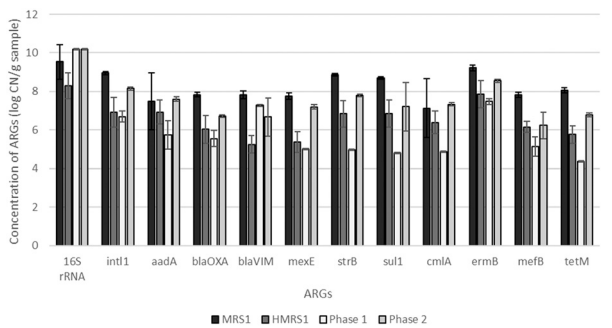

Fig. 6 Abundance of ARGs in sludge samples before (MRS1) and after THP (HMRS1) treatment and comparison of AD samples with (phase 1) and without (phase 2) THP pretreatment.

Importantly, the concentration of ARGs was mainly reduced by THP, with a maximum reduction of 3.5 log units. Further reduction is caused by AD, where the reduction is up to 1.5 log units. A trend was observed across all studied ARGs except blaVIM, where regrowth was observed. This observation is consistent with findings from studies such as that of Ju *et al.*,[56] which highlighted the persistence of ARGs during anaerobic digestion of wastewater sludge. Compared with the digested sludge sample from the pilot scale with THP pretreatment and the sample from full-scale AD, the combination of THP–TAD was most beneficial for reducing ARGs. Mechanistically, the reduction of ARGs during THP–TAD is likely governed primarily by thermal and biochemical degradation processes. During thermal hydrolysis, exposure to high temperatures (typically 160–170 °C) disrupts microbial cell membranes and denatures intracellular proteins, leading to extensive cell lysis and release of genetic material. The harsh thermal conditions are reported to cause fragmentation of plasmid and chromosomal DNA carrying ARGs, thereby diminishing their structural integrity and reducing the likelihood of horizontal gene transfer.[21] In the thermophilic anaerobic digestion (TAD) stage, these residual DNA fragments and lysed cellular components undergo further biodegradation and stabilization under sustained high temperatures. Nonetheless, complete ARG elimination is rarely achieved, as extracellular DNA and heat-tolerant mobile genetic elements can persist under thermophilic conditions, acting as potential reservoirs for resistant genes.[56]

Energy feasibility of THP–TAD

The energy performance of both stages (Table 4) was evaluated based on sludge composition and methane yield. The results show that when THP–TAD is operated without prior sludge thickening, as is the case during Phase 1, the thermal energy required to heat the sludge during THP exceeds the energy recovered from the produced biogas. In contrast, applying sludge thickening substantially improves the overall energy balance by reducing the water content and, consequently, the heat demand.[12] Under these conditions, the net specific energy balance of THP–TAD reached +4.1 kWh kg^{-1} $VS_{removed}$ (+335.7 MJ m^{-3}) while TAD (phase 2) achieved +1.5 kWh kg^{-1} $VS_{removed}$ (+111.6 MJ m^{-3}). These results are consistent with the findings of Balasundaram *et al.* (2024), who reported a negative net energy balance for TAD (−22 MJ m^{-3}) and slightly higher positive balance for THP–MAD (+384 MJ m^{-3}).[57]

Implementation of results

This study unequivocally demonstrated that the combination of THP with TAD is a viable and effective concept for treating MRS. By integrating THP, biogas production or organic reduction improved compared with TAD, but the feasibility of the pilot plant operation was confirmed under realistic operational conditions. The findings also establish that THP–

Table 4 Net energy balance of thermophilic anaerobic digestion (TAD), and thermal hydrolysis pretreatment followed by TAD (THP–TAD) under different feed conditions. Negative values represent energy demand, and positive values represent recoverable energy. The thickened THP–TAD scenario assumes 16.5% total solids prior to THP

	THP–TAD (phase 1)	THP–TAD (phase 1, thickened 16.5% TS)	TAD (phase 2)
Thickening	0	−0.030	0
THP-Heating	−16.4	−10.1	0.0
THP-Heat recovery flash evaporation	4.2	2.6	0.0
THP-SUM	−12.3	−7.6	0.0
AD-Heating	0.0	0.0	−2.4
Energy biogas	4.0	11.6	3.9
AD-SUM	4.0	11.6	1.5
Net balance (kWh kg^{-1} $VS_{removed}$)	−8.2	4.1	1.5
Net balance (MJ m^{-3})	−454.5	335.7	111.6

TAD can effectively handle fluctuating sludge qualities and short hydraulic retention times (HRTs) of 8–12 days, ensuring stable performance and robust outcomes.

As proven in this study, shortening the HRT in TAD offers transformative benefits. It allows for a reduction in digester size, which directly minimises capital investments (reactor size). This feature is especially critical for WWTPs seeking to expand their capacity or optimise costs without additional space requirements. Furthermore, THP–TAD represents a sustainable solution by enabling existing infrastructure to manage higher sludge loads, improving energy efficiency, and reducing the environmental footprint of sludge treatment. Despite the improved performance and intensification of the TAD process, this study highlights a trade-off: the enhanced performance of THP–TAD is accompanied by increased energy demands associated with the THP and increased temperature in AD. Energy integration and optimisation at the plant level are thus essential for maximising efficiency and ensuring the long-term viability of this approach, which was also demonstrated in the energy net balance.

The results also emphasise the importance of sludge characteristics in process outcomes. While the study achieved stable operation under pilot-scale conditions, a greater degree of raw sludge thickening before THP has yet to be tested. Further investigations are necessary to prove that THP–TAD is a working concept even when the MRS is thickened to 16.5% TS, with a primary focus on the ammonia concentration, methane yield and volatile fatty acid (VFA) profile. These factors could significantly influence the operational stability and economic feasibility of THP–TAD systems.

To conclude, this study contributes critical insights into the optimisation of anaerobic digestion processes. The data underscore the need for further research into sludge thickening and energy optimisation strategies to fully realise the potential of THP–TAD as a sustainable and efficient sludge management technology. Addressing these knowledge gaps will facilitate a more robust understanding of the system's dynamics, ultimately supporting its broader adoption in wastewater treatment practices.

Conclusions

This study provides a comprehensive analysis of the integration of THP with TAD in the treatment of MRS.

- The pilot-scale operation validates THP–TAD as a technically and operationally viable concept, capable of significantly enhancing sludge treatment efficiency under real-world conditions. Our THP–TAD operated at short HRTs of 8 and 12 days and achieved a VSR, biogas production, and methane yield comparable to those of TAD alone with a standard 20-day HRT. This improvement can reduce the required digester size to 60–40% or offer the potential to manage increased sludge volumes without additional investments in reactor capacity.
- Additionally, THP pretreatment of MRS improved the reduction in ARGs by 3.5 log units, whereas hygienization of sludge can be achieved only by TAD. To the best of the authors' knowledge, this is the first study realistically appreciating the benefits of THP–TAD with an appropriately adapted inoculum.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5ew00456j.

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