Bioresource Technology 340 (2021) 125705

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Thermophilic (55 °C) and hyper-thermophilic (70 °C) anaerobic digestion as novel treatment technologies for concentrated black water

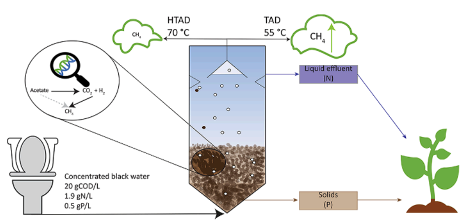
Marinus J. Moerland [a], Laura Castañares Pérez [a], Maria E. Ruiz Velasco Sobrino [a], Paraschos Chatzopoulos [b], Brendo Meulman [b], Vinnie de Wilde [a], Grietje Zeeman [c], Cees J. N. Buisman [a], Miriam H.A. van Eekert [a,*]

[a] Department of Environmental Technology, Wageningen University and Research, Bornse Weilanden 9, 6706 WG Wageningen, the Netherlands
[b] DeSaH B.V, Pieter Zeemanstraat 6, 8606 JR Sneek, the Netherlands
[c] LeAF B.V, Bornse Weilanden 9, 6706 WG Wageningen, the Netherlands

HIGHLIGHTS

- Thermophilic AD of black water (BW) results in 70% CODt removal.
- Compared to mesophilic AD, higher loading rates can be applied during TAD of BW.
- A thermophilic UASB treating BW can be started up in 12 days.
- Hyper-thermophilic AD of BW results in 38% methanisation of CODt.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Thermophilic anaerobic digestion
Hyper-thermophilic anaerobic digestion
Resource recovery
Black water
New sanitation
Methanogenesis

ABSTRACT

Thermophilic and hyper-thermophilic anaerobic digestion (AD) are promising techniques for the treatment of concentrated black water (toilet fraction of domestic wastewater collected by low flush volume toilets; BW), recovery of nutrients and simultaneous pathogen removal for safe recovery and reuse of those nutrients. This study showed that thermophilic AD (55 °C) of concentrated BW reaches the same methanisation and COD removal as mesophilic anaerobic treatment of BW (conventional vacuum toilets) and kitchen waste while applying a higher loading rate (OLR) (2.5–4.0 kgCOD/m^3/day). With a retention time of 8.7 days, and an OLR of >3 kgCOD/m^3/day, COD removal of 70% and a methanisation of 62% (based on COD$_t$) was achieved during thermophilic AD. Hyper-thermophilic (70 °C) reached lower levels of methanisation (38%). Start-up time of thermophilic AD was 12 days. And during thermophilic AD, a shift from acetoclastic methanogenesis towards syntrophic acetate oxidation was observed.

1. Introduction

The growing world population causes increasing pressure on the environment and on agriculture for food supply, and increases the demand for nutrients as nitrogen (N), phosphorous (P) and potassium (K). Recovery and reuse of these nutrients from domestic waste water is still

* Corresponding author.
E-mail addresses: merijn.moerland@wur.nl (M.J. Moerland), lcastanares@aguasigma.com (L. Castañares Pérez), emma.ruiz@nijhuisindustries.com (M.E. Ruiz Velasco Sobrino), p.chatzopoulos@desah.nl (P. Chatzopoulos), b.meulman@wmd.nl (B. Meulman), vinnie.dewilde@wur.nl (V. de Wilde), grietje.zeeman@wur.nl (G. Zeeman), cees.buisman@wur.nl (C.J.N. Buisman), miriam.vaneekert@wur.nl (M.H.A. van Eekert).

https://doi.org/10.1016/j.biortech.2021.125705
Received 17 June 2021; Received in revised form 27 July 2021; Accepted 28 July 2021
Available online 4 August 2021
0960-8524/

limited whilst alternative resources than the current natural rock reserves or atmospheric nitrogen for NPK fertilizers are needed for a more sustainable agrofoodsystem (Kujawa-Roeleveld and Zeeman, 2006).

Worldwide, as was estimated by Cordell et al. (2009), the amount of P excreted by humans equalled 21% of the annual P used as artificial fertilizer, however in most countries current legislative restrictions, social acceptance and technological challenges prevent the utilization of these nutrients present in domestic waste streams. Legislative restrictions mainly concern the presence of pathogens and heavy metals in faecal matter (Collivignarelli et al., 2019; Harder et al., 2019; Zeeman et al., 2008), whereas technological challenges are mainly caused by dilute waste streams (Verstraete et al., 2009; Zeeman and Kujawa-Roeleveld, 2011), which are unfavourable for nutrient recovery and energy production through AD. Within the Horizon2020 project Run4-Life (www.run4life-project.eu), a new approach is proposed based on source separation of domestic wastewater to obtain concentrated waste streams in combination with (hyper-)thermophilic anaerobic digestion ((H)TAD) for simultaneous treatment and disinfection (Bisschops et al., 2019).

Source-separated collection of domestic waste water results in different waste streams and excludes rainwater and industrial wastewater along with its contaminants (Tervahauta et al., 2014). Treatment methods for nutrients, water and energy recovery can be tailor-made for each stream (Hammes et al., 2000; Zeeman and Kujawa-Roeleveld, 2011; Zeeman et al., 2008).

The toilet fraction (black water; BW) has a high organics concentration, which can be converted to methane during AD (de Graaff et al., 2011; Tervahauta et al., 2013). Vacuum collection by ultra-low flush volume vacuum toilets, resulting in a concentrated BW stream, makes the stream suitable for energy and nutrient recovery (NPK) through (hyper)thermophilic AD (de Graaff et al., 2011; Verstraete et al., 2009), whilst minimizing the energy input for heating purposes (Tervahauta et al., 2013; Verstraete et al., 2009; Zeeman et al., 2008). By implementing these ultra-low flush volume vacuum toilets (<1 L) it is expected to obtain a concentrated BW stream (20–30 gCOD/L) which contains enough COD to match energy required for heating purposes of (H)TAD (based on 60% methanisation, and the heat capacity of water). Decentralised treatment of source-separated BW is already applied in multiple places in The Netherlands (de Wit et al., 2018) and other countries around the world (Abdel-Shafy et al., 2009; Gao et al., 2019).

Anaerobic treatment of BW (conventional vacuum toilets) has been successfully demonstrated by de Graaff et al. (2010) in an Upflow Anaerobic Sludge Blanket (UASB) reactor at 25 °C with a hydraulic retention time (HRT) of 8 days, and organic loading rate (OLR) of 1 kgCOD/m^3/day resulting in 78% COD removal and 54% COD conversion to methane, which indicates the potential of anaerobic treatment of separately collected BW. However, to ensure safe reuse of recovered nutrients, sufficient pathogen removal is required, for which thermophilic (55 °C) and hyper-thermophilic (70 °C) AD are proposed in this study. A previous study shows that efficient pathogen removal under these conditions is feasible (Moerland et al., 2020). Furthermore, (hyper-)thermophilic AD has the potential of higher removal efficiencies and lower retention times (Ryue et al., 2020; Wu et al., 2020) as compared to mesophilic AD. High efficiencies and low retention times are beneficial in terms of decreased treatment time and thus decreased reactor size. Also, energy costs are lower in these smaller reactors (Ho et al., 2014). On the other hand, (hyper-)thermophilic conditions are often associated with VFA accumulation and ammonia inhibition, which is a challenge for the development of (H)TAD, especially since the concentrated BW potentially has a high nitrogen concentration which could lead to increased ammonia toxicity effects (Ahring et al., 2001; Zinder et al., 1984). Zhang et al. (2020) showed that despite these challenges, thermophilic treatment of concentrated BW in a UASB reactor performed by could result in 84% total COD removal and 57% methanisation with a long HRT of 20 days and an OLR of 3.5 kgCOD/m^3/day. In the current study, shorter HRTs and lower COD:N and COD:P ratios are applied compared to Zhang et al. (2020) in order to fully optimise (H)TAD processes for BW treatment in terms of decreased operation time and thus efficient treatment. Per capita 3.9, 0.47 and 1.4 kg N, P and K could respectively be recovered annually and in this light (hyper)thermophilic AD has certain benefits over mesophilic treatment, amongst which pathogen removal for safe nutrient recovery, potentially higher microbial conversion rates which result in higher possible loading rates, and shorter HRTs.

In this paper we aim to show the potential of thermophilic (55 °C) and hyper-thermophilic (70 °C) anaerobic digestion of vacuum-collected BW with UASB reactors. In this study short HRTs will be employed at (hyper-)thermophilic conditions with long-term operation, aiming at the development of a robust treatment process in order to improve the reuse potential of the nutrients in concentrated BW.

2. Materials and methods

2.1. Seed sludge, BW collection and feeding strategy

BW was collected at the Department of Environmental Technology of Wageningen University and Research through ultra-low flush volume vacuum toilets (Qua-vac B.V., Almere, The Netherlands, type: EVAC VT910). Flush volumes were set to 0.2 and 0.5 L/flush for small and big flushes respectively. Collected BW was stored in a stirred tank with a volume of 200L with an estimated retention time of 7 days at a temperature of 4 to 10 °C. From this tank, BW was transported batch-wise to a cooled (4–7 °C, HRT: 7 days) buffer tank and the reactors were supplied with BW from this buffer tank. The reactors were placed in an experimental cabin that was not temperature controlled, but the average temperature was 25 °C ± 5 °C as was logged by an analogue thermometer. During the stable operation phases I, II and III of the thermophilic and hyper-thermophilic reactors and the start-up phase of the TAD start-up reactor (See section 2.2), the BW was circulated by a Watson Marlow Qdos 120 pump with a flow of 4–6 L/h. From this

Table 1
Average composition of BW influent during the different stable operation phases.

	Phase[a]	TAD start-up	Phase I	Phase II	Phase III[b]
	Unit				
pH	[-]	7.4 ± 0.11	7.4 ± 0.18	7.5 ± 0.12	7.6 ± 0.08
COD	[gCOD/L]	14 ± 2.8	14 ± 2.4	16 ± 1.8	20 ± 3.6
$COD_{suspended/colloidal}$	[gCOD/L]	9.9 ± 2.7	10 ± 2.4	12 ± 1.9	15 ± 3.4
$COD_{soluble}$	[gCOD/L]	4.4 ± 0.39	4.4 ± 0.44	3.7 ± 0.34	4.4 ± 0.79
VFA	[gCOD/L]	3.0 ± 0.44	3.8 ± 1.0	2.3 ± 0.48	2.4 ± 0.78
N_T	[gN/L]		1.4 ± 0.16	n.d.	1.9 ± 0.15
P_T	[gP/L]		0.51 ± 0.30	0.27 ± 0.01	0.44 ± 0.06

[a] For detailed description of operational phases refer to section 2.2.
[b] During Phase III, influent BW consisted of a 1:1 mix of BW from DeSaH offices in Sneek, The Netherlands and the department of Environmental Technology of Wageningen University and Research.

circulation stream the UASB reactors were pulse-fed with three separate Masterflex L/S® peristaltic pumps. Biogas was quantified at 25 °C with a Ritter(®) drumtype gasflow meter, through which biogas was pumped with a pressure-controlled gas pump. The composition of the vacuum collected BW during the three different operational phases is shown in Table 1. The total run time of the system was 719 days. During Phase I and the start-up phase BW was more dilute, because flushing volumes were not yet optimised. During Phase III, where the accessibility to the department was still limited due to COVID-19, the COD concentration was increased with concentrated BW from DeSaH B.V., Sneek, The Netherlands.

All three reactors were inoculated with thermophilic digestate (55 °C) from a sludge digester in Echten, The Netherlands. This digestate was selected, because from all available digestate sources it was most adapted to similar conditions as the (hyper-)thermophilic BW reactors.

Each reactor was inoculated with 2L sludge (15 g/L VS, 35 g/L TS).

2.2. UASB reactor operation

To assess the feasibility of TAD and HTAD, three glass UASB reactors (internal diameter 110 mm, height 675 mm) with a working volume of 4.9L were operated at 55 °C (two reactors) and 70 °C respectively. The reactors were heated by a mantle, through which heated water (55 °C) or oil (70 °C) was circulated. The functioning of the temperature control was confirmed by manual temperature measurements inside the reactor. One of the 55 °C reactors was started up at a later stage (after 279 days) to establish a quick start-up protocol for the thermophilic anaerobic digestion (TAD start-up). Different phases, which are summarised in Table 2, can be distinguished from the period of operation. The relatively long start-up phase (Phase 0) is characterized by low organic loading rates and low methanisation levels. Low organic loading rates were a consequence of multiple factors, but first and foremost of unexpectedly low influent COD concentrations, and knowingly low pumping rates to prevent overloading and other factors associated with the start-up of a reactor with high suspended solids concentrations in combination with real-life BW.

Steady states were achieved during Phase I, II and III. These steady states are defined as periods after a minimum start-up of 6 solid retention times (SRTs) in which the average organic loading rate (OLR) is at least 2 kgCOD/m^3/day and has a standard deviation which is lower than 50% of the average OLR. The phases can be distinguished in phases with low (Phase I) and high (Phase II and III) OLRs, being 2.5 and 3–4 kgCOD/m^3/day respectively. During the COVID-19 lock-down (day 499–607) access to the laboratory was limited and thus the amount of produced black water was decreased. As a consequence, the OLR had to be decreased by decreasing the flow rate.

2.3. Analytical methods

Twice a week, grab samples with a volume of 50 mL were taken for COD_{total}, $COD_{soluble}$, VFA. Additionally, grab samples were taken on a regular basis for N_T, P_T, total ammonia nitrogen, *ortho*-phosphate. TS and VS were analysed monthly. Total and soluble COD were measured with Hach-Lange COD kits (LCK314). For soluble COD samples were centrifuged at 10,000 rpm for 10 min and the supernatant was filtrated over a washed 0.45 µm filter (CHROMAFIL®, MACHERY-NAGEL, Düren, Germany). Other centrifuged samples were stored at −20 °C for later VFA analysis. The dry matter content of the sludge was determined using standard methods (US-EPA, 2001 method 1684). Small- and medium-chain fatty acids (up to C-8) were analysed by gas chromatography (Agilent 7890B GC, USA) in liquid samples, which were centrifuged at 10,000 rpm for 10 min to remove suspended material and subsequently diluted with a formic acid water solution (to a final 1.5% v/v concentration) and milli-Q water and analysed as described by Sudmalis et al. (2018). Occasionally, samples were filtered over 0.45 µm filters when solids were observed. Oxygen, nitrogen, methane and carbon dioxide content in the biogas was measured twice a week with a GC (Shimadzu GC-2010, Japan) with thermal conductivity detection (TCD) and a GC (HP5890, USA) with TCD for hydrogen as described by Chen et al. (2016). The pH was measured with an electrode (PHM210/HQ440D). Nutrients were analysed with Hach-Lange kits, LCK338 for total nitrogen, LCK303 for total ammonia nitrogen, and LCK350 for both total- and orthophosphorous. Samples for total ammonia and *ortho*-phosphate were centrifuged for 10 min at 10,000 rpm prior to analysis. Inductive Coupled Plasma – Optical Emission spectrometry (ICP-OES, PerkinElmer Avio 500, Ohio, United States) was used for magnesium and calcium measurements.

2.4. Microbial community analysis

A sample of the sludge bed at different heights (150 and 220 mm) in each reactor after 209 and 16 days for the (H)TAD and TAD-start-up reactors respectively and from the seed sludge were used for microbial community analysis. Samples were centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and the pellet was stored at −20 °C. DNA extraction, using the Powersoil DNA isolation kit and sequencing, was performed as described by de Leeuw et al. (2019). Selected operational taxonomic unit (OTU) sequences were used in a blast search in the NCBI nucleotide database. Microbiota raw sequencing data are submitted to the ENA database (https://www.ebi.ac.uk/ena) under acces-

Table 2

Characterisation of the defined operational phases and average applied OLR.

TAD/HTAD			TAD start-up		
Phase	Characteristic	Average OLR (kgCOD/m^3/day)	Phase	Characteristic	Average OLR (kgCOD/m^3/day)
Phase 0 (Day 0–180)	Start-up	TAD: 1.6 ± 1.0 HTAD: 1.6 ± 1.2	Start-up	Quick start-up at medium OLR	2.2 ± 0.95
Phase I (Day 181–357)	Low OLR	TAD: 2.5 ± 0.89 HTAD: 2.5 ± 1.2			
Transition phase (Day 358–430)	Gradual increase of OLR				
Phase II (Day 431–498)	High OLR	TAD: 3.1 ± 1.0 HTAD: 3.8 ± 1.3			
COVID-19 (Day 499–607)	Low OLR due to decreased collection of BW	TAD: 2.0 ± 0.72 HTAD: 2.3 ± 0.84			
Phase III (Day 608–719)	Recovered OLR	TAD: 3.7 ± 1.1 HTAD: 3.3 ± 0.82	Phase III	High OLR	3.9 ± 1.6

sion number PRJEB43366.

2.5. Calculations

The main parameters that were obtained to assess the reactor performance were COD removal, methanisation, pH and VFA effluent concentrations. Both COD removal and methanisation were calculated as a percentage of the total incoming COD.

The COD balance was determined for each phase in the TAD and HTAD reactor. The load for total, soluble and VFA load was determined as the sum of products of the total influent mass and average concentrations of the respective fractions. Additionally, the total methane production was calculated as a product of the average methane fraction of the biogas and average normalised gas production per day and the duration of the respective phase. Subsequently, the produced methane load was converted to COD. All values were expressed as a fraction of the

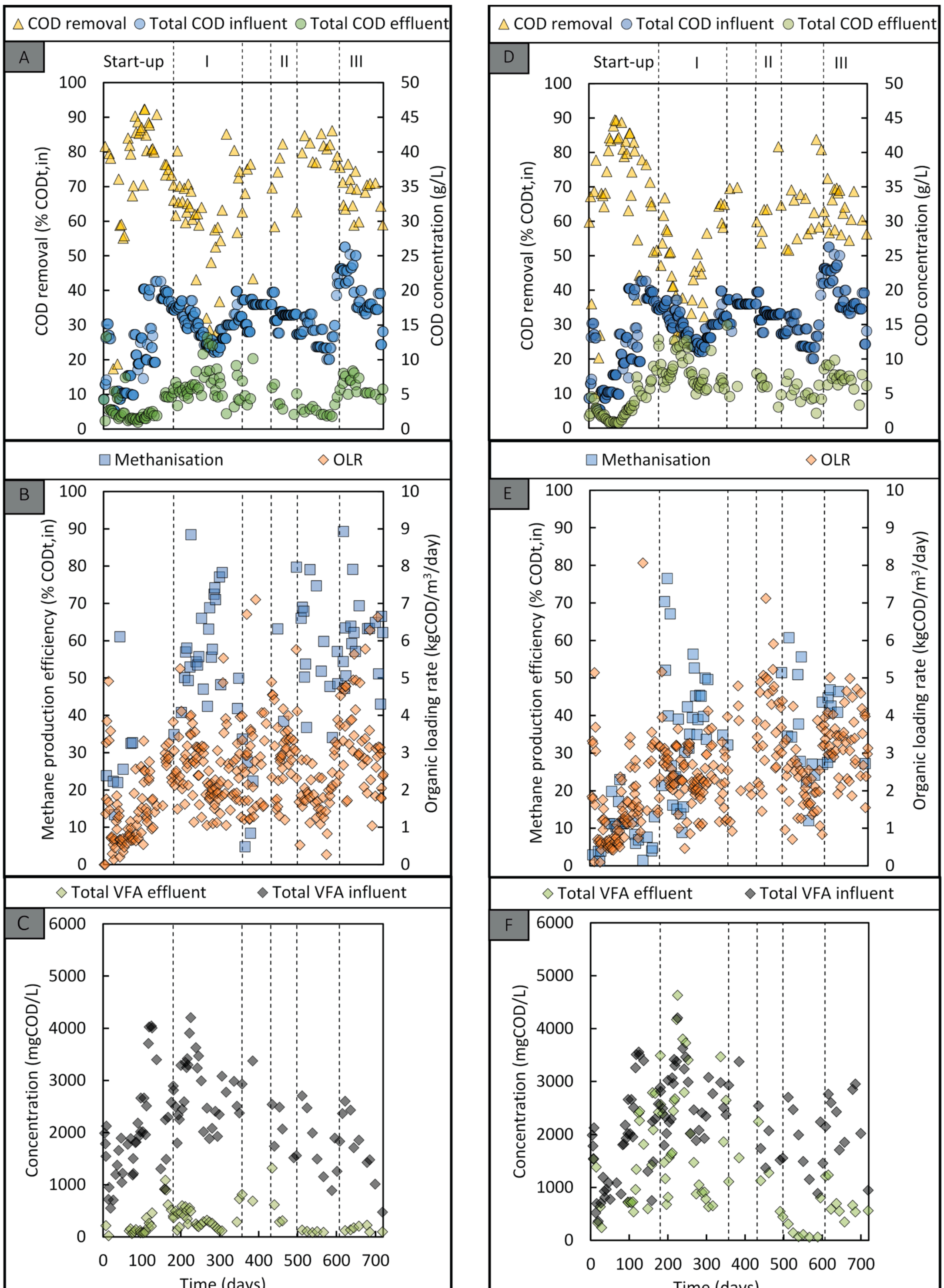


Fig. 1. Operation data for UASB reactors: TAD (ABC) and HTAD (DEF), containing influent and effluent total COD concentrations and resulting COD removal (A and D), methane production and organic loading rate (B and E) and total influent and effluent VFA concentrations (C and F).

Table 3
Overview of operational parameters and performance data for the 55 and 70 °C reactor during each phase. Data obtained by de Graaff et al. (2010) is shown in the last column as a reference.

Operation days		Phase 0 – start-up 0-180		Phase I 181-357		Phase II 431-498		COVID-19 499-607		Phase III 608- 719			De Graaff
Temperature	°C	55	70	55	70	55	70	55	70	55	55 (start-up reactor)	70	25
OLR	[kgCOD/m^3/day]	1.6 ± 1.0	1.6 ± 1.2	2.5 ± 0.89	2.5 ± 1.2	3.1 ± 1.0	3.8 ± 1.3	2.0 ± 0.72	2.3 ± 0.84	3.7 ± 1.1	3.9 ± 1.6	3.3 ± 0.82	1.0
HRT	[days]	7.5 ± 3.2	7.8 ± 3.3	6.5 ± 2.3	6.8 ± 3.5	6.0 ± 2.1	5.0 ± 2.0	8.7 ± 6.3	7.3 ± 4.2	6.1 ± 1.4	5.4 ± 2.2	6.2 ± 0.93	8.7
COD removal	[%COD$_{t,in}$]	75 ± 17	68 ± 19	60 ± 13	47 ± 12	71 ± 8.3	63 ± 8.3	81 ± 3.2	64 ± 9.8	70 ± 4.4	63 ± 9.1	63 ± 5.5	78
COD$_{col+SS}$ removal	[%COD$_{col+SS, in}$]	76 ± 21	76 ± 16	47 ± 31	61 ± 10	78 ± 9.8	74 ± 9.3	85 ± 39	65 ± 12	71 ± 4.6	70 ± 8.2	68 ± 8.9	
Methanisation	[%COD$_{t,in}$]	29 ± 13	9.3 ± 5.5	57 ± 14	37 ± 16	NA[a]	NA	57 ± 13	35 ± 14	62 ± 13	47 ± 8.9	38 ± 7.5	54[b]
VFA effluent	[gCOD/L]	0.37 ± 0.31	1.5 ± 0.90	0.36 ± 0.18	1.9 ± 1.2	0.61 ± 0.42	1.1 ± 0.64	0.12 ± 0.04	0.15 ± 0.11	0.20 ± 0.06	0.38 ± 0.24	0.57 ± 0.09	0.25
pH effluent	[-]	8.3 ± 0.29	8.3 ± 0.34	8.1 ± 0.19	8.0 ± 0.63	8.5 ± 0.10	8.3 ± 0.25	8.2 ± 0.09	8.3 ± 0.09	8.4 ± 0.12	8.4 ± 0.12	8.5 ± 0.09	

[a] Not Available, only a single measurement for methanisation was performed in phase II for the HTAD, and two for TAD. Therefore, no methanisation data is shown for Phase II; [b]Methanisation was based on the total COD mass balance over the entire operation period.

total COD load in the influent.

3. Results and discussion

3.1. Reactor performance

Two UASB reactors were operated for over 700 days, one at thermophilic (TAD) and one at hyper-thermophilic conditions (HTAD). Fig. 1 shows the COD removal (a, d), OLR and methanisation (b, e) and VFA profile for the influent and effluent (c, f). All relevant data are summarized per operational phase in Table 3. The thermophilic reactor outperformed the hyper-thermophilic reactor in terms of COD removal and methanisation. Additionally, the thermophilic reactor more adequately recovered from increased loading rates and suffered VFA accumulations to a lower extent.

3.1.1. Reactor performance under thermophilic conditions

During start-up, the COD removal was 80–90% in the TAD reactor, however methanisation was only 30%. Possibly, low organic loading rates and sub-optimal quantification methods (e.g. gas flow meters with low counterpressure) caused this low methanisation. As consequence of the gradual OLR increase to 2–3 kgCOD/m^3/d during the start-up, the COD removal dropped slightly to 75% at day 160. This also resulted in a VFA peak, but the total VFA effluent concentration had a maximum of 1.1 gCOD/L. This indicates that the methanisation was limiting anaerobic digestion at least during an increase in organic loading.

After the start-up phase, stable operation (in terms of OLR and methanisation) was achieved during Phases I, II and III. Phases I and II were separated by an intermediary phase to adapt to high OLRs. Phase II was abruptly ended by the COVID-19 pandemic (days 499–607) during which only low loading rates solely for the survival of the biomass could be applied. During COVID-19 the OLR was decreased to the level of the start-up phase, however methanisation was 57% with 81% COD removal, indicating that the biomass had adapted in the meantime. Throughout all phases the OLR increased, resulting in OLRs of 2.5, 3.1 and 3.7 kgCOD/m^3/day during Phase I, II and III respectively.

The methanisation was 57% on average during Phase I. Between day 252 and 293, the COD removal dropped to 30%, but VFA effluent concentrations remained below 0.5 gCOD/L, which consisted mainly of acetate and propionate, 65% and 34% of the total effluent VFAs respectively. The drop in COD removal is likely a consequence of fluctuations in the OLR. The COD removal recovered to approximately 80% on day 316 and was 60% on average during Phase I. The transition phase resulted in a slight increase in VFA concentration as a consequence of the increase in loading rate. With this increased loading rate of 3.1 kgCOD/m^3/day in Phase II, the COD removal was 71%. At the end of Phase II the effluent VFA concentration recovered to a concentration of 0.25 gCOD/L, which was similar to de Graaff et al. (2010). Phase III had the highest loading rate with 3.7 kgCOD/m^3/day on average resulting in 70% COD removal and 62% methanisation and a low VFA effluent concentration of 0.20 gCOD/L. The study of de Graaff et al. (2010) showed that 78% of the COD can be removed and 54% of the total COD can be converted to methane at mesophilic conditions.

Cunha et al. (2018) achieved 87% COD removal and 65% methanisation at similar conditions. Cunha and de Graaff employed an operation temperature of 25 °C, HRT of 8 days and an OLR of 1 kgCOD/m^3/day (Cunha et al., 2018; de Graaff et al., 2010). More recently, two studies on BW TAD were performed (Zhang et al., 2020; Zhang et al., 2021). Zhang et al. (2020) found 56.7% methanogenesis with BW (33.5 gCOD/L) treatment in a 2L UASB, but with a retention time of 20 days. In another study, Zhang et al. (2021) found similar methanogenesis in the same UASB reactor, but at a high loading rate (12.3 kgCOD/m^3/day) and a low HRT (2.5 days). The TAD in the current study achieved similar performance with regards to COD removal (70–71% or 81% at low loading rates during the COVID-19 phase which was a consequence of increased COD$_{colloidal+ss}$ removal) and methanisation rates (57-62%) as previous BW studies. However, our results were obtained by operating at a lower HRT (6–8 days) or at 3 times higher loading rates compared previous anaerobic digestion studies of (concentrated) BW, except for the study performed by Zhang et al. (2021). However, results in the latter study were based on short steady states (20 days) and the effects on long term operations were not studied. The current study shows that TAD of concentrated BW is stable for a long period under the applied OLRs and HRTs, which is essential for full-scale application. To fully explore the potential of thermophilic anaerobic digestion of concentrated BW, future experiments should focus on the further decrease of the HRT and SRT and subsequent increase in loading rate during long-term steady state operation. For instance, thermophilic treatment in CSTRs was successfully applied with HRTs of 3–4 days as described by Ho et al. (2014) for waste activated sludge. Based on studies with other thermophilic CSTRs treating (dewatered) sludge, the minimum SRT that should be maintained is 10–15 days (Ferrer et al., 2010; Nges and Liu, 2010). In the thermophilic UASB reactor used in the current study, the SRT during Phase III in the TAD reactor was estimated to be 30 days based on a model as proposed by Zeeman and Lettinga (1999). If the SRT can be reduced to 10 days, a maximum OLR of 10 kgCOD/m^3/day can be achieved, based on the assumption that hydrolysis rates and biomass concentrations remain stable.

For future implementation of decentralized sanitation and treatment, TAD is a promising technique, because of the high COD removal and conversion rate to methane. The achieved methanisation of 60% matches with the demand for an energy neutral treatment process for black water based on assumed energy consumption for heating and energy recovery through methane.

3.1.2. Lower methane production during hyper-thermophilic anaerobic digestion

During start-up the OLR of the HTAD reactor was increased to 2–3 kgCOD/m^3/day to gradually transit to Phase I. Peaks in OLR resulted in a drop in COD removal and effluent VFA concentrations during this start-up phase.

The COD removal dropped to 30% in the beginning of Phase I during HTAD, resulting in effluent VFA concentrations as high as the influent VFA concentrations. Mainly acetate and propionate accumulated in the reactor, while methanisation levels dropped to 10%. Around day 280 the reactor was stabilised, VFA effluent concentrations were below 1 gCOD/L again and the COD removal and methanisation recovered to 50–60% and 30–40% respectively. On average the methanisation was 37% with a maximum of 55%. During Phase II the effluent VFA concentration dropped to 0.7 gCOD/L and COD removal increased to an average of 63%, methanisation was 51%, based on a single measurement. The high VFA concentrations in the reactor, combined with lower methane production and COD removal compared to TAD indicate that HTAD is less suitable for concentrated BW treatment. Furthermore, an increase in OLR resulted in a temporary drop in COD removal and was accompanied by high effluent VFA concentrations indicating that the HTAD is more sensitive towards increasing the OLR.

Both the temperature and increased ammonia toxicity could be responsible for the decreased performance of HTAD. Ho et al. (2014) found that a negative effect of increased temperatures in the range of 55–65 °C at short HRTs on acetoclastic methanogens. In the current study, decreased viability of (acetoclastic) methanogens at hyper-thermophilic conditions could explain the decreased methane production and increased VFA concentrations as compared to TAD. Additionally, an increase in temperature leads to an equilibrium shift towards free ammonia (NH_3). At the conditions of the HTAD reactor (70 °C and a pH of 8.3) roughly 50% of the total ammonium is present in the form of free ammonia (FA) which means that concentrations in the HTAD reached 750 mg/L NH_3 (Hafner et al., 2006). Furthermore, it is known that especially methanogens are sensitive towards FA (Wu et al., 2020). The inferior performance of HTAD is probably thus a consequence of decreased microbial activity combined with the short HRTs that were applied and increased FA toxicity.

3.2. Thermophilic anaerobic digestion of BW reaches stable and good performance after 12 days

Thermophilic AD had good performance and outcompeted hyper-thermophilic AD, however the start-up time could be optimised for which another reactor was operated to determine the minimum start-up time for TAD only. During the start-up of the long-term TAD reactor, the loading rate was low as a consequence of operational problems and low concentration of BW. Also, a state-of-the-art start-up methodology for thermophilic BW treatment was lacking. Although some work is done on thermophilic AD processes, most studies focus on the comparison between direct and step-wise increasing from mesophilic to thermophilic temperatures (Boušková et al., 2005; Palatsi et al., 2009; Shin et al., 2019). Since the start-up strategy consisted of inoculating with thermophilic sludge, a step-wise temperature increase was not considered for both TAD and HTAD. Additionally, the OLR management is in general considered to be crucial for a successful start-up of a thermophilic reactor (de la Rubia et al., 2013). An OLR of 2–3 kgCOD/m^3/day was applied from the start. This was demonstrated in a second start-up reactor, showing a more rapid start-up as compared to the long-term TAD reactor as a consequence of a more stable but foremost higher OLR from the start. In Fig. 2 the performance of the TAD start-up reactor is shown. High COD removal and methanisation was obtained after 12 days, the reactor performance was followed up to 80 days to confirm stable operation after the rapid start-up. The obtained results indicate that a high and stable OLR results in a quick start-up. Feeding started directly after inoculation with the same seed sludge as in the (H)TAD reactors, which shows that the appropriate seed sludge was selected.

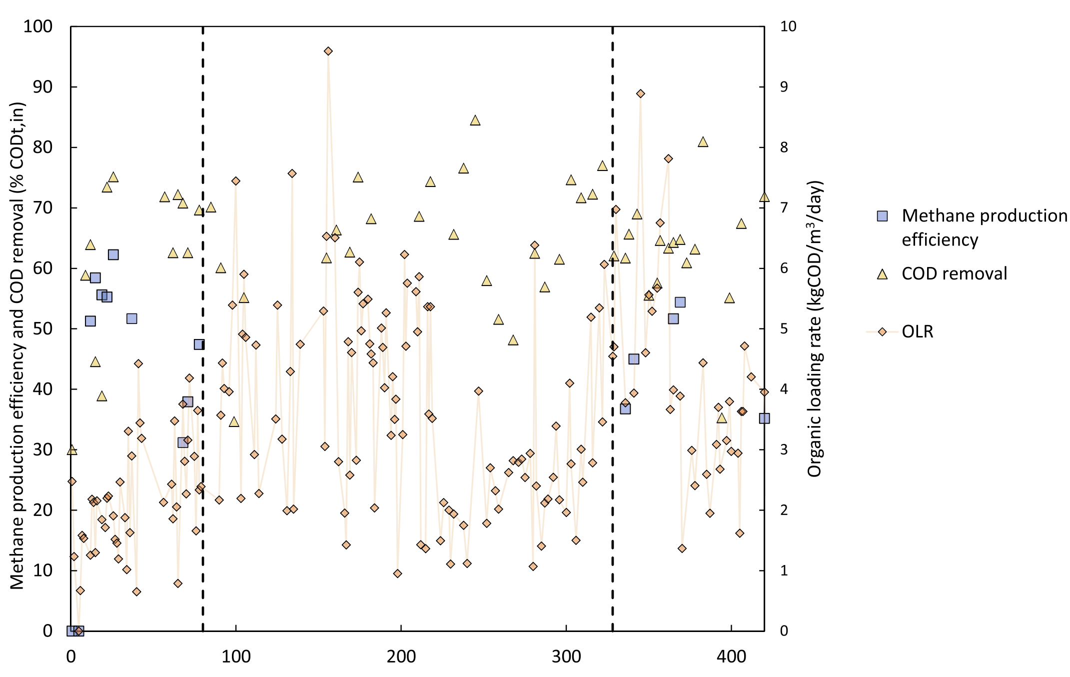


Fig. 2. Performance of the TAD start-up reactor. The first 80 days (indicated with the first dashed line) represent the start-up. After 328 days (second dashed line) biogas quantification was recovered and the loading rate was increased (Phase III).

After one day, the COD removal was 30%, probably as an effect of solids retainment. The COD removal doubled to roughly 60% after 12 days at the point when also the first biogas was produced, which resulted in 50% methanisation. This percentage increased slightly to 50–60%, similar to what was achieved during the long-term TAD. A short drop in methane production efficiency was observed, which was possibly caused by a short drop in loading rate. While the COD removal remained stable, the methane production efficiency recovered. Comparable continuous reactor studies with thermophilic seed sludge resulted in start-up periods of 36 days (Poh and Chong, 2014), or recovery from a single step increase from mesophilic to thermophilic conditions in 20 days (Palatsi et al., 2009).

After an intermediary phase without biogas quantification in the TAD start-up reactor, the loading rate was increased (from day 328 onwards). During this phase, the loading rate was 3.9 kgCOD/m^3/day on average with 47% conversion to methane. Like in the long-term TAD reactor, the TAD start-up reactor showed a slight drop in COD removal as a consequence of the increased loading rate, but methanisation efficiencies remain high and are comparable to methanisation efficiencies achieved at lower OLR in the long-term TAD reactor.

3.3. COD balance

During Phase I over 90% of the influent COD load is recovered in the effluent or biogas. The TAD mass balance (Fig. 3) of Phase III also shows 90% recovery of COD, however during Phase II (41 and 38% of missing COD for TAD and HTAD respectively) and Phase III (22% missing COD) in the HTAD the COD mass balance is incomplete. During Phase II, this is possibly a result of the increased suspended COD in the influent, which might have been entrapped in the sludge bed. In this case the missing COD could be lost through peak losses in the effluent. No sludge was removed from the reactors other than sludge required for batch experiments, which does not explain the mass balance during Phase II. Also sludge growth does not explain the gap, since the 40% COD mass would result in roughly 20 L of sludge (based on 1.4 gCOD/gVS and 15 gVS/L for the sludge solids content), which is not realistic since that is 4 times the total reactor volume. During Phase I the effluent VFA was higher for the HTAD compared to the TAD. Similar results were obtained in Phase II and III, but these could also be a consequence of the incomplete COD balance. However, previous studies reported increased accumulation of VFAs at elevated temperatures (Ahring et al., 2001; Nges and Liu, 2010).

3.4. Methanisation and COD removal is independent of the applied OLR

Multiple OLRs were applied during the different stable operational phases of the long-term TAD and HTAD within the range of 1.5–4 kgCOD/m^3/day. In Fig. 4 it can be seen that over this range of OLRs the methanisation and COD removal are stable for the thermophilic reactor, indicating that both process parameters are independent of the loading rate within the range of tested OLRs (The start-up phase was disregarded in this analysis). It could be the case that during the whole operation period the hyper-thermophilic reactor was underloaded and the system overall requires a higher loading rate, since increased temperatures lead to increased microbial growth rates (van Lier, 1995) but also higher decay rates (Kim et al., 2002). The higher NH_3 concentrations in the reactor could have had an inhibitory effect as discussed above in section 3.1.2 Also, longer periods of underloading could lead to insufficient net growth of methanogens and subsequently higher VFA in the effluent. For instance Liu et al. (2017) found that the optimal loading rate for thermophilic AD was higher than for mesophilic AD for the treatment of food waste. Additional experiments at higher loading rates could further explore the potential of (hyper-)thermophilic AD of BW and the relation

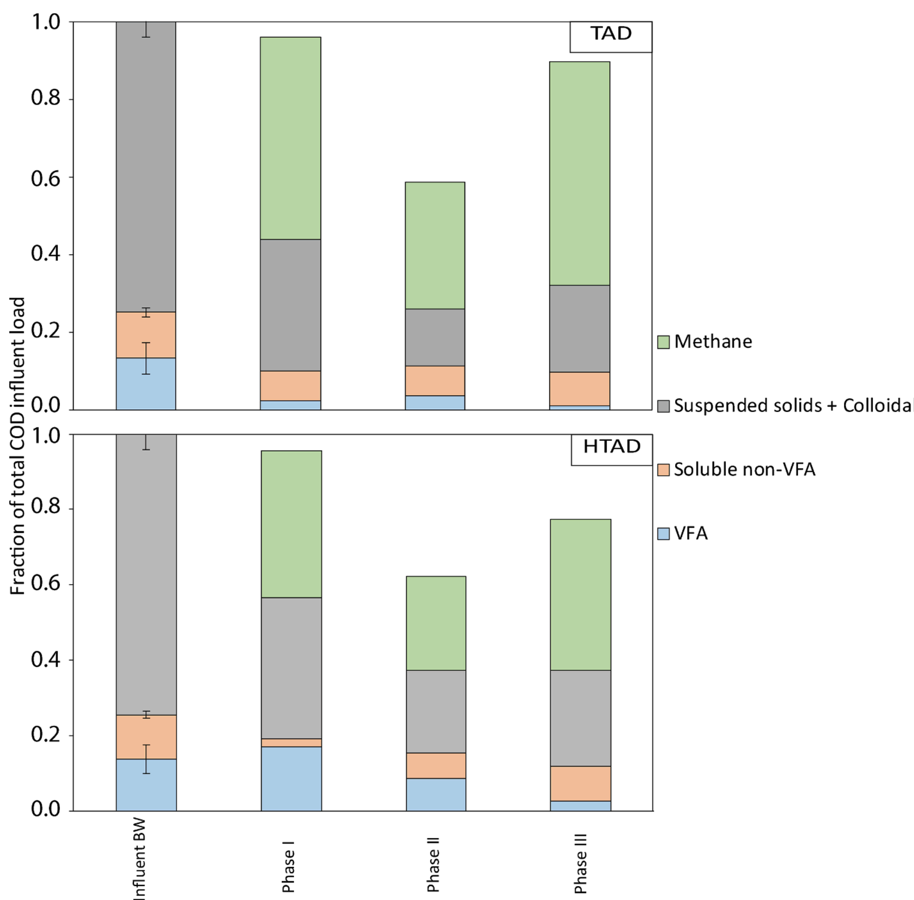


Fig. 3. COD mass balances (as fraction of the total COD load) for TAD (top) and HTAD (bottom) during the different operational phases.

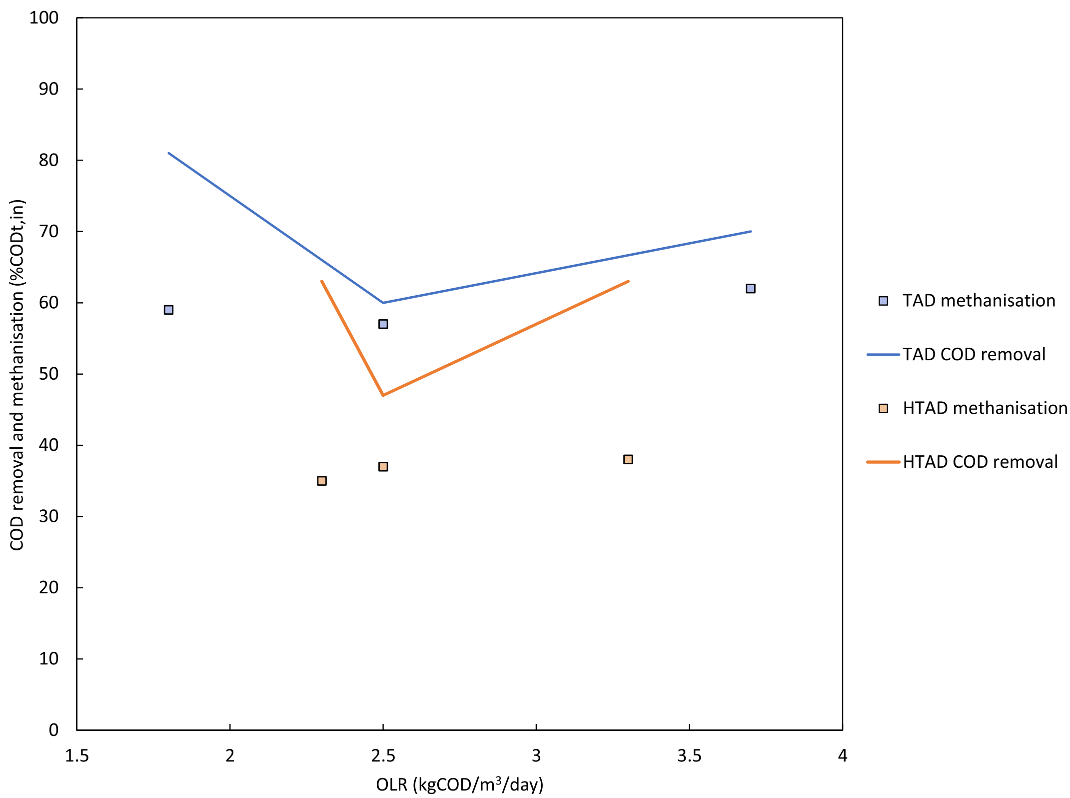


Fig. 4. COD removal and methanisation efficiency for the thermophilic and hyper-thermophilic reactors at the different loading rates of the stable operational phases.

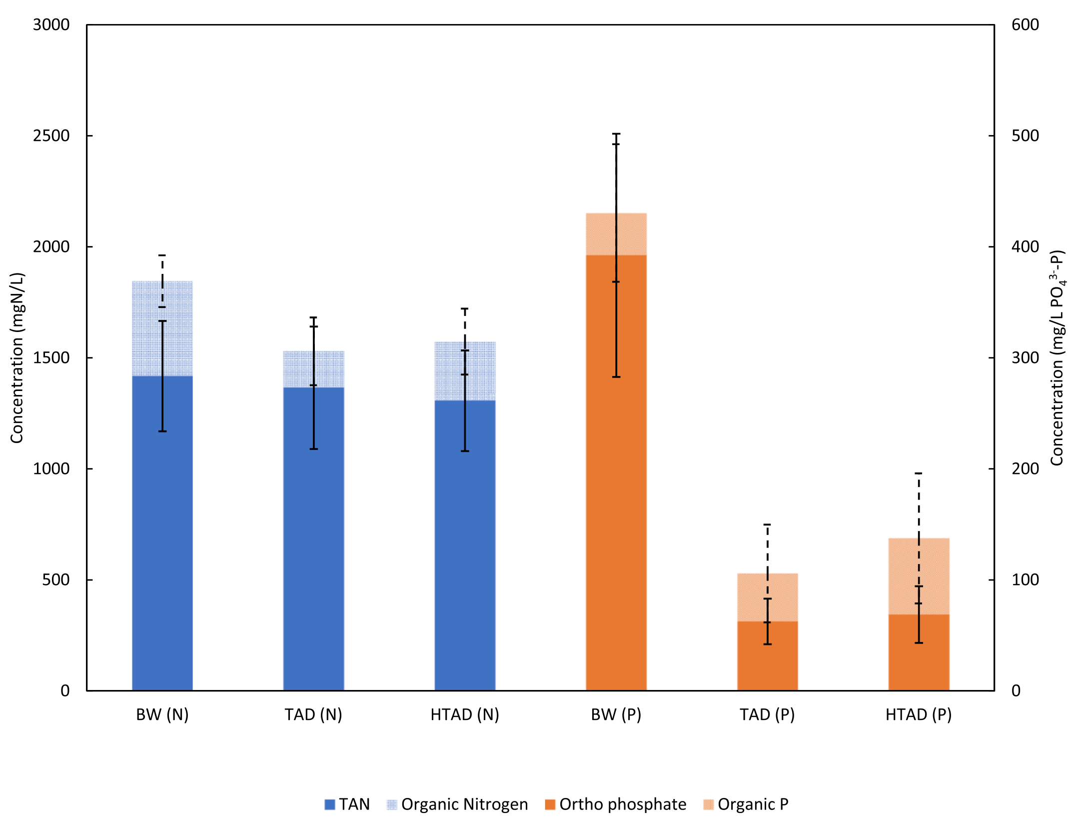


Fig. 5. Nutrient concentrations in influent and effluent from samples between day 594 and 644 during TAD and HTAD.

with the OLR. An increased OLR results in smaller reactors, which would also decrease the capital costs (Ho et al., 2014). Furthermore, the envisioned application of (H)TAD in a decentralized domestic waste treatment approach would benefit from reduced reactor sizes as consequence of an increased OLR, since these reactors are placed within the community areas as was done in for instance Sneek, The Netherlands (de Wit et al., 2018).

3.5. Potential for nutrient recovery

The nutrient concentrations in both the BW as in the (H)TAD effluents have been monitored over time. Typical results (day 594–644) are shown in Fig. 5. For both conditions almost all nitrogen was present in the effluent. The organic fraction (determined as the difference between total nitrogen and total ammonia nitrogen) decreased after treatment in both reactors. There is no net ammonia nitrogen accumulation in the reactors, possibly as consequence of the escape of gaseous ammonia. The high concentration of (dissolved) ammonia provides a high potential for ammonia stripping (Bisschops et al., 2019), especially since the pH and temperature shift the equilibrium more towards the gaseous FA. Ammonia stripping during (hyper-)thermophilic AD is an efficient strategy, since the BW is already heated to 55 or 70 °C. Additionally, minimal amounts of chemicals (e.g. alkaline solution for pH manipulation) are required for ammonia stripping under these conditions as consequence of the decreased pKa.

With regards to the total phosphorous, a strong decrease in the effluent concentration compared to the influent concentration was observed. During HTAD 68% of phosphorous was removed. Higher removal was observed in the TAD, where 75% of the incoming phosphorous was retained in the reactor (Fig. 5). Accordingly, based on ICP-OES measurements, magnesium and calcium concentrations were reduced by 8 and 6 mM respectively. These reductions, along with a removal of 11 mM P suggest precipitation of phosphorous, e.g. in the form of struvite (molar Mg:P ratio 1:1) or calcium phosphate (molar Ca:P ratio 1.5:1), especially since biomass growth could only account for 30 mgP/L removal based on the assumption that 10% of the incoming COD is converted to biomass (based on $C_{60}H_{87}O_{23}N_{12}P$ as chemical formula for biomass with a COD of 1.46 gO_2/g) (Henze et al., 2008). Similarly, for nitrogen 170 mgN/L can be attributed to biomass formation resulting in a negligible difference between influent and effluent nitrogen concentrations. At mesophilic conditions, 40–50% of the phosphorous is retained in the reactor during AD of (diluted) BW (Cunha et al., 2018; de Graaff et al., 2011) , which is 20–35 percentage point lower than found in this study. Phosphorous removal rates of 75%, as obtained in this study without calcium dosing, are comparable to those obtained by Zhang et al. (2021). Cunha et al. (2019) showed that utilisation of an internal gas lift combined with calcium dosing resulted in 57% of the influent P in calcium phosphate granules with a phosphorous content of 7.8%wt in the sludge bed and over 85% total P removal in the reactor with BW containing 200 mgP/L (Cunha et al., 2019). High phosphorous removal during thermophilic AD of concentrated BW, combined with the results obtained with calcium dosing and an internal gas lift by Cunha et al. (2019) indicate the potential for efficient phosphorous recovery during TAD. However, large phosphorous precipitates are required for efficient separation of calcium phosphate and this process is yet to be developed under thermophilic conditions during BW treatment.

3.6. Microbial community changes at thermophilic and hyper-thermophilic conditions

The biomass from the reactors at different temperatures was collected and analysed using 16 s rRNA gene microbial analysis, and all sequences belonging to a specific taxonomic unit were counted to determine total OTU counts and relative abundances. Samples from the seed sludge were also sequenced for comparison. The results show that the diversity in orders present in the inoculum decreases over time in both the TAD and the HTAD reactor (Table 4). In comparison, the TAD start-up reactor had the highest diversity of orders compared to the TAD and HTAD samples, which can be explained by the fact that the analysis was performed on day 16 after the start-up and the microbial consortium was still adapting.

In general, Clostridiales, Bacteroidales and Thermoanaerobacterales dominated both the TAD and HTAD. Previously, Clostridiales emerged in high temperature and ammonia conditions which is in line with the current study (de Vrieze et al., 2015). In the same study by de Vrieze et al. (2015), Bacteroidales were dominant in all tested anaerobic conditions.

Thermoanaerobacterales have previously been found to emerge in thermophilic AD reactors (Lim et al., 2020). Two syntrophic acetate

Table 4
Heat map for all relative abundances of the most common orders. Data is shown for the inoculum which is analysed twice (once in duplicate and once in a single sample), 70 °C and 55 °C reactors after 209 days from different sampling heights and R1 (55 °C) shortly after start-up in duplicate.

		Seed sludge			HTAD		TAD		TAD start-up	
Order	t (days)				209		209		16	
	Main characteristic of most abundant OTU(s)	A	B		Bottom	Middle	Bottom	Middle	A	B
Bacteroidales	Protein and carbohydrate conversion	1%	1%	1%	10%	11%	4%	3%	9%	9%
Campylobacterales	Nitrate reduction	0%	0%	0%	8%	7%	1%	1%	0%	0%
Clostridiales	Reduction of fatty acids, sugars and proteins	9%	9%	14%	31%	34%	20%	20%	26%	29%
MBA03	Glycerol degradation	5%	4%	9%	1%	0%	9%	9%	1%	2%
Methanobacteriales	Hydrogenotrophic methanogenesis	0%	1%	0%	4%	5%	4%	4%	1%	1%
Methanosarcinales	Acetoclastic methanogenesis	3%	3%	1%	3%	3%	5%	5%	2%	2%
Sphingobacteriales	Carbohydrate conversion	18%	18%	20%	2%	2%	11%	11%	7%	7%
Synergistales	Organic and amino acids degradation	3%	3%	1%	6%	6%	8%	8%	2%	2%
Thermoanaerobacterales	Syntrophic acetate oxidation	19%	18%	18%	12%	12%	14%	15%	13%	12%
Sum of orders		58%	57%	65%	77%	79%	76%	77%	61%	63%
Other orders		42%	43%	35%	23%	21%	24%	23%	39%	37%

<1% 1 - 34%

oxidizers belonging to the Thermoanaerobacterales *(Thermacetogenium phaeum* (100% query cover and 91.39% identity) and *Syntrophaceticus schinkii* (94% query cover, 91.05% identity) are very abundant in both the TAD and HTAD reactors, whilst having no or very little OTUs in the seed sludge, which indicates a shift to syntrophic acetate oxidation coupled to hydrogenotrophic methanogenesis (SAO-HM).

Members of the order of Methanobacteriales (hydrogenotrophic methanogens) emerged in both the TAD and the HTAD reactor, which was not yet the case in the TAD start-up reactor after 16 days. This means that a quick start-up was achieved without high abundance of hydrogenotrophic methanogens. Additionally, despite the persistence of a species related to a strict acetoclastic methanogen (*Methanosarcina thermophila*), the increase in relative abundance in the TAD and HTAD reactors of syntrophic acetate oxidizers and hydrogenotrophic methanogens in comparison to the seed sludge indicates a shift towards SAO-HM during both TAD and HTAD. The SAO-HM is not yet established in the TAD start-up reactor after 16 days, probably as consequence of the low growth rates of syntrophic acetate oxidizers. These results, along with the quick start-up as described before, indicate that the used seed sludge was suitable to quickly start-up a reactor treating concentrated BW at low loading rates, however development of the SAO-HM pathway is essential for longterm stable operation.

A shift towards SAO-HM dominance during anaerobic digestion at (hyper-)thermophilic conditions has been observed before (Hao et al., 2011; Ho et al., 2013). Tang et al. (2008) found the same metabolic shift at a temperature of 65 °C, during treatment of synthetic wastewater containing glucose in CSTRs. This synthetic wastewater contained 337 mg/L ammonium (Tang et al., 2008), which is lower than the ammonium concentrations in the current study. This might explain why the methanogenic pathway shift is observed at a lower temperature in our systems with high ammonia concentrations. Elevated ammonia concentrations could also cause a shift in the metabolic pathway since acetoclastic methanogens are more susceptible to toxicity of ammonia (Lü et al., 2013; Schnürer and Nordberg, 2008). An increased FA concentration is a direct consequence of increased temperature due to a shift in the pKa. So results in this study support the theory that temperature directly and/or indirectly through increased ammonia toxicity, steers the methanogenic pathway towards SAO-HM.

4. Conclusions

Thermophilic anaerobic digestion is a suitable technology for concentrated BW treatment. It achieves similar results as mesophilic AD and outperforms HTAD. Additionally, TAD has potential for pathogen-free nutrient recovery through precipitation of phosphorous and ammonia stripping. TAD of concentrated BW results in in 75% P removal and 70–80% COD removal. Furthermore, 60% of the total COD in the concentrated BW is converted to CH_4 at a SRT of 30 days and lower HRTs (6–9 days) as shown in previous long-term mesophilic and thermophilic studies on AD of concentrated BW.

CRediT authorship contribution statement

Marinus J. Moerland: Formal analysis, Writing – original draft, Methodology, Conceptualization. **Laura Castañares Pérez:** Formal analysis. **Maria E. Ruiz Velasco Sobrino:** Formal analysis. **Paraschos Chatzopoulos:** Writing - review & editing. **Brendo Meulman:** Writing - review & editing. **Vinnie Wilde:** Methodology, Resources. **Grietje Zeeman:** Funding acquisition, Conceptualization, Writing - review & editing. **Cees J.N. Buisman:** Funding acquisition, Conceptualization, Supervision, Writing - review & editing. **Miriam H.A. Eekert:** Funding acquisition, Supervision, Project administration, Conceptualization, Writing - review & editing.

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.

Acknowledgments

This work was funded by the Run4Life project (http://run4life-project.eu). Run4Life receives funding from the EU Horizon 2020 Research and Innovation program under grant agreement number 730285.

The authors want to acknowledge Zichen Xu for watching the reactors during the holiday.

Furthermore, discussions with Hamse Kjerstadius and Daniel Todt during the Run4Life project general assemblies were highly appreciated and valuable for the research. The authors wish to thank Dainis Sudmalis and Bert Willemsen for their help with practical issues with the reactors in general and for sharing knowledge and apparatus for the pressuresteered gas measurement system in particular. Pieter Gremmen, Livio Carlucci, Katja Grolle, Zoe Spencer and Julian Zamudio Pineres are thanked for their valuable contributions during the analytical work. Inge van der Velde of Waterboard Drents Overijsselse Delta is acknowledged for providing the inoculum.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2021.125705.

References

Abdel-Shafy, H.I., El-Khateeb, M., Regelsberger, M., El-Sheikh, R., Shehata, M., 2009. Integrated system for the treatment of blackwater and greywater via UASB and constructed wetland in Egypt. Desal. Wat. Treat. 8 (1–3), 272–278. https://doi.org/10.5004/dwt.2009.788.

Ahring, B.K., Ibrahim, A.A., Mladenovska, Z., 2001. Effect of temperature increase from 55 to 65°C on performance and microbial population dynamics of an anaerobic reactor treating cattle manure. Wat. Res. 35 (10), 2446–2452. https://doi.org/10.1016/S0043-1354(00)00526-1.

Bisschops, I., Kjerstadius, H., Meulman, B., van Eekert, M.H.A., 2019. Integrated nutrient recovery from source-separated domestic wastewaters for application as fertilisers. Curr. Opin. Environ. Sustain. 40, 7–13. https://doi.org/10.1016/j.cosust.2019.06.010.

Boušková, A., Dohányos, M., Schmidt, J.E., Angelidaki, I., 2005. Strategies for changing temperature from mesophilic to thermophilic conditions in anaerobic CSTR reactors treating sewage sludge. Wat. Res. 39 (8), 1481–1488. https://doi.org/10.1016/j.watres.2004.12.042.

Chen, W.S., Ye, Y., Steinbusch, K.J.J., Strik, D.P.B.T.B., Buisman, C.J.N., 2016. Methanol as an alternative electron donor in chain elongation for butyrate and caproate formation. Biomass Energy 93, 201–208. https://doi.org/10.1016/j.biombioe.2016.07.008.

Collivignarelli, M., Abbà, A., Frattarola, A., Carnevale Miino, M., Padovani, S., Katsoyiannis, I., Torretta, V., 2019. Legislation for the reuse of biosolids on agricultural land in Europe: overview. Sustainability 11, 6015. https://doi.org/10.3390/su11216015.

Cordell, D., Drangert, J.-O., White, S., 2009. The story of phosphorus: global food security and food for thought. Global Environ. Chang. 19 (2), 292–305. https://doi.org/10.1016/j.gloenvcha.2008.10.009.

Cunha, J.R., Schott, C., van der Weijden, R.D., Leal, L.H., Zeeman, G., Buisman, C., 2019. Recovery of calcium phosphate granules from black water using a hybrid upflow anaerobic sludge bed and gas-lift reactor. Environ. Res. 178, 108671. https://doi.org/10.1016/j.envres.2019.108671.

Cunha, J.R., Tervahauta, T., van der Weijden, R.D., Hernández Leal, L., Zeeman, G., Buisman, C.J.N., 2018. Simultaneous recovery of calcium phosphate granules and methane in anaerobic treatment of black water: Effect of bicarbonate and calcium fluctuations. J. Environ. Manage. 216, 399–405. https://doi.org/10.1016/j.jenvman.2017.09.013.

de Graaff, M.S., Temmink, H., Zeeman, G., Buisman, C.J.N., 2010. Anaerobic treatment of concentrated black water in a UASB reactor at a short HRT. Water 2 (1), 101. https://doi.org/10.3390/w2010101.

de Graaff, M.S., Temmink, H., Zeeman, G., Buisman, C.J.N., 2011. Energy and phosphorus recovery from black water. Wat. Sci. Technol. 63 (11), 2759–2765. https://doi.org/10.2166/wst.2011.558.

de la Rubia, M.A., Riau, V., Raposo, F., Borja, R., 2013. Thermophilic anaerobic digestion of sewage sludge: focus on the influence of the start-up. A review. Crit. Rev. Biotechnol. 33 (4), 448–460. https://doi.org/10.3109/07388551.2012.72696 2.

de Leeuw, K.D., Buisman, C.J.N., Strik, D.P.B.T.B., 2019. Branched medium chain fatty acids: iso-caproate formation from Iso-butyrate broadens the product spectrum for microbial chain elongation. Environ. Sci. Technol. 53 (13), 7704–7713. https://doi.org/10.1021/acs.est.8b07256.

de Vrieze, J., Saunders, A.M., He, Y., Fang, J., Nielsen, P.H., Verstraete, W., Boon, N., 2015. Ammonia and temperature determine potential clustering in the anaerobic digestion microbiome. Wat. Res. 75, 312–323. https://doi.org/10.1016/j.watres.2015.02.025.

de Wit, J.B., de Graaf, R., Elzinga, N., Debucquoy, W., Rodenhuis, J., Stutterheim, E., Piekema, L., 2018. Evaluatie nieuwe sanitatie Noorderhoek/Waterschoon. STOWA.

Ferrer, I., Vázquez, F., Font, X., 2010. Long term operation of a thermophilic anaerobic reactor: Process stability and efficiency at decreasing sludge retention time. Bioresour. Technol. 101 (9), 2972–2980. https://doi.org/10.1016/j.biortech.2009.12.006.

Gao, M., Zhang, L., Florentino, A.P., Liu, Y., 2019. Performance of anaerobic treatment of blackwater collected from different toilet flushing systems: can we achieve both energy recovery and water conservation? J. Hazard. Mater. 365, 44–52. https://doi.org/10.1016/j.jhazmat.2018.10.055.

Hafner, S.D., Bisogni, J.J., Jewell, W.J., 2006. Measurement of un-ionized ammonia in complex mixtures. Environ. Sci. Technol. 40 (5), 1597–1602. https://doi.org/10.1021/es051638j.

Hammes, F., Kalogo, Y., Verstraete, W., 2000. Anaerobic digestion technologies for closing the domestic water, carbon and nutrient cycles. Wat. Sci. Technol. 41 (3), 203–211. https://doi.org/10.2166/wst.2000.0073.

Hao, L.-P., Lü, F., He, P.-J., Li, L., Shao, L.-M., 2011. Predominant contribution of syntrophic acetate oxidation to thermophilic methane formation at high acetate concentrations. Environ. Sci. Technol. 45 (2), 508–513. https://doi.org/10.1021/es102228v.

Harder, R., Wielemaker, R., Larsen, T.A., Zeeman, G., Öberg, G., 2019. Recycling nutrients contained in human excreta to agriculture: Pathways, processes, and products. Crit. Rev. Environ. Sci. Technol. 49 (8), 695–743. https://doi.org/10.1080/10643389.2018.1558889.

Henze, M., van Loosdrecht, M.C., Ekama, G.A., Brdjanovic, D., 2008. Biological wastewater treatment. First ed. IWA publishing, London. https://doi.org/10.2166/9781780401867.

Ho, D., Jensen, P.D., Batstone, D.J., 2014. Effects of temperature and hydraulic retention time on acetotrophic pathways and performance in high-rate sludge digestion. Environ. Sci. Technol. 48 (11), 6468–6476. https://doi.org/10.1021/es500074j.

Ho, D.P., Jensen, P.D., Batstone, D.J., 2013. Methanosarcinaceae and acetate-oxidizing pathways dominate in high-rate thermophilic anaerobic digestion of waste-activated sludge. Appl. Environ. Microbiol. 79 (20), 6491–6500. https://doi.org/10.1128/aem.01730-13.

Kim, M., Ahn, Y.-H., Speece, R.E., 2002. Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. Wat. Res. 36 (17), 4369–4385. https://doi.org/10.1016/S0043-1354(02)00147-1.

Kujawa-Roeleveld, K., Zeeman, G., 2006. Anaerobic treatment in decentralised and source-separation-based sanitation concepts. Rev. Environ. Sci. Biotechnol. 5 (1), 115–139. https://doi.org/10.1007/s11157-005-5789-9.

Lim, J.W., Kelvin Wong, S.W., Dai, Y., Tong, Y.W., 2020. Effect of seed sludge source and start-up strategy on the performance and microbial communities of thermophilic anaerobic digestion of food waste. Energy 203, 117922. https://doi.org/10.1016/j.energy.2020.117922.

Liu, C., Wang, W., Anwar, N., Ma, Z., Liu, G., Zhang, R., 2017. Effect of organic loading rate on anaerobic digestion of food waste under mesophilic and thermophilic conditions. Energy Fuels 31 (3), 2976–2984. https://doi.org/10.1021/acs.energyfuels.7b00018.

Lü, F., Hao, L., Guan, D., Qi, Y., Shao, L., He, P., 2013. Synergetic stress of acids and ammonium on the shift in the methanogenic pathways during thermophilic anaerobic digestion of organics. Wat. Res. 47 (7), 2297–2306. https://doi.org/10.1016/j.watres.2013.01.049.

Moerland, M.J., Borneman, A., Chatzopoulos, P., Fraile, A.G., van Eekert, M.H.A., Zeeman, G., Buisman, C.J.N., 2020. Increased (Antibiotic-Resistant) pathogen indicator organism removal during (Hyper-)thermophilic anaerobic digestion of concentrated black water for safe nutrient recovery. Sustainability 12 (22), 9336. https://doi.org/10.3390/su12229336.

Nges, I.A., Liu, J., 2010. Effects of solid retention time on anaerobic digestion of dewatered-sewage sludge in mesophilic and thermophilic conditions. Renew. Energy 35 (10), 2200–2206. https://doi.org/10.1016/j.renene.2010.02.022.

Palatsi, J., Gimenez-Lorang, A., Ferrer, I., Flotats, X., 2009. Start-up strategies of thermophilic anaerobic digestion of sewage sludge. Wat. Sci. Technol. 59 (9), 1777–1784. https://doi.org/10.2166/wst.2009.180.

Poh, P.E., Chong, M.F., 2014. Upflow anaerobic sludge blanket-hollow centered packed bed (UASB-HCPB) reactor for thermophilic palm oil mill effluent (POME) treatment. Biomass Energy 67, 231–242. https://doi.org/10.1016/j.biombioe.2014.05.007.

Ryue, J., Lin, L., Kakar, F.L., Elbeshbishy, E., Al-Mamun, A., Dhar, B.R., 2020. A critical review of conventional and emerging methods for improving process stability in thermophilic anaerobic digestion. Energy Sustain. Dev. 54, 72–84. https://doi.org/10.1016/j.esd.2019.11.001.

Schnürer, A., Nordberg, Å., 2008. Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. Wat. Sci. Technol. 57 (5), 735–740. https://doi.org/10.2166/wst.2008.097.

Shin, J., Jang, H.M., Shin, S.G., Kim, Y.M., 2019. Thermophilic anaerobic digestion: Effect of start-up strategies on performance and microbial community. Sci. Total Environ. 687, 87–95. https://doi.org/10.1016/j.scitotenv.2019.05.428.

Sudmalis, D., Gagliano, M., Pei, R., Grolle, K., Plugge, C., Rijnaarts, H., Zeeman, G., Temmink, H., 2018. Fast anaerobic sludge granulation at elevated salinity. Wat. Res. 128, 293–303. https://doi.org/10.1016/j.watres.2017.10.038.

Tang, Y.-Q., Matsui, T., Morimura, S., Wu, X.-L., Kida, K., 2008. Effect of temperature on microbial community of a glucose-degrading methanogenic consortium under hyperthermophilic chemostat cultivation. J. Biosci. Bioeng. 106 (2), 180–187. https://doi.org/10.1263/jbb.106.180.

Tervahauta, T., Hoang, T., Hernández Leal, L., Zeeman, G., Buisman, C.J.N., 2013. Prospects of source-separation-based sanitation concepts: a model-based study. Water 5 (3), 1006. https://doi.org/10.3390/w5031006.

Tervahauta, T., Rani, S., Hernández Leal, L., Buisman, C.J.N., Zeeman, G., 2014. Black water sludge reuse in agriculture: are heavy metals a problem? J. Hazard. Mater. 274, 229–236. https://doi.org/10.1016/j.jhazmat.2014.04.018.

van Lier, J.B., 1995. Thermophilic anaerobic wastewater treatment: temperature aspects and process stability, Landbouwuniversiteit Wageningen.

Verstraete, W., Van de Caveye, P., Diamantis, V., 2009. Maximum use of resources present in domestic "used water". Bioresour. Technol. 100 (23), 5537–5545. https://doi.org/10.1016/j.biortech.2009.05.047.

Wu, Z.-L., Lin, Z., Sun, Z.-Y., Gou, M., Xia, Z.-Y., Tang, Y.-Q., 2020. A comparative study of mesophilic and thermophilic anaerobic digestion of municipal sludge with high-solids content: reactor performance and microbial community. Bioresour. Technol. 302, 122851. https://doi.org/10.1016/j.biortech.2020.122851.

Zeeman, G., Kujawa-Roeleveld, K., 2011. Resource recovery from source separated domestic waste(water) streams; full scale results. Wat. Sci. Technol. 64 (10), 1987–1992. https://doi.org/10.2166/wst.2011.562.

Zeeman, G., Kujawa, K., de Mes, T., Hernandez, L., de Graaff, M., Abu-Ghunmi, L., Mels, A., Meulman, B., Temmink, H., Buisman, C., van Lier, J., Lettinga, G., 2008. Anaerobic treatment as a core technology for energy, nutrients and water recovery from source-separated domestic waste(water). Wat. Sci. Technol. 57 (8), 1207–1212. https://doi.org/10.2166/wst.2008.101.

Zeeman, G., Lettinga, G., 1999. The role of anaerobic digestion of domestic sewage in closing the water and nutrient cycle at community level. Wat. Sci. Technol. 39 (5), 187–194. https://doi.org/10.2166/wst.1999.0238.

Zhang, L., Guo, B., Mou, A., Li, R., Liu, Y., 2020. Blackwater biomethane recovery using a thermophilic upflow anaerobic sludge blanket reactor: Impacts of effluent recirculation on reactor performance. J. Environ. Manage. 274, 111157. https://doi.org/10.1016/j.jenvman.2020.111157.

Zhang, L., Mou, A., Guo, B., Sun, H., Anwar, M.N., Liu, Y., 2021. Simultaneous phosphorus recovery in energy generation reactor (SPRING): high rate thermophilic blackwater treatment. Resour. Conserv. Recycl. 164, 105163. https://doi.org/10.1016/j.resconrec.2020.105163.

Zinder, S., Anguish, T., Cardwell, S., 1984. Effects of temperature on methanogenesis in a thermophilic (58 C) anaerobic digestor. Appl. Environ. Microbiol. 47 (4), 808–813. https://doi.org/10.1128/AEM.47.4.808-813.1984.