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Research Paper

High-solid digestion – A comparison of completely stirred and plug-flow reactor systems

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ABSTRACT

High-solid digestion (HSD) for biogas production is a resource-efficient and sustainable method to treat organic wastes with high total solids content and obtain renewable energy and an organic fertiliser, using a lower dilution rate than in the more common wet digestion process. This study examined the effect of reactor type on the performance of an HSD process, comparing plug-flow (PFR) type reactors developed for continuous HSD processes, and completely stirred-tank reactors (CSTRs) commonly used for wet digestion. The HSD process was operated in thermophilic conditions (52 °C), with a mixture of household waste, garden waste and agricultural residues (total solids content 27–28 %). The PFRs showed slightly better performance, with higher specific methane production and nitrogen mineralisation than the CSTRs, while the reduction of volatile solids was the same in both reactor types. Results from 16S rRNA gene sequencing showed a significant difference in the microbial population, potentially related to large differences in stirring speed between the reactor types (1 rpm in PFRs and 70–150 rpm in CSTRs, respectively). The bacterial community was dominated by the genus *Defluviitoga* in the PFRs and order MBA03 in the CSTRs. For the archaeal community, there was a predominance of the genus *Methanoculleus* in the PFRs, and of the genera *Methanosarcina* and *Methanothermobacter* in the CSTRs. Despite these shifts in microbiology, the results showed that stable digestion of substrates with high total solids content can be achieved in both reactor types, indicating flexibility in the choice of technique for HSD processes.

1. Introduction

Anaerobic digestion (AD) is a well-established, sustainable method for treating various organic waste streams, such as household waste, sewage sludge from wastewater treatment and residues from the food industry and agriculture, and for utilising these as resources to obtain renewable energy (biogas) and to enable nutrient recycling (Scarlat et al. 2018; Mignogna et al. 2023; Subbarao et al. 2023). During the AD process, organic material is degraded in an oxygen free environment, with methane (CH_4) and carbon dioxide (CO_2) as final degradation products (Anukam et al. 2019). The residual material, the digestate, is typically rich in nutrients and can be used as organic fertiliser (Möller & Müller 2012). Anaerobic digestion is carried out by a complex community of microorganisms in a step-wise process (Anukam et al. 2019), initiated by a hydrolysis step, followed by fermentation and then anaerobic oxidation and methanogenesis (Schnürer & Jarvis 2018). During the first steps, complex polymers (carbohydrates, proteins and fat) are degraded into monomers that are fermented into various volatile fatty acids (VFAs), alcohols, H_2 and CO_2 (Anukam et al. 2019). The VFAs are converted by bacteria during anaerobic oxidation, a process that is only thermodynamically favourable at low H_2 partial pressure, meaning that H_2-consuming partners are needed (syntrophy) (Schnürer & Jarvis 2018). Finally, methanogenic archaea utilise either acetate (acetotrophic), CO_2 and H_2 (hydrogenotrophic) or other single-carbon molecules to produce CH_4 (Costa & Leigh 2014).

An important factor in AD is the water content in the process, which determines the technique and equipment that can be used for the digestion. The most well-established commercial process for AD is so called wet digestion, which operates with total solids (TS) content < 15 % and can be applied to an array of different substrates (André et al. 2018; Wang et al. 2023). However, high-solid digestion (HSD) processes, which operate with > 15 % TS in the reactor material, are receiving increased attention as they require less substrate dilution compared with wet digestion (André et al. 2018; Wang et al. 2023). This

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result in requirements of comparably small reactor volumes and transportation of lesser volumes of substrates and digestate, combined having the potential to reduce costs (Duan et al. 2012; Wang et al. 2023). Thus, HSD could be an important technology to access the future potential of biogas from high TS substrates, such as solid manure and crop residues (Brown et al. 2012; Chiumenti et al. 2018), as well as food waste and dewatered fractions of wastewater sludge (Duan et al. 2012; Westerholm et al. 2020; Nordell et al. 2021).

HSD is often performed in plug-flow reactors (PFRs), which have *e.g.* mixing and feeding systems that are suitable for bulky and highly viscous substrates (Li et al. 2011; Akinbomi et al. 2022, Kothari et al. 2014). In an ideal PFR, material moves slowly from inlet to outlet without horizontal mixing, keeping all the input material within the reactor over one full hydraulic retention time (HRT) (Toson et al. 2019). This is in contrast to completely stirred-tank reactors (CSTRs), typically used for wet digestion, which ideally have completely homogenous reactor content (Toson et al. 2019). Theoretically, a higher productivity can be achieved from a PFR than a CSTR since removal of a fraction of the substrate shortly after feeding, *i.e.* short-circuiting, is unavoidable in a continuous CSTR process, representing a loss in methane potential (Das et al. 2016). In addition to retaining material within the system, plug flow should also in theory enable separation of different microbial steps along the reactor and allow for more optimised and stable degradation, a concept previously demonstrated with sequential CSTRs (Srisowmeya et al., 2020; Moestedt et al. 2020; Roy et al. 2009; Donoso-Bravo et al. 2018). Although a few previous studies have reported plug-flow behaviour or indications of phase separation in AD reactors without a compartmentalisation or sequential set-up (Nordell et al. 2021; Rossi et al. 2022), data show that plug flow is not necessarily obtained in PFRs at laboratory or industrial scale (Li et al. 2014; Perman et al. 2024). In a previous study by our research group (Perman et al. 2024), two laboratory-scale PFRs were observed to behave like completely stirred reactors, based on comparisons between sections and a tracer test. This raises the question of whether the HSD process could be operated equally well in CSTRs. The advantage of a CSTR is the simplicity of the reactor design and that it is a common type of reactor, readily available for both industrial and laboratory scale processes. If efficient HSD can be achieved in CSTRs in addition to PFRs, this could provide greater versatility in the choice of reactor technology and allow for alternative operational strategies of HSD processes.

In our previous study (Perman et al. 2024), the PFRs were operated with recirculation of digestate from the outlet back to the feed inlet. This is a commonly used practice in PFRs, with the main aim of re-inoculating the reactor section at the feed inlet to keep the microbial community in this section fresh and active (Li et al. 2011; Donoso-Bravo et al. 2018). In reactors of PFR type, high recirculation ratio has been observed to be beneficial both for biogas yield (Ratanatamskul & Saleart 2016; Chen et al. 2020) and process stability (Gómez et al. 2019; Nordell et al. 2021). In single CSTRs, recirculation of either the liquid or solid fraction of the digestate can be beneficial in order to dilute the substrate or increase the retention time of solid material and microorganisms (Nordberg et al. 2007; Rico et al. 2011; Peng et al. 2016; Singh et al. 2019, Brémond et al. 2021). However, recirculation of whole digestate in a CSTR will not result in the same effect as in a phase-separated process. Thus, if PFRs behave more like completely stirred reactors than plug-flow systems, the question is whether recirculation of whole digestate is necessary to maintain a stable process in these reactors and if not, does it even reduce the potential to obtain separate microbial communities across PFRs.

In HSD, high TS content and viscosity involves a risk of process disturbances due to poor mass transfer and challenges in achieving sufficient mixing (Bollon et al. 2013; Rocamora et al. 2020; Li et al. 2023). It is therefore of interest to evaluate whether stable digestion of high TS substrates can be achieved in different reactor types with different mixing equipment. So far, only a few studies have evaluated both PFRs and CSTRs in the same experiment (Li et al. 2007; Yue et al. 2011; Lee et al. 2017) and among those only one made direct comparisons using the same substrate and operating parameters (manure, TS<13 %) in both reactor types (Li et al. 2007). However, no other study has yet compared PFRs and CSTRs operating under HSD conditions (>15 %). Given the unique advantages of PFRs and CSTRs respectively, the present study aimed to explore the possibility of using different reactor technologies for a HSD process and assess to which extent the reactor type affects biogas production efficiency, degree of substrate degradation as well as the microbial population. This was studied by transferring a thermophilic laboratory-scale HSD process, operated using substrate with high TS content (27–28 %), from PFRs to CSTRs. The process performance of both reactor types was assessed by running the processes in parallel while monitoring process parameters and assessing biogas and methane yields, reduction of volatile solids (VS) and nitrogen mineralisation, as well as microbial community structure. Additionally, an evaluation of recirculation was conducted to test its necessity for maintaining a stable and efficient process and microbial community within a PFR.

2. Material and methods

2.1. Laboratory-scale reactors and substrate characteristics

Three CSTRs (CR1, CR2 and CR3) and two PFRs (PR1 and PR2) were operated during a total of 38 weeks. The CSTR processes were carried out in three laboratory-scale tank reactors (active volume 5 L) stirred with rotor blades (Belach Bioteknik, Stockholm, Sweden). The stirring speed of the CSTRs was first set to 70 rpm, but was increased to 150 rpm during week 30 to avoid build-up of solids due to sedimentation. The design parameters of the two laboratory-scale PFRs (referred to as LR1 and LR2) were as described previously (Perman et al. 2024). These reactors, built to mimic an industrial-scale plant, each had an active volume of 45 L and were stirred at a speed of 1 rpm by vertical paddles. Also, like the industrial-scale process, all laboratory-scale reactors were operated at thermophilic temperature (52–53 °C), which is an approved method for hygienisation if the material resides within the reactor during a sufficient period of time (10 h) (Jordbruksverket, 2016). The substrate, a mixture of food waste, garden residues, horse manure, olive cake and potato waste (51, 17, 26, 6 and < 1 % volatile solids (VS), respectively), was fed to all reactors six days per week. These substrate fractions were collected at an industrial-scale plant every 3–4 months and stored at 4 °C until use. All fractions were pre-treated by grinding to particle size ~ 10 mm and then analysed for TS and VS content. Additionally, total nitrogen (N), carbon (C), trace elements, organic N, ammonium-N (NH_4^+-N) and concentration of macromolecules were analysed in the final substrate mixes (Table 1).

2.2. Experiment start-up and time-line

The study started with inoculation of the three CSTRs with digestate from one of the PFRs (PR2). At this time-point, the PFRs had already been operated in parallel for 302 days at an average OLR of 5.2 g VS/L/d and HRT of 42 days, and with recirculation of whole digestate (30 % of ingoing material). The inoculum was strained to obtain a maximum particle size of 7 mm. During the start-up phase (three weeks; Table S1), the CSTRs were run with low and step-wise increasing loading rate up to an OLR of 5.2 g VS/L/d. During the first eight weeks of operation at full OLR, all CSTRs were fed a substrate mix diluted to a TS content of 19 %. The HRT during this time was 30 days in all CSTRs. Reactor CR1 was operated under these conditions during the whole experiment, while operating parameters in reactors CR2 and CR3 were changed to mimic conditions in the PFRs. From experiment week 9, the TS content in the substrate (TS_{in}) was increased to 27–28 % in CR2, by reducing the amount of added water, and from week 20 the same was done also in CR3 (Table 2). In the CSTRs with high TS_{in}, the volumetric HRT was around 42 days, the same as in the PFRs (Table 2). The whole

Table 1
Chemical composition of substrate mixes (diluted to 22% volatile solids, 27–28% total solids) used in all completely stirred-tank reactors (CSTRs) and plug-flow reactors (PFRs) during experiment weeks 1–26, 27–34 and 35–38 respectively. All values are given in g/kg (ww) substrate.

Week	1–26	27–34	35–38
Total N	5.2	5.3	5.2
Organic N [1]	5.0	4.9	4.9
NH_4^+-N [2]	0.1	0.4	0.4
Total C [1]	90.3	108.0	113.2
Total P [2]	0.7	0.7	0.7
Total K [2]	3.1	3.0	3.3
Total Mg [2]	0.6	0.6	0.6
Total Ca [2]	4.9	4.3	4.5
Total Na [2]	0.9	1.0	0.8
Total S [2]	0.5	0.5	0.5
Raw protein [3]	26.8	30.6	29.4
Raw fat [4]	19.6	19.2	18.4
Carbohydrates [5]	146.1	152.0	146.0

[1] Relative measurement uncertainty (RMU) 5%.
[2] RMU 10%.
[3] RMU 20%.
[4] RMU 30%.
[5] RMU not determined.

Table 2
Operating parameters for reactors (PR1, PR2 and CR1-CR3) during experiment week 20–38.

Reactor	PR1	PR2	CR1	CR2	CR3
Type	PFR	PFR	CSTR	CSTR	CSTR
Volume (L)	45	45	5	5	5
Recirculation (%)[1]	30	–	*NA*	*NA*	*NA*
OLR (g VS/L/d)	5.3 ± 0.0	5.3 ± 0.0	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
HRT (days)	42	42	30	43	42
TS_{in} (%)	27–28	27–28	19–20	27–28	27–28
VS_{in} (%)	22–23	22–23	16	22–23	22–23

NA: not applicable.
[1] Percent of ingoing wet weight.

experiment was run for 262 days, corresponding to approximately 9 HRTs for CR1 which was operated under the same conditions during the whole experiment. Reactors CR2 and CR3 were operated at high TS_{in} for 209 and 132 days, corresponding to 5 and 3 HRTs, respectively (Table S1).

In the PFRs, process disturbances were experienced until experiment week 8, due to high NH_4^+-N levels that had accumulated during a previous experimental phase. The disturbance was alleviated by operation at temporarily reduced loading rate (Table S1). However, from week 9 onwards, both PFRs were operated at full OLR, 5.3 g VS/L/d. At the start of the experimental period, whole digestate was recirculated (30 % of ingoing material) in both PFRs, but from week 15 onwards recirculation was stopped in PR2 to evaluate the effect of recirculation on the process. PR1 and PR2 were operated at these conditions during more than 5 and 3 HRTs, respectively. An overview of the experimental phases in the different reactors is given in Table S1. The final experimental set-up and operating parameters effective from week 20 are listed in Table 2.

During the whole experimental period, digestate samples were taken from all reactors for chemical and microbial analyses (see section 2.3). For the PFRs, samples were taken from section S3 (outgoing digestate). In addition, to evaluate plug-flow behaviour, samples from the PFRs were also taken from section S1 (close to feeding inlet) during weeks 18–31.

2.3. Analytical methods

In the PFRs, gas volume was measured continuously with RITTER Drum-type TG0.5 m (RITTER Apparatebau GmbH & Co. KG, Bochum, Germany). In the CSTRs, gas production was measured continuously by built-in gas counters (Belach Bioteknik, Stockholm, Sweden), calibrated using a RITTER Drum-type meter of the same sort as above. Gas composition (CH_4, CO_2, O_2, H_2, H_2S) in all reactors was determined using AwiFLEX (Awite Bioenergie Gmbh, Langenbach). For the CSTRs, gas composition was measured in samples collected during an entire day, while instantaneous gas composition just before feeding was measured for the PFRs. The TS- and VS content in substrate fractions and digestate samples were analysed in triplicate according to standard methods (APHA, 1998). In the substrate samples organic N (SS-ISO 13 878), NH_4^+-N (Foss Tecator, Application Note, AN 5226, based on ISO 11732) and total C (SS-ISO 10 694) content were measured by Agrilab AB (Uppsala, Sweden). In digestate samples the total concentration of N in the form of ammonium (NH_4^+) and ammonia (NH_3), here denoted as NH_4^+-N concentration, was measured using an LCK 302 Ammonium kit (Hach Lange Gmbh, Düsseldorf, Germany) as described previously (Perman et al. 2024). The concentration of N in the form of NH_3 (NH_3-N) was calculated based on temperature, pH and the measured NH_4^+-N concentration (Hansen et al. 1998). Sample preparation and VFA measurements in digestate by high-performance liquid chromatography (HPLC) (Agilent 1100 Series Agilent Technologies, Waldbronn, Germany) were carried out as described previously (Westerholm et al. 2012; Perman et al. 2024). Alkalinity and ratio between VFA and alkalinity (FOS/TAC) were measured on fresh, sieved digestate samples as described previously (Perman et al. 2024). Protein (based on total Kjeldahl-N (EN 13342), NH_4^+-N (Standard Methods 1998, 4500 mod.)) and raw fat (NMKL 160 mod.) content in substrate were measured by Eurofins Food & Feed Testing Sweden (Lidköping). Carbohydrate content (SLVFS 1993:21) in substrate was measured by Eurofins Environment Testing Sweden AB.

2.4. Analyses of microbial community

Sequencing of 16S rRNA genes was carried out to evaluate microbial community structures. Sampling of digestate for DNA extraction was performed at five time-points for the PFRs (week 26, 27, 28, 31 and 36) and six time-points for the CSTRs (25, 27, 28, 30, 31 and 36). Samples were stored at −20 °C before extraction. For the first three time-points in the PFRs, samples were taken from two different reactor sections (S1 and S3). DNA extraction was carried out in duplicate using the FastDNA Spin Kit for Soil (MP Biomedicals Europe), as described previously (Danielsson et al. 2017) and extracted DNA was stored at −20 °C. Libraries of the 16S rRNA gene were prepared using primers for amplification of the V4 region (515′F/806R). Library preparation and sequencing (Illumina Novaseq platform) were carried out by Novogene (UK) Company Limited, Cambridge, United Kingdom. The procedure for processing raw sequences and subsequent data analyses was as described previously (Perman et al. 2024).

2.5. Calculations and statistical analyses

Volatile solids reduction was calculated as (VS_{in} (%) − (1 − VR) * VS_{out} (%)) / VS_{in} (%) and nitrogen mineralisation as ((1 − VR) * NH_4^+-$N_{digestate}$ (g/L) − NH_4^+-$N_{substrate}$ (g/L)) / Org-$N_{substrate}$ (g/L), where VR is volume reduction of reactor material and VS is percentage of wet weight. VR was estimated based on daily volumetric gas production (GP_v) and gas density (ρ_g; 1.2 g/L), and was calculated as (GP_v (L/d) * ρ_g (g/L)) / ww_{in} (g/d), where ww_{in} is the wet weight of ingoing substrate.

Differences between reactors in terms of specific methane and gas production, VS reduction and nitrogen mineralisation were evaluated using data collected during weeks 25–38. Differences between reactors in VFA, pH and NH_4^+-N levels were investigated during weeks 31–38. Statistical analyses were carried out with ANOVA, using the *lm* function in R v4.1.2. Pairwise comparisons between reactors were carried out using the *emmeans* function in the emmeans package v1.7.3.

3. Results & discussion

3.1. Process performance, yield and degree of degradation

3.1.1. Dynamics of process parameters during the experiment

Substrate with high TS_{in} content (27–28 %) was digested using two different reactor technologies, horizontal reactors of PFR type and CSTRs, applying similar operating parameters. The operating parameters applied (temperature, HRT, OLR, TS_{in}) were based on an industrial-scale HSD process operated with a similar substrate mixture (Perman et al. 2024). The OLR was 5.2–5.3 g VS/L/d, which is within the range or slightly lower than reported previously for HSD processes (Fagbohungbe et al. 2015; Rocamora et al. 2020; Westerholm et al. 2020; Wang et al. 2023). Average specific methane production (SMP) during the whole experiment was 317–328 NL CH_4/kg VS in the PFRs and 241–253 NL CH_4/kg VS in the CSTRs. This was comparable to values obtained in a previous experiment in the same PFRs (338 ± 57 and 339 ± 47 NL CH_4/kg VS in PR1 and PR2 respectively), using a similar substrate composition (Perman et al. 2024). The SMP level in the PFRs became relatively stable after some initial disturbances during the first 10 weeks caused by high NH_4^+-N levels. In the CSTRs, SMP varied more between weeks throughout the experiment, but with no clear increasing or decreasing trend (Fig. 1a, 1b).

During the course of the experiment, the NH_4^+-N level ranged between 1.1 and 2.4 g/L in the CSTRs and between 2.0 and 3.8 g/L in the PFRs, depending on the operating regime (Fig. 1c, 1d). The pH range in CSTRs and PFRs was 7.5–8.4 and 8.0–8.6, respectively (Fig. 1e, 1f), which resulted in a NH_3-N level of 0.2–1.0 and 0.5–1.9 g/L, respectively (Fig. 1c, 1d). Some changes in the process parameters occurred over time, coupled to changes in operation during the experiment. During the first eight weeks of operation, the substrate for all CSTRs was diluted with water to TS_{in} 19 % (VS_{in} 16 %). This dilution clearly had an effect on the NH_4^+-N concentration and on the pH, which decreased compared with that in the inoculum during the first eight weeks of operation. When first CR2 and later CR3 were fed higher TS_{in} and the HRT was increased from 30 to 42 days (Table S1), pH and NH_4^+-N increased (from week 9 and 20, respectively). In CR3, a similar NH_4^+-N level as in the duplicate reactor (CR2) was reached after approximately one HRT and after that it was considered that these two reactors had similar process performance. In the PFRs, VFA concentration, pH and NH_4^+-N level were initially high from the previous experimental phase, as mentioned above. However, after a short period of temporarily reduced OLR (Table S1), ammonia stress was alleviated, the reactors recovered and all parameters remained relatively stable from week 10 onwards (Fig. 1b, 1d, 1f, 1h). During the last period of the experiment (week 31–38), there were no significant differences in NH_4^+-N and NH_3-N levels between the PFRs and the two high-solid CSTRs (CR2 and CR3) ($p > 0.05$), while reactor CR1, still receiving the diluted substrate mix, had significantly lower levels than all other reactors ($p < 0.05$). During the same time-period, pH was significantly lower in CR1 compared with the two PFRs ($p < 0.05$), but no significant differences could be seen comparing the other reactors to each other.

It is well known that high ammonia concentrations are toxic for the microbial community (Rajagopal et al. 2013), but the threshold for inhibition varies widely between different systems. A range of 0.6–1.5 g NH_3-N/L has previously been reported as potentially inhibiting in thermophilic processes (Westerholm et al. 2016). However, in the previous experimental phase of the PFRs, disturbances were experienced when the NH_4^+-N level reached > 4 g/L, in those processes equal to ~ 2 g NH_3-N/L (Perman et al. 2024). A similar trend has also been observed in industrial thermophilic HSD processes (Westerholm et al. 2020; Perman et al. 2024). In line with this, no trend for accumulating VFA levels (Fig. 1g, 1 h) or other signs of inhibition were detected in any of the reactors in the present study after the initial 10 weeks, as NH_4^+-N concentration remained < 3 g/L (<1.5 g NH_3-N/L). However, VFA levels fluctuated more and also reached higher peaks in CR2 and CR3 than in CR1, potentially as a result of the comparably higher NH_4^+-N concentrations in these two reactors (Fig. 1g, Fig. S1). Among the PFRs, VFA levels fluctuated more in the non-recirculated reactor PR2 than in PR1 (Fig. 1h, Fig. S1), but in general stayed at a low level in relation to that in PR1 before week 10 and compared with levels expected during a disturbance (Perman et al. 2024).

3.1.2. Reactor comparisons; degree of degradation and specific gas and methane production

The PFRs were fed substrate mix with 27–28 % TS_{in} throughout the entire experiment. Initially, high TS_{out} (17–23 %) relative to VS_{out} (12–14 %) were observed in the digestate from these reactors, but after week 10 the values stabilised and thereafter remained at 14–18 % TS_{out} and 11–13 % VS_{out} (Fig. 2b). Possibly, the high initial values were related to the observed process disturbance during this period, and the corresponding adjustment of operation. All CSTRs were, during the start of the experiment, fed the same diluted substrate mix (TS_{in} 19 %) and therefore had similar TS_{out} (8–9 %) and VS_{out} (6–7 %) during the first 10 weeks (Fig. 2b). However, when the TS_{in} was increased (to 27–28 %) in CR2 and CR3, the outgoing TS and VS levels increased slowly and reached the same levels as in the PFRs around experiment week 25–30 (approximately one HRT after the increase in TS_{in}), but still showed an increasing trend throughout the experiment (Fig. 2b). Surprisingly, reactor CR1, still receiving the diluted substrate, showed the same increasing trend in TS_{out}. Since the TS_{out} also increased in the PFRs during the same period, this could indicate a difference in degradability of different substrate batches introduced week 27 and week 35 (Table 1). The more fluctuating TS_{out} and VS_{out} in the CSTRs, especially during the last weeks of the experiment (Fig. 2b), could also indicate build-up of TS in the reactors due to insufficient mixing. Another possible explanatory factor is that it was more difficult to obtain representative digestate samples from the CSTRs, since the digestate discharge openings in those reactors were narrower than in the PFRs.

Reduction in VS during the digestion process is an important measure of degree of substrate degradation. During weeks 25–38, the inter-quartile range of the VS reduction was 50–54 %, which is low in comparison with values reported for other HSD processes operated with food waste (Karthikeyan & Visvanathan 2013; Rocamora et al. 2020; Westerholm et al. 2020). However, the substrate mix in the present study comprised food waste together with a large fraction of recalcitrant lignocellulosic material, so the VS reduction observed is still reasonable (Ahlberg-Eliasson et al. 2017; Perman et al. 2024). Comparing the reactors operated under HSD conditions (CR2, CR3, PR1 and PR2), there were no significant differences in VS reduction ($p > 0.05$), based on data from weeks 25–38 (Fig. 2d). However, the control reactor operated at wet AD conditions (CR1) had significantly lower VS reduction compared with CR3 and the two PFRs ($p < 0.05$) (Fig. 2d).

Another measure of degree of degradation is N mineralisation, which was seemingly high at the start of the experiment due to initially high levels of NH_4^+-N in the PFRs and the CSTR inoculum (Fig. 2a). After week 10, N mineralisation stabilised and remained relatively constant throughout the remainder of the experiment, within the inter-quartile range 29–34 % (Fig. 2a). Pairwise comparisons of N mineralisation (from week 25) in different reactors showed significantly higher values in PR1 than in CR2 and CR3 ($p < 0.05$). However, there were no significant differences between any of the other reactors ($p > 0.05$) (Fig. 2c). In summary, the VS reduction was not clearly affected by reactor type, while the N mineralisation was lower in the CSTRs operating under HSD conditions, indicating somewhat better protein degradation in the PFRs.

There were no significant differences in specific gas production (SGP) or SMP between the two PFRs ($p > 0.05$) (Fig. 3). However, there was a general trend of significantly lower SGP and SMP in the CSTRs than the PFRs ($p < 0.05$) (Fig. 3). The wet AD reactor (CR1) had the lowest specific gas production among the CSTRs, although the difference was not significant (compared with CR2 and CR3) (Fig. 3). As mentioned

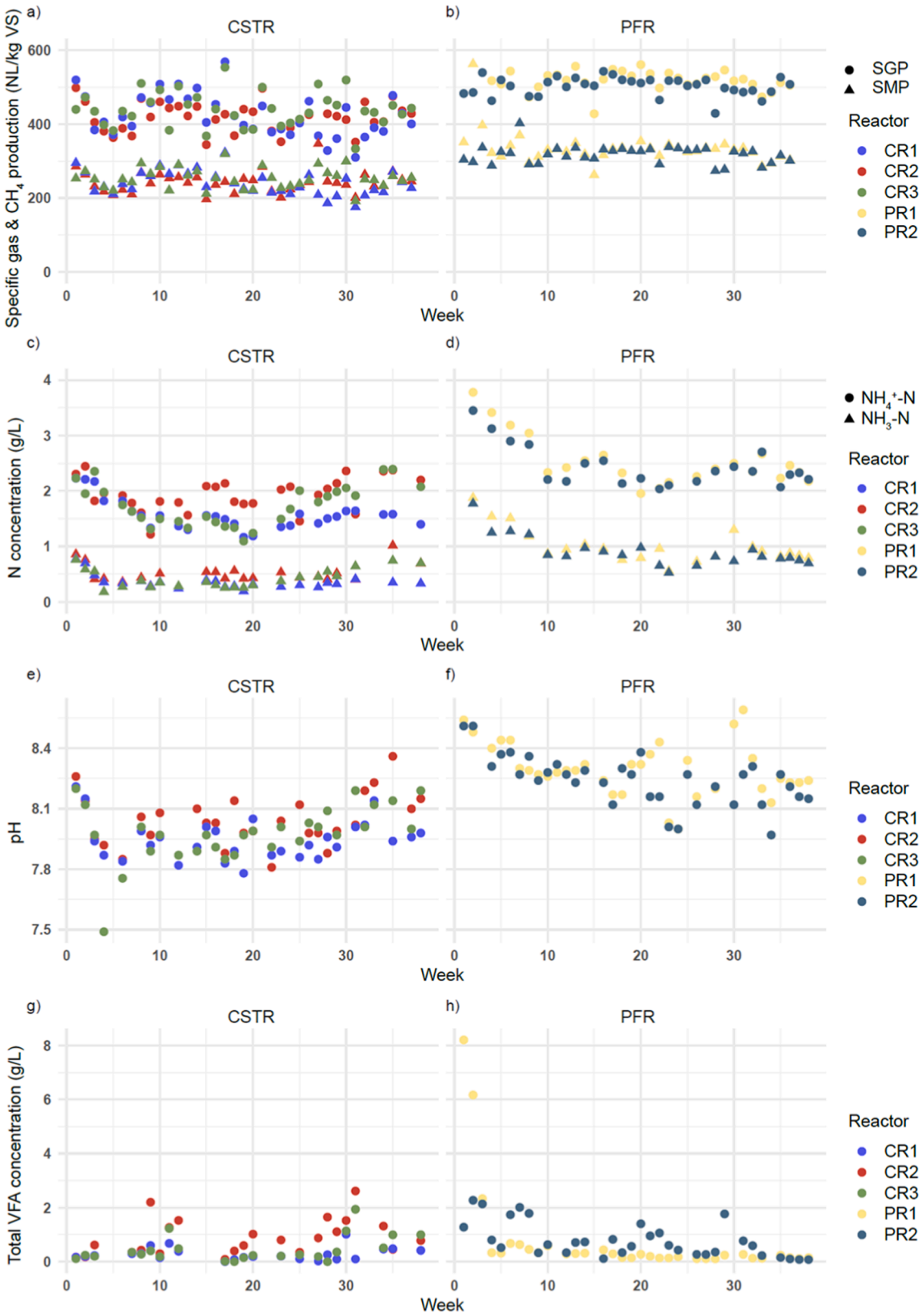

Fig. 1. Process parameters during the 38-week study period in reactors CR1, CR2 and CR3 (completely stirred-tank reactors (CSTR)) and PR1 and PR2 (plug-flow reactors (PFR)). Specific gas production (SGP) and specific methane production (SMP) in (a) CSTRs and (b) PFRs; total ammonium-N (including ammonia-N) (NH_4^+-N) and ammonia-N (NH_3-N) concentration in (c) CSTRs and (d) PFRs; pH in (e) CSTRs and (f) PFRs; and total volatile fatty acid (VFA) concentration in (g) CSTRs and (h) PFRs.

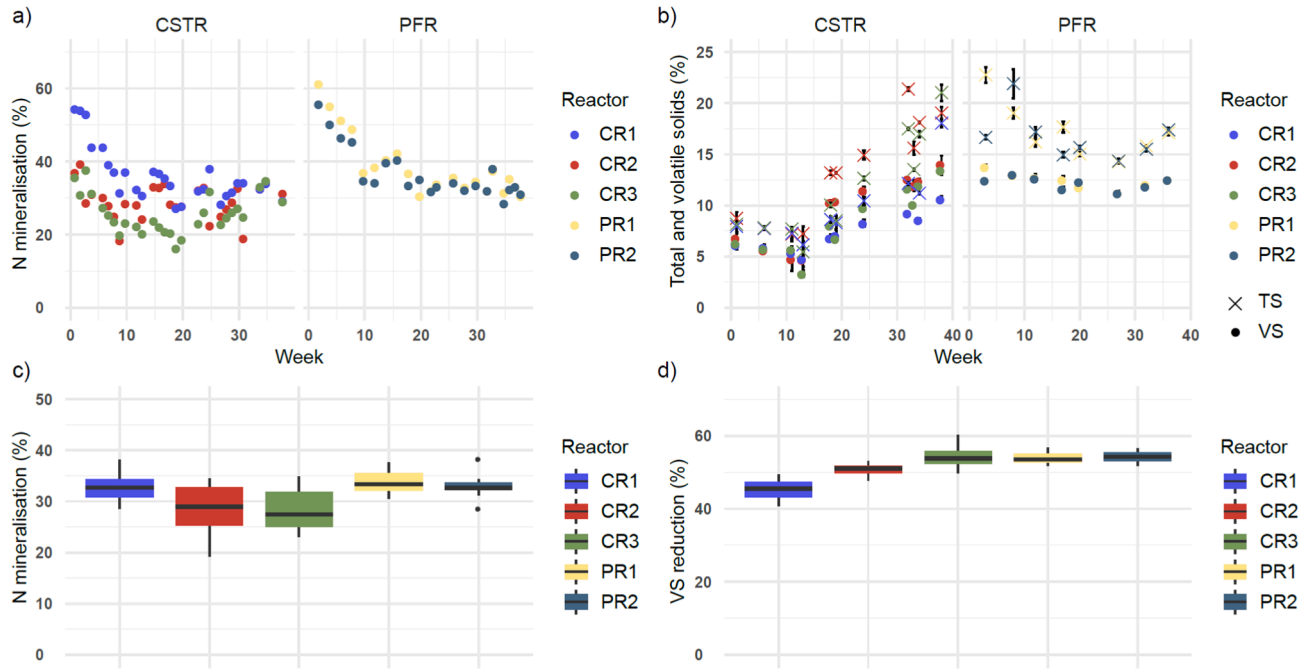

Fig. 2. (a) Nitrogen (N) mineralisation and (b) total solids (TS) and volatile solids (VS) content in digestate in completely stirred-tank (CSTR) reactors (CR1, CR2, CR3) and plug-flow (PFR) reactors (PR1, PR2) during the 38-week study period and (c) nitrogen (N) mineralisation and (d) VS reduction in the reactors during weeks 25–38.

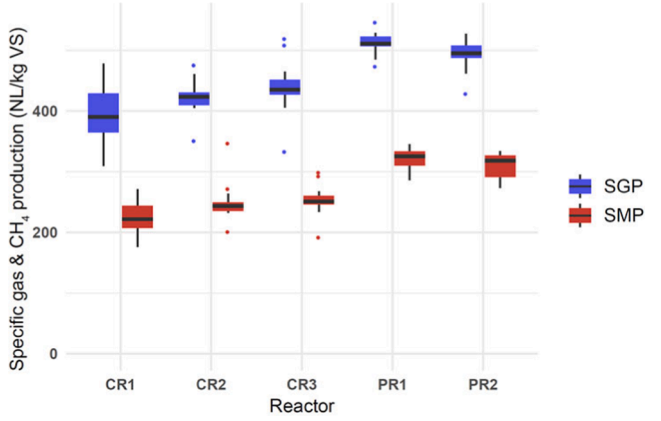

Fig. 3. Specific gas production (SGP) and specific methane production (SMP) in completely stirred-tank (CSTR) reactors (CR1, CR2, CR3) and plug-flow (PFR) reactors (PR1, PR2). Measurements from experiment weeks 25–38.

above, the lower N mineralisation in CR2 and CR3 indicated less effective protein degradation in these reactors compared with the PFRs, which could be a contributing factor to the comparably lower SGP and SMP. In CR1, the relatively low VS reduction indicated overall poorer substrate degradation, which could be one reason for the comparably low gas and methane production in this reactor. However, a possible source of error was that gas production in the CSTRs was measured using a different instrument and strategy than in the PFRs. Gas analysis for the PFRs was an instantaneous measure at a time-point before feeding, while gas analysis for the CSTRs was performed on gas collected during a whole day. This gas sampling strategy also could have affected the methane content (and thus SMP), which was generally higher in the PFRs (63 %) compared with the CSTRs (57–58 %) (Table S2).

To more thoroughly evaluate the difference in SGP and SMP observed between the two reactor types, theoretical values of SGP, SMP and TS_{out} were calculated based on assumptions of degree of degradation (VS reduction) and theoretical methane potential (TMP) values for the different substrate fractions (Table S3). With the settings given in Table S3a, the theoretical calculation matched the gas production obtained in practice from the CSTRs, *i.e.* SGP of 415 and SMP of 250 NL/kg VS. For the PFR, a comparably higher VS reduction of food waste, horse manure and garden waste had to be assumed (Table S3b) in order to reach values similar to those obtained in practice. Here it was possible to match the SGP (495 NL/kg VS), while the theoretical SMP (290 NL/kg VS) was lower than what was obtained from the reactor experiment. This discrepancy is likely related to the comparably higher methane concentration measured in the PFRs, than obtained from the calculations. The higher SGP and SMP obtained in the PFRs, compared with the CSTRs, theoretically should result in a corresponding increase in overall VS reduction. Based on the calculations, this would give a TS_{out} of 16 % in the PFRs, compared with 18 % in the CSTRs. In conclusion, the theoretical calculations show that higher SGP and SMP from the PFRs compared with the CSTRs is reasonable if assuming that VS_{out} was slightly lower for the former. From this exercise it was also clear that an increased degradation of a single substrate component alone could not result in the difference in SMP observed between the PFRs and CSTRs (data not shown).

3.1.3. *Effect of digestate recirculation*

Comparing the two PFRs to each other, the process parameters, degree of degradation and gas yield (*i.e.* VFA, pH, NH_4^+-N level, N mineralisation, VS reduction, SGP and SMP) indicated no significant differences between the reactors ($p > 0.05$). Thus, there was no significant effect of digestate recirculation in the plug-flow system. This was further confirmed by comparative analysis (pH, VFA and NH_4^+-N level) of the first and third reactor sections (S1 and S3) in the PFRs (data not shown), which showed no significant difference ($p > 0.05$). This suggests that the PFRs were not plug flow systems, either with or without digestate recirculation. This result is in line with findings in our previous study using the same reactors where plug flow was evaluated in the PFRs with recirculation (Perman et al. 2024).

3.2. Microbial community structure and links to the process and operating parameters

3.2.1. Differences in microbial community between reactors and reactor sections

16S rRNA sequencing was carried out to examine how the microbial community was affected by transition from PFR to CSTR. To gain an initial overview of β-diversity in the different reactors, weighted principal coordinate analysis (PCoA) was used (including samples from weeks 25–28). The results showed clear differences between the CSTRs and PFRs (Fig. 4). Moreover, comparing the CSTRs to each other revealed that the community in the low-TS reactor (CR1) was slightly separated from the two others, as further discussed below. Despite the significant difference in the TS level between CR1 and the two high-TS CSTRs (CR2 and CR3), the two reactor types still formed two clear clusters where CR2 and CR3 were more similar to CR1 than to the PFRs with corresponding TS level. This suggests that the TS level of the process was not the strongest deterministic factor for the microbial community structure. For the PFRs, the PCoA results illustrated no significant differences between individual reactors or between reactor sections which confirms that recirculation of digestate had a low influence on the development of the microbial community and that separation of microbial phases did not occur, irrespective of whether digestate was recycled or not. As said above, this is in line with previous findings for the same reactors (Perman et al. 2024), but contradictory to other reports where differences in community structure were seen across PFRs with recirculation ratios of 45–60 % (Chen et al. 2020; Rossi et al. 2022). In general, few previous studies have investigated the microbial stages in different reactor sections of a PFR and how they are affected by different operating strategies such as digestate recirculation. Thus, it is difficult to draw conclusions on how separation can be achieved.

3.2.2. Dominating microbial groups and relationship to differences in process parameters

Further analysis of community structure indicated that two groups in particular, MBA03 (phylum Bacillota) and *Defluviitoga* (phylum Thermotogota), dominated and differed in relative abundance between the CSTRs and PFRs (Fig. 5). These two groups have been observed previously to dominate in various thermophilic HSD processes (Dyksma et al. 2020; Westerholm et al. 2020; Zhang et al. 2022; Perman et al. 2024). In this study, *Defluviitoga* was the genus with the highest relative abundance in the PFRs, representing 15–41 % of the total community, while lower abundance (0–19 %) was observed in the CSTRs. Instead MBA03 dominated in the CSTRs, with relative abundance 30–73 %, while in the PFRs 10–17 % of the community belonged to this group. Another highly abundant genus was *Lentimicrobium* (phylum Bacteroidota), with relative abundance of 10–20 % in the PFRs and 1–12 % in the CSTRs. Interestingly, both *Defluviitoga* and *Lentimicrobium* decreased in abundance over time in the CSTRs, while MBA03 became increasingly dominant, especially in CR2 and CR3 (Fig. 5).

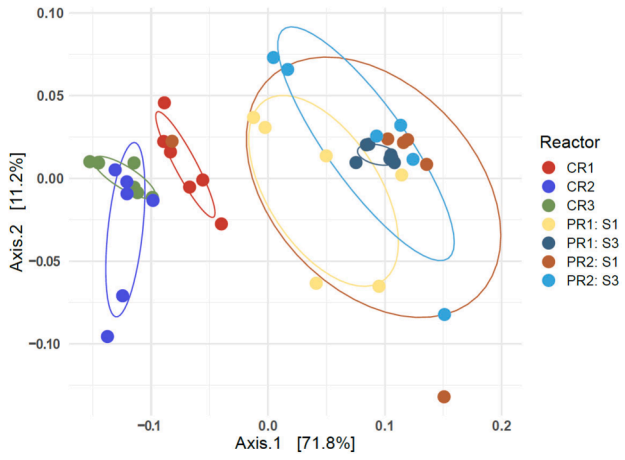

Fig. 4. Weighted principal coordinate analysis (PCoA) plot illustrating β-diversity in completely stirred-tank (CSTR) reactors (CR1, CR2, CR3) and in two different sections (S1 and S3) of plug-flow (PFR) reactors (PR1 and PR2). Samples taken during week 25–28.

Defluviitoga is known as an important degrader of complex carbohydrates in thermophilic AD and as a producer of acetate, ethanol, H_2 and CO_2 as fermentation products (Ben Hania et al. 2012). MBA03, on the other hand, is relatively uncharacterised, since no species from this group has yet been isolated, despite the high abundance of MBA03 in many processes. Otto et al. (2024) identified MBA03 as member of a core community in 80 industrial-scale digesters (present in 100 % of the samples), indicating its importance in AD. Recently, Puchol-Royo et al. (2023) suggested MBA03 as a new candidate order named Darwinibacterales with two subfamilies: "*Ca.* Darwinibacteriaceae" and "*Ca.* Wallacebacteriaceae". Based on metagenomic data, "*Ca.* family Darwinibacteriaceae" is suggested to have the ability to act as a syntrophic acetate-oxidising bacteria (SAOB) and it also possesses several hydrolytic enzymes, *e.g.* for starch, cellobiose and pectin degradation (Puchol-Royo et al. 2023). This is in line with previous observations suggesting that the MBA03 group may be involved in all these processes (Zheng et al. 2019; Dyksma et al. 2020). Although its exact function is not yet known, a previous study point towards a correlation between high abundance of MBA03 and high methane production in digestion of grass silage using CSTR (FitzGerald et al. 2019). Somewhat contradictory results were found in the present study, as MBA03 showed higher relative abundance in the CSTRs, where methane production was lower than in the PFRs.

Both *Defluviitoga* and the known cellulose degrader *Halocella* (phylum Halanaerobiaeota) (Simankova et al. 1993) were present at comparatively lower relative abundance in the CSTRs (<1%) than in the PFRs (2–5 %). In addition, the relative abundance of both *Defluviitoga* and *Halocella* decreased over time in all three CSTRs, indicating that the environment in the CSTRs was less favourable for these cellulolytic bacteria. All CSTRs were initially diluted with water (more than the PFR processes), which reduced the pH and NH_4^+-N levels (see Fig. 1). Throughout the majority of the experiment (week 5–30) the ammonia level was < 0.5 g NH_3-N/L in the CSTRs, while it was around 0.5–1.0 g NH_3-N/L in the PFRs. Although these levels were below inhibitory concentrations (Perman et al. 2024), ammonia is still a strong driver for the microbial community composition (Lee et al. 2017; Lv et al. 2019), and thus the differences in ammonia level might still explain some of the differences observed between the reactor types. For instance, both *Halocella* and *Defluviitoga* are known to be tolerant to high ammonia- and high pH-levels (Simankova et al. 1993; Ben Hania et al. 2012; Maus et al. 2017; Westerholm et al. 2020), and could have had an advantage in the PFR systems.

Genera identified in the reactors that are known to have protein-degrading members were *Proteiniphilum* (phylum Bacteroidota), *Acetomicrobium* (phylum Synergistota), *Keratinibaculum* and *Tepidimicrobium* (phylum Bacillota) (Chen & Dong 2005; Slobodkin et al. 2006; Huang et al. 2013; Hania et al. 2016). Among these, *Proteiniphilum* was the only genus showing higher abundance in the CSTRs (3–13 %) than the PFRs (1–2 %). *Tepidimicrobium* showed similar relative abundance in all reactors (around 1 %), while *Acetomicrobium* showed a higher relative abundance (2–5 %) in the PFRs compared with the CSTRs (<1–3 %), and *Keratinibaculum* showed a similar pattern, with relative abundance of 1–4 % in the PFRs and < 1–2 % in the CSTRs. These trends in relative abundance of protein-degrading genera could explain the lower N mineralisation in CR2 and CR3 compared with the PFRs (see Fig. 2). However, this is contradicted by the results for CR1, which showed similar N mineralisation as the PFRs, but similar microbial community structure as the other CSTRs.

As in other thermophilic HSD processes with relatively high ammonia levels (Tang et al. 2011; Dyksma et al. 2020; Westerholm et al.

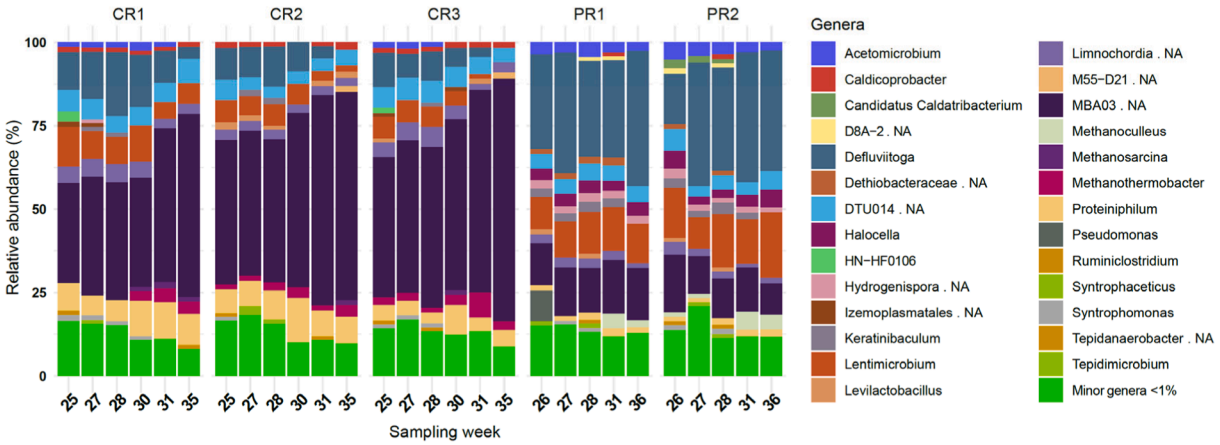

Fig. 5. Relative abundance of genera in completely stirred-tank (CSTR) reactors (CR1, CR2, CR3) and plug-flow (PFR) reactors (PR1 (section 3) and PR2 (section 3)). Genera with < 1 % relative abundance grouped as minor genera.

2020), methane was primarily produced through the hydrogenotrophic pathway, as illustrated by dominance of the hydrogenotrophic genera *Methanoculleus* (phylum Halobacteriota) and *Methanothermobacter* (phylum Euryarchaeota) within the archaeal community. Comparison of the methanogenic community in the two reactor types revealed a shift from higher relative abundance of *Methanoculleus* in the PFRs (<1–5 %) to higher abundance of *Methanothermobacter* in the CSTRs (<1–8 %). In the CSTRs, also the genus *Methanosarcina* (phylum Halobacteriota) was detected (<1–2 %), while this methanogen was absent in most of the samples from the PFRs. *Methanosarcina* has a broad substrate spectrum and can grow as a hydrogenotroph, acetotroph or methylotroph (De Vrieze et al. 2012). Acetate-consuming methanogens are generally more sensitive to ammonia inhibition than hydrogenotrophic methanogens (Fischer et al. 2019; Dyksma et al. 2020), which might explain the higher relative abundance of *Methanosarcina* in the CSTR processes. At high NH_3-N concentration, acetate conversion typically occurs through syntrophic acetate oxidation (SAO) in collaboration with hydrogenotrophic methanogenesis (Westerholm et al. 2016). In the studied reactors, ammonia concentrations did not cause disturbance, but were high enough to promote SAO activity in all reactors. Two potential SAOB observed within the community were *Syntrophaceticus* (phylum Bacillota) and *Caldicoprobacter* (phylum Bacillota) (Westerholm et al. 2018; Campanaro et al. 2020) and these were slightly affected by the transition from PFR to CSTR. The relative abundance of *Syntrophaceticus* was higher in most of the samples from the PFRs than in samples from the CSTRs, while the opposite was observed for *Caldicoprobacter*. However, the differences between reactor types were small, since the relative abundances of these genera were < 1–2 % in all samples. In conclusion, the results indicate that SAO was the predominant acetate degrading pathway in all investigated reactors, facilitated by *e.g. Syntrophaceticus* in collaboration with *Methanoculleus* or *Methanothermobacter*. However, in the CSTRs, acetoclastic methanogenesis by *Methanosarcina* might also have contributed to acetate consumption.

3.2.3. *Effect of reactor mixing speed on microbial community structure*

Although most operating parameters were identical in both reactor types, certain factors, such as reactor stirring speed, had to be different to fit the reactor design. In the CSTRs, a higher speed was necessary to avoid sedimentation of the solid material and enable good heat transfer. These factors were also the reason for further increasing the stirring speed from 70 to 150 rpm at week 30 in the CSTRs. In the PFRs, the inner walls were scraped by stirrer blades to prevent sedimentation and, compared with the CSTRs, a greater volume of material was moved in each revolution in the PFRs, which allowed use of much lower stirring speed (1 rpm). Stirring frequency can have a significant effect on the microbial population, *i.e.* the observed difference between the PFRs and CSTRs could be related to differences in stirring rate. For example, higher mixing speed has been shown to impede the interaction between bacteria performing SAO and hydrogenotrophic methanogens (Singh et al. 2020). In line with this, the higher abundance of *Methanosarcina* in the CSTRs could be a result of higher stirring speed since acetoclastic methanogens such as *Methanosarcina* are reported to be present in higher abundance in more agitated systems, while less agitation favours hydrogenotrophic methanogens such as *Methanoculleus* (Zhang et al. 2019; Sekine et al. 2022). In contrast, the hydrogenotrophic methanogen *Methanothermobacter* was present in higher relative abundance in the CSTRs. A trend for higher relative abundance of *Methanothermobacter* at higher stirring speed has been observed previously (Ghanimeh et al. 2018) and could be related to improved distribution of H_2 in the material at higher mixing speed (Singh et al. 2020), promoting growth of *Methanothermobacter* in these reactors. Slower mixing, and thereby lower H_2 transfer in the PFRs, appears to have been more favourable for *Methanoculleus*, which can grow at low H_2 levels (Neubeck et al. 2016; Kato et al. 2020).

The VFA profiles were similar throughout the experiment, with acetate and propionate as the main VFA species in all reactors (Fig. S1). No significant differences in propionate levels (during week 31–38) could be observed between the reactors ($p > 0.05$). The level of acetate however was significantly higher in the CTSRs operated at HSD conditions (CR2 and CR3), than in the PFRs (during week 31–38, $p < 0.05$). The reason could be a higher rate of acetate generation and/or a decreased SAO activity in the CSTRs. The latter potentially as a result of the higher stirring speed inhibiting the interaction between methanogens and SAOB. Several studies have shown that lower mixing frequency and intensity are beneficial for methane yield and process stability (Latha et al. 2019; Zhang et al. 2019; Sekine et al. 2022). In line with this, acetate concentration in all CSTRs peaked during, or just after, week 30 when the stirring speed was increased. The indication of decreased SAO activity in the CSTRs contradicts the hypothesis that MBA03 function as SAOB. If MBA03 were indeed SAOB, its high abundance would likely correlate with efficient acetate conversion, which was not observed.

Mixing intensity and speed have also been observed to affect cellulose degradation which is dependent on microbes attaching or coming into close proximity to the substrate (Schwarz 2001), interactions that can be disrupted at higher stirring rate. Kim et al. (2017) found that low or intermittent mixing in a semi-continuous CSTR was beneficial for cellulose degradation while an increase in mixing speed (50–150 rpm) caused severe disturbance and a significant decrease in methane production from rice straw. This is consistent with the reduction in the highly abundant cellulose-degrading genera (*Defluviitoga* and *Halocella*) observed in the CSTRs in comparison with the PFRs, and suggests that

the stirring intensity could be a contributing factor to the lower SMP in the CSTRs.

A potential reason for the high relative abundance of MBA03 observed in the CSTRs could be related to the substrate dispersion rate which likely was improved by the high stirring speed (Singh et al. 2020), meaning that new substrate was distributed more rapidly to the entire reactor volume. The initially higher water content in the CSTRs might also have simplified distribution of soluble organic matter in these reactors which may have favoured fast-growing microbes and microbes that easily can take up and metabolise soluble substrates. This could have favoured MBA03 and *Proteiniphilum* that both have members with the ability to ferment soluble carbohydrates (Hahnke et al. 2016; Puchol-Royo et al. 2023), and be a reason for the marked increase in relative abundance of these genera. *Lentimicrobium* also has members that can grow on soluble carbohydrates (Sun et al., 2016). However, this genus decreased in abundance in the CSTRs, possibly outcompeted by *e.g.* MBA03.

4. Conclusions

This study investigated the performance of an HSD process transferred from laboratory-scale reactors of PFR type to CSTRs. Theoretically, a process operated in a PFR has an advantage over a CSTR process, since short-circuiting of material is avoided in a well-functioning PFR. However, no evidence of plug-flow behaviour was observed and thus both the PFRs and CSTRs were effectively completely stirred reactor systems. Also, recirculation of whole digestate (30 %) in the PFR could not be shown to affect plug-flow behaviour and process performance significantly, nor influence the potential to obtain separate microbial communities across the length of the reactor.

In line with the lack of plug-flow characteristics, results from the comparative analysis in the present study could not clearly confirm theoretical benefits of PRF over CSTR for HSD, as no clear difference in degree of degradation could be shown. Still, the significantly higher SGP and SMP obtained from the PFRs indicated a better degradation capacity in this system. The higher NH_4^+-N level in the PFRs compared with the CSTRs suggests improved protein degradation in the PFRs, which might have contributed to the higher gas yield. However, as indicated by theoretical calculations as well as the microbial analysis this may not be the sole explanation for the comparably higher gas production in the PFRs. Most likely a combination of factors interplayed, affecting not only protein degradation but also conversion of other components. Such factors included differences in stirring frequency and ammonia level, leading to reduced activity of *e.g.* SAOB and cellulose degrading microbes in the CSTRs. Analysis of microbial community structure revealed a shift in both the potential cellulolytic and proteolytic genera between the reactor types with, *Defluviitoga* and *Lentimicrobium* dominating in the PFRs and the to the yet-to-be cultivated order MBA03 and the genus *Proteiniphilum* in the CSTRs. The methanogenic community also shifted, from dominance of *Methanoculleus* in the PFRs to *Methanothermobacter* and *Methanosarcina* in the CSTRs.

In addition, although process disturbances were not experienced in the present study, it is possible that the intense stirring could make the CSTR process more susceptible to *e.g.* inhibiting ammonia levels, as the SAO pathway is sensitive to high mixing speed. This could be an important disadvantage when operating HSD processes in CSTRs, as high ammonia concentration is a common problem in thermophilic HSD. Thus, HSD in CSTR processes could likely benefit from optimisation of operating parameters such as stirring intensity and mechanism. Despite differences in methane yield, this reactor comparison demonstrated that stable operation could be maintained while digesting substrates with high TS level (TS_{in} 27–28 %) in both PFRs and CSTRs, indicating flexibility in the choice of reactor technique. This could help in making informed decisions about selection of reactor system when designing and planning HSD experiments and processes.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 3.5 in order to find synonyms and improve language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Ebba Perman: Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Anna Karlsson:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Maria Westerholm:** Writing – review & editing, Supervision, Funding acquisition. **Simon Isaksson:** Investigation, Data curation. **Anna Schnürer:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

16S rRNA-gene sequence data can be accessed at BioProject accession number PRJNA1126088 at Sequence Read Archive (SRA), National Centre for Biotechnology Information (NCBI).

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2024.08.025.

References

Ahlberg-Eliasson, K., Nadeau, E., Leven, L., Schnurer, A., 2017. Production efficiency of Swedish farm-scale biogas plants. Biomass Bioenergy 97, 27–37. https://doi.org/10.1016/j.biombioe.2016.12.002.

Akinbomi, J.G., Patinvoh, R.J., Taherzadeh, M.J., 2022. Current challenges of high-solid anaerobic digestion and possible measures for its effective applications: a review. Biotechnol. Biofuels Bioprod. 15 (1) https://doi.org/10.1186/s13068-022-02151-9.

André, L., Pauss, A., Ribeiro, T., 2018. Solid anaerobic digestion: state-of-art, scientific and technological hurdles. Bioresour. Technol. 247, 1027–1037. https://doi.org/10.1016/j.biortech.2017.09.003.

Anukam, A., Mohammadi, A., Naqvi, M., Granström, K., 2019. A review of the chemistry of anaerobic digestion: methods of accelerating and optimizing process efficiency. Processes 7 (8), 504. https://doi.org/10.3390/pr7080504.

Ben Hania, W., Godbane, R., Postec, A., Hamdi, M., Ollivier, B., Fardeau, M.-L., 2012. *Defluviitoga tunisiensis* gen. nov., sp nov., a thermophilic bacterium isolated from a mesothermic and anaerobic whey digester. Int. J. Syst. Evol. Microbiol. 62, 1377–1382. https://doi.org/10.1099/ijs.0.033720-0.

Bollon, J., Benbelkacem, H., Gourdon, R., Buffiere, P., 2013. Measurement of diffusion coefficients in dry anaerobic digestion media. Chem. Eng. Sci. 89, 115–119.

Brémond, U., Bertrandias, A., De Buyer, R., Latrille, E., Jimenez, J., Escudié, R., Steyer, J.-P., Bernet, N., Carrere, H., 2021. Recirculation of solid digestate to enhance energy efficiency of biogas plants: strategies, conditions and impacts. Energ. Conver. Manage. 231, 113759 https://doi.org/10.1016/j.enconman.2020.113759.

Brown, D., Shi, J., Li, Y., 2012. Comparison of solid-state to liquid anaerobic digestion of lignocellulosic feedstocks for biogas production. Bioresour. Technol. 124, 379–386.

Campanaro, S., Treu, L., Rodriguez-R, L.M., Kovalovszki, A., Ziels, R.M., Maus, I., Zhu, X., Kougias, P.G., Basile, A., Luo, G., Schlüter, A., Konstantinidis, K.T., Angelidaki, I., 2020. New insights from the biogas microbiome by comprehensive genome-resolved metagenomics of nearly 1600 species originating from multiple anaerobic digesters. Biotechnol. Biofuels 13 (1). https://doi.org/10.1186/s13068-020-01679-y.

Chen, S., Dong, X., 2005. *Proteiniphilum acetatigenes* gen. nov., sp. nov., from a UASB reactor treating brewery wastewater. Int. J. Syst. Evol. Microbiol. 55, 2257–2261.

Chen, R., Li, Z., Feng, J., Zhao, L., Yu, J., 2020. Effects of digestate recirculation ratios on biogas production and methane yield of continuous dry anaerobic digestion. Bioresour. Technol. 316, 123963.

Chiumenti, A., da Borso, F., Limina, S., 2018. Dry anaerobic digestion of cow manure and agricultural products in a full-scale plant: Efficiency and comparison with wet fermentation. Waste Manage. 71, 704–710.

Costa, K.C., Leigh, J.A., 2014. Metabolic versatility in methanogens. Curr. Opin. Biotechnol. 29, 70–75. https://doi.org/10.1016/j.copbio.2014.02.012.

Danielsson, R., Dicksved, J., Sun, L., Gonda, H., Müller, B., Schnürer, A., Bertilsson, J., 2017. Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure. Front. Microbiol. 8 https://doi.org/10.3389/fmicb.2017.00226.

Das, A., Mondal, C., Chatterjee, S.G., 2016. Time-lag models for continuous stirred tank and plug flow digesters for biogas production. Energy Fuels, 10404–10416. Doi: 10.1021/acs.energyfuels.6b01320.

De Vrieze, J., Hennebel, T., Boon, N., Verstraete, W., 2012. *Methanosarcina*: the rediscovered methanogen for heavy duty biomethanation. Bioresour. Technol. 112, 1–9. https://doi.org/10.1016/j.biortech.2012.02.079.

Donoso-Bravo, A., Sadino-Riquelme, C., Gómez, D., Segura, C., Valdebenito, E., Hansen, F., 2018. Modelling of an anaerobic plug-flow reactor. Process analysis and evaluation approaches with non-ideal mixing considerations. Bioresour. Technol. 260, 95–104.

Duan, N., Dong, B., Wu, B., Dai, X., 2012. High-solid anaerobic digestion of sewage sludge under mesophilic conditions: feasibility study. Bioresour. Technol. 104, 150–156.

Dyksma, S., Jansen, L., Gallert, C., 2020. Syntrophic acetate oxidation replaces acetoclastic methanogenesis during thermophilic digestion of biowaste. Microbiome 8 (1). https://doi.org/10.1186/s40168-020-00862-5.

Fagbohungbe, M.O., Dodd, I.C., Herbert, B.M.J., Li, H., Ricketts, L., Semple, K.T., 2015. High solid anaerobic digestion: operational challenges and possibilities. Environ. Technol. Innov. 4, 268–284. https://doi.org/10.1016/j.eti.2015.09.003.

Fischer, M.A., Ulbricht, A., Neulinger, S.C., Refai, S., Wassmann, K., Kuenzel, S., Schmitz, R.A., 2019. Immediate effects of ammonia shock on transcription and composition of a biogas reactor microbiome. Front. Microbiol. 10 https://doi.org/10.3389/fmicb.2019.02064.

FitzGerald, J.A., Wall, D.M., Jackson, S.A., Murphy, J.D., Dobson, A.D.W., 2019. Trace element supplementation is associated with increases in fermenting bacteria in biogas mono-digestion of grass silage. Renew. Energy 138, 980–986. https://doi.org/10.1016/j.renene.2019.02.051.

Ghanimeh, S.A., Al-Sanioura, D.N., Saikaly, P.E., El-Fadel, M., 2018. Correlation between system performance and bacterial composition under varied mixing intensity in thermophilic anaerobic digestion of food waste. J. Environ. Manage. 206, 472–481. https://doi.org/10.1016/j.jenvman.2017.10.062.

Gómez, Ramos, S., Fernández, Muñoz, Tey, Romero, G., Hansen, 2019. Development of a modified plug-flow anaerobic digester for biogas production from animal manures. Energies, 12(13), 2628. Doi: 10.3390/en12132628.

Hahnke, S., Langer, T., Koeck, D.E., Klocke, M., 2016. Description of *Proteiniphilum saccharofermentans* sp. nov., *Petrimonas mucosa* sp. nov. and *Fermentimonas caenicola* gen. nov., sp. nov., isolated from mesophilic laboratory-scale biogas reactors, and emended description of the genus *Proteiniphilum*. Int. J. Syst. Evol. Microbiol. 66, 1466–1475. https://doi.org/10.1099/ijsem.0.000902.

Hania, W.B., Bouanane-Darenfed, A., Cayol, J.-L., Ollivier, B., Fardeau, M.-L., 2016. Reclassification of Anaerobaculum mobile, Anaerobaculum thermoterrenum, Anaerobaculum hydrogeniformans as Acetomicrobium mobile comb. nov., Acetomicrobium thermoterrenum comb. nov. and Acetomicrobium hydrogeniformans comb. nov., respectively, and emendati. Int. J. Syst. Evol. Microbiol. 66 (3), 1506–1509. https://doi.org/10.1099/ijsem.0.000910.

Hansen, K.H., Angelidaki, I., Ahring, B.K., 1998. Anaerobic digestion of swine manure: inhibition by ammonia. Water Res. 32, 5–12.

Huang, Y., Sun, Y., Ma, S., Chen, L., Zhang, H., Deng, Y., 2013. Isolation and characterization of Keratinibaculum paraultunense gen. nov., sp. nov., a novel thermophilic, anaerobic bacterium with keratinolytic activity. FEMS Microbiol. Lett. 345 (1), 56–63.

Jordbruksverket (2016). *Rötning av animaliska biprodukter*. https://jordbruksverket.se/download/18.16c992c517644f0089398dab/1685446982064/Rotning-av-animaliska-biprodukter-tga.pdf.

Karthikeyan, O.P., Visvanathan, C., 2013. Bio-energy recovery from high-solid organic substrates by dry anaerobic bio-conversion processes: a review. Rev. Environ. Sci. Biotechnol. 12 (3), 257–284. https://doi.org/10.1007/s11157-012-9304-9.

Kato, S., Takashino, M., Igarashi, K., Mochimaru, H., Mayumi, D., Tamaki, H., 2020. An iron corrosion-assisted H2-supplying system: a culture method for methanogens and acetogens under low H2 pressures. Sci. Rep. 10 (1) https://doi.org/10.1038/s41598-020-76267-z.

Kim, M., Kim, B.-C., Choi, Y., Nam, K., 2017. Minimizing mixing intensity to improve the performance of rice straw anaerobic digestion via enhanced development of microbe-substrate aggregates. Bioresour. Technol. 245, 590–597.

Kothari, R., Pandey, K.A., Kumar, S., Tyagi, V.V., Tyagi, S.K., 2014. Different aspects of dry anaerobic digestion for bio-energy: an overview. Renew. Sust. Energ. Rev. 39, 174–195.

Latha, K., Velraj, R., Shanmugam, P., Sivanesan, S., 2019. Mixing strategies of high solids anaerobic co-digestion using food waste with sewage sludge for enhanced biogas production. J. Clean. Prod. 210, 388–400.

Lee, J., Shin, S.G., Han, G., Koo, T., Hwang, S., 2017. Bacteria and archaea communities in full-scale thermophilic and mesophilic anaerobic digesters treating food wastewater: key process parameters and microbial indicators of process instability. Bioresour. Technol. 245, 689–697. https://doi.org/10.1016/j.biortech.2017.09.015.

Li, W., Gupta, R., Zhang, Z., Cao, L., Li, Y., Show, P.L., Gupta, V.K., Kumar, S., Lin, K.-Y.-A., Varjani, S., Connelly, S., You, S., 2023. A review of high-solid anaerobic digestion (HSAD): from transport phenomena to process design. Renew. Sustain. Energy Rev. 180, 113305.

Li, R., Li, X., Chen, S., 2007. Performance evaluation of anaerobic digestion of dairy manure in plug flow reactor and continuous stirred tank reactor. Trans. Chinese Soc. Agric. Eng. 23, 186–190.

Li, Y., Park, S.Y., Zhu, J., 2011. Solid-state anaerobic digestion for methane production from organic waste. Renew. Sustain. Energy Rev. 15 (1), 821–826. https://doi.org/10.1016/j.rser.2010.07.042.

Li, Y., Chen, P., Yu, Z., 2014. Spatial and temporal variations of micobial community in a mixed plug-flow loop reactor fed with dairy manure. J. Microbial. Biotechnol. 7, 332–346. https://doi.org/10.1111/1751-7915.12125.

Lv, Z., Leite, A.F., Harms, H., Glaser, K., Liebetrau, J., Kleinsteuber, S., Nikolausz, M., 2019. Microbial community shifts in biogas reactors upon complete or partial ammonia inhibition. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-018-9444-0.

Maus, I., Kim, Y.S., Wibberg, D., Stolze, Y., Off, S., Antonczyk, S., Puehler, A., Scherer, P., Schlueter, A., 2017. Biphasic study to characterize agricultural biogas plants by high-throughput 16S rRNA gene amplicon sequencing and microscopic analysis. J. Microbiol. Biotechnol. 27 (2), 321–334. https://doi.org/10.4014/jmb.1605.05083.

Mignogna, D., Ceci, P., Cafaro, C., Corazzi, G., Avino, P., 2023. Production of biogas and biomethane as renewable energy sources: a review. Appl. Sci. 13 (18), 10219. https://doi.org/10.3390/app131810219.

Moestedt, J., Müller, B., Nagavara Nagaraj, Y., Schnürer, A., 2020. Acetate and lactate production during two-stage anaerobic digestion of food waste driven by lactobacillus and aeriscardovia. Front. Energy Res. 8 https://doi.org/10.3389/fenrg.2020.00105.

Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. Eng. Life Sci. 12 (3), 242–257. https://doi.org/10.1002/elsc.201100085.

Neubeck, A., Sjöberg, S., Price, A., Callac, N., Schnürer, A., 2016. Effect of nickel levels on hydrogen partial pressure and methane production in methanogens. PLoS One 11, e0168357.

Nordberg, Å., Jarvis, Å., Stenberg, B., Mathisen, B., Svensson, B.H., 2007. Anaerobic digestion of alfalfa silage with recirculation of process liquid. Bioresour. Technol. 98, 104–111.

Nordell, E., Moestedt, J., Österman, J., Yekta, S.S., Björn, A., Sun, L., Schnürer, A., 2021. Post-treatment of dewatered digested sewage sludge by thermophilic high-solid digestion for pasteurization with positive energy output. Waste Manage. 119, 11–21.

Otto, P., Puchol-Royo, R., Ortega-Legarreta, A., Tanner, K., Tideman, J., De Vries, S.-J., Pascual, J., Porcar, M., Latorre-Perez, A., Abendroth, C., 2024. Multivariate comparison of taxonomic, chemical and operational data from 80 different full-scale anaerobic digester-related systems. Biotechnol. Biofuels Bioproducts 17, 84. https://doi.org/10.1186/s13068-024-02525-1.

Peng, X., Nges, I.A., Liu, J., 2016. Improving methane production from wheat straw by digestate liquor recirculation in continuous stirred tank processes. Renew. Energy 85, 12–18.

Perman, E., Westerholm, M., Liu, T., Schnürer, A., 2024. Comparative study of high-solid anaerobic digestion at laboratory and industrial scale – Process performance and microbial community structure. Energ. Conver. Manage. 300, 117978.

Puchol-Royo, R., Pascual, J., Ortega-Legarreta, A., Otto, P., Tideman, J., De Vries, S.-J., Abendroth, C., Tanner, K., Porcar, M., Latorre-Perez, A., 2023. Unveiling the ecology, taxonomy and metabolic capabilities of MBA03, a potential key player in anaerobic digestion [Preprint]. Cold Spring Harbor Laboratory. https://doi.org/10.1101/2023.09.08.556800.

Rajagopal, R., Massé, D.I., Singh, G., 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour. Technol. 143, 632–641. https://doi.org/10.1016/j.biortech.2013.06.030.

Ratanatamskul, C., Saleart, T., 2016. Effects of sludge recirculation rate and mixing time on performance of a prototype single-stage anaerobic digester for conversion of food wastes to biogas and energy recovery. Environ. Sci. Pollut. Res. 23 (8), 7092–7098. https://doi.org/10.1007/s11356-015-4448-0.

Rico, C., Rico, J.L., Muñoz, N., Gómez, B., Tejero, I., 2011. Effect of mixing on biogas production during mesophilic anaerobic digestion of screened dairy manure in a pilot plant. Eng. Life Sci. 11 (5), 476–481. https://doi.org/10.1002/elsc.201100010.

Rocamora, I., Wagland, S.T., Villa, R., Simpson, E.W., Fernández, O., Bajón-Fernández, Y., 2020. Dry anaerobic digestion of organic waste: A review of operational parameters and their impact on process performance. Bioresour. Technol. 299, 122681.

Rossi, E., Becarelli, S., Pecorini, I., Di Gregorio, S., Iannelli, R., 2022. Anaerobic digestion of the organic fraction of municipal solid waste in plug-flow reactors: focus on bacterial community metabolic pathways. Water 14 (2), 195. https://doi.org/10.3390/w14020195.

Roy, C.S., Talbot, G., Topp, E., Beaulieu, C., Palin, M.-F., Masse, D.I., 2009. Bacterial community dynamics in an anaerobic plug-flow type bioreactor treating swine manure. Water Res. 43 (1), 21–32. https://doi.org/10.1016/j.watres.2008.09.034.

Scarlat, N., Dallemand, J.F., Fahl, F., 2018. Biogas: developments and perspectives in Europe. Renew. Energy 129, 457–472.

Schnürer, A., Jarvis, A., 2018. Microbiology of the biogas process. ISBN 978-91-576-9546-8.

Schwarz, W.H., 2001. The cellulosome and cellulose degradation by anaerobic bacteria. Appl. Microbiol. Biotechnol. 56 (5–6), 634–649. https://doi.org/10.1007/s002530100710.

Sekine, M., Mizuno, N., Fujiwara, M., Kodera, T., Toda, T., 2022. Improving methane production from food waste by intermittent agitation: Effect of different agitation frequencies on solubilization, acidogenesis, and methanogenesis. Biomass Bioenergy 164, 106551.

Simankova, M.V., Chernych, N.A., Osipov, G.A., Zavarzin, G.A., 1993. *Halocella cellulolytica* gen. nov., sp. nov., a new obligately anaerobic, halophilic, cellulolytic bacterium. Syst. Appl. Microbiol. 16 (3), 385–389. https://doi.org/10.1016/s0723-2020(11)80270-5.

Singh, B., Szamosi, Z., Siménfalvi, Z., 2019. State of the art on mixing in an anerobic digester: a review. Renew. Energy 141, 922–936.

Singh, B., Szamosi, Z., Siménfalvi, Z., 2020. Impact of mixing intensity and duration on biogas production in an anaerobic digester: a review. Crit. Rev. Biotechnol. 40 (4), 508–521. https://doi.org/10.1080/07388551.2020.1731413.

Slobodkin, A.I., Tourova, T.P., Kostrikina, N.A., Lysenko, A.M., German, K.E., Bonch-Osmolovskaya, E.A., Birkeland, N.K., 2006. Tepidimicrobium ferriphilum gen. nov., sp. nov., a novel moderately thermophilic, Fe(III)-reducing bacterium of the order Clostridiales. Int. J. Syst. Evol. Microbiol. 56 (2), 369–372. https://doi.org/10.1099/ijs.0.63694-0.

Subbarao, P.M.V., D' Silva, T.C., Adlak, K., Kumar, S., Chandra, R., Vijay, V.K., 2023. Anaerobic digestion as a sustainable technology for efficiently utilizing biomass in the context of carbon neutrality and circular economy. Environ. Res., 234, 116286.

Srisowmeya, G., Chakravarthy, M., Nandhini Devi, G., 2020. Critical considerations in two-stage anaerobic digestion of food waste – a review. Renew. Sustain. Energy Rev. 119, 109587.

Sun, L., Toyonaga, M., Ohashi, A., Tourlousse, D.M., Matsuura, N., Meng, X.-Y., Tamaki, H., Hanada, S., Cruz, R., Yamaguchi, T., Sekiguchi, Y., 2016. Lentimicrobium Saccharophilum gen. nov., sp. nov., a strictly anaerobic bacterium representing a new family in the phylum Bacteroidetes, and proposal of Lentimicobiaceae fam. nov. Int. J. Syst. Evol. Micobiol. 66, 2542–2635.

Tang, Y.-Q., Ji, P., Hayashi, J., Koike, Y., Wu, X.-L., Kida, K., 2011. Characteristic microbial community of a dry thermophilic methanogenic digester: its long-term stability and change with feeding. Appl. Microbiol. Biotechnol. 91 (5), 1447–1461. https://doi.org/10.1007/s00253-011-3479-9.

Toson, P., Doshi, P., Jajcevic, D., 2019. Explicit residence time distribution of a generalised cascade of continuous stirred tank reactors for a description of short recirculation time (bypassing). Processes 7 (9), 615. https://doi.org/10.3390/pr7090615.

Wang, Z., Hu, Y., Wang, S., Wu, G., Zhan, X., 2023. A critical review on dry anaerobic digestion of organic waste: characteristics, operational conditions, and improvement strategies. Renew. Sustain. Energy Rev. 176, 113208.

Westerholm, M., Hansson, M., Schnürer, A., 2012. Improved biogas production from whole stillage by co-digestion with cattle manure. Bioresour. Technol. 114, 314–319. https://doi.org/10.1016/j.biortech.2012.03.005.

Westerholm, M., Moestedt, J., Schnurer, A., 2016. Biogas production through syntrophic acetate oxidation and deliberate operating strategies for improved digester performance. Appl. Energy 179, 124–135. https://doi.org/10.1016/j.apenergy.2016.06.061.

Westerholm, M., Müller, B., Singh, A., Karlsson Lindsjö, O., Schnürer, A., 2018. Detection of novel syntrophic acetate-oxidising bacteria from biogas processes by continuous acetate enrichment approaches. J. Microbial. Biotechnol. 11, 680–693. https://doi.org/10.1111/1751-7915.13035.

Westerholm, M., Liu, T., Schnürer, A., 2020. Comparative study of industrial-scale high-solid biogas production fromfood waste: process operation and microbiology. Bioresour. Technol. 304, 122981.

Yue, Z., Teater, C., MacLellan, J., Liu, Y., Liao, W., 2011. Development of a new bioethanol feedstock – anaerobically digested fiber from confined dairy operations using different digestion configurations. Biomass Bioenergy 35, 1946–1953.

Zhang, J., Mao, L., Nithya, K., Loh, K.-C., Dai, Y., He, Y., Tong, Y.W., 2019. Optimizing mixing strategy to improve the performance of an anaerobic digestion waste-to-energy system for energy recovery from food waste. Appl. Energy 249, 28–36.

Zhang, S., Xiao, M., Liang, C., Chui, C., Wang, N., Shi, J., Liu, L., 2022. Multivariate insights into enhanced biogas production in thermophilic dry anaerobic co-digestion of food waste with kitchen waste or garden waste: Process properties, microbial communities and metagenomic analyses. Bioresour. Technol. 361, 127684.

Zheng, D., Wang, H., Gou, M., Nubo, M.K., Narihiro, T., Hu, B., Nie, Y., Tang, Y.Q., 2019. Identification of novel potential acetate-oxidizing bacteria in thermophilic methanogenic chemostats by DNA stable isotope probing. Appl. Microbiol. Biotechnol. 103, 8631–8645. https://doi.org/10.1007/s00253-019-10078-9.