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Full-scale study on high-rate low-temperature anaerobic digestion of agro-food wastewater: process performances and microbial community

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ABSTRACT

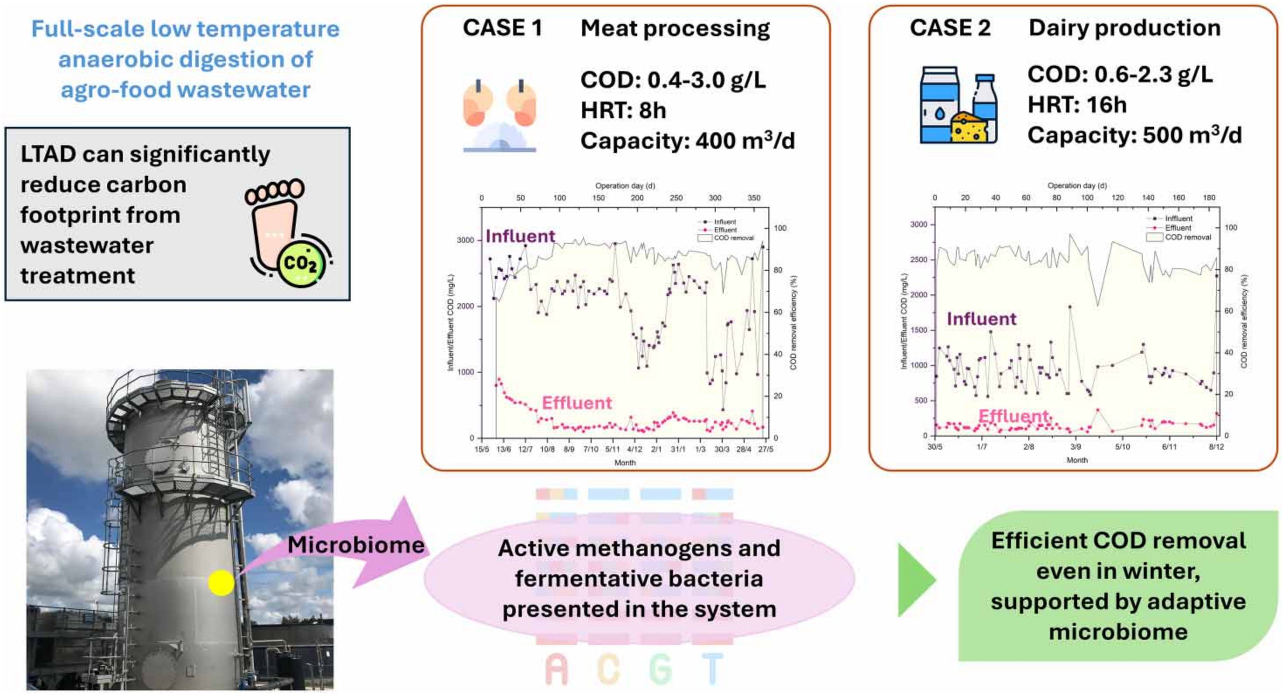
The fast-growing global population has led to a substantial increase in food production, which generates large volumes of wastewater during the process. Despite most industrial wastewater being discharged at lower ambient temperatures (<20 °C), majority of the high-rate anaerobic reactors are operated at mesophilic temperatures (>30 °C). High-rate low-temperature anaerobic digestion (LtAD) has proven successful in treating industrial wastewater both at laboratory and pilot scales, boasting efficient organic removal and biogas production. In this study, we demonstrated the feasibility of two full-scale high-rate LtAD bioreactors treating meat processing and dairy wastewater, and the microbial communities in both reactors were examined. Both reactors exhibited rapid start-up, achieving considerable chemical oxygen demand (COD) removal efficiencies (total COD removal >80%) and generating high-quality biogas (CH_4% in biogas >75%). Long-term operations (6–12 months) underscored the robustness of LtAD bioreactors even during winter periods (average temperature <12 °C), as evidenced by sustained high COD removal rates (total COD removal >80%). The stable performance was underpinned by a resilient microbial community comprising active acetoclastic methanogens, hydrolytic, and fermentative bacteria. These findings underscore the feasibility of high-rate low-temperature anaerobic wastewater treatment, offering promising solutions to the zero-emission wastewater treatment challenge.

Key words: agro-food wastewater, biogas, low-temperature anaerobic digestion, microbial community, wastewater treatment

HIGHLIGHTS

- First study on full-scale low-temperature anaerobic digestion of industrial wastewater.
- Efficient COD removal and biogas production even at cold temperatures.
- Stable performances during long-term operations supported by a robust microbiome.
- This technology could significantly reduce the energy consumption in wastewater treatment.

GRAPHICAL ABSTRACT



1. INTRODUCTION

The rapid increase in the world's population has led to substantial growth in food production to meet the demand (D'Odorico *et al.* 2018). However, a large amount of water is required during the manufacturing processes, subsequently, generating high volumes of wastewater. The agro-food wastewater contains abundant biodegradable constituents and nutrients (Bokhary *et al.* 2023). The direct discharge of this wastewater poses serious threats to the environment, polluting land and water reservoirs, and endangering human and ecosystem health. Additionally, wastewater has been reported to contribute to up to 6% of all anthropogenic methane emissions (Tauseef *et al.* 2013). In this context, the development of an efficient, low-carbon wastewater treatment process becomes imperative to mitigate the environmental impacts arising from agro-food wastewater.

Anaerobic digestion (AD) is an efficient and well-established technology for wastewater treatment that links organic matter degradation with energy production in the form of biogas. During AD, complex organic matter is sequentially converted to smaller molecules through the activity of several groups of microorganisms in a four-step process: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Almansa *et al.* 2023). A previous study showed that a dairy production facility with a full-scale AD unit can significantly reduce the environmental impacts of the processing facility by providing 20% of the energy consumption of the factory and reducing the total carbon footprint by 13% (Stanchev *et al.* 2020). Besides biogas production, the main benefits of AD are its high bioconversion efficiency (~90%), low sludge production, high organic loads, low operational costs and efficient removal of pathogens (Almansa *et al.* 2023).

High-rate anaerobic reactors are designed for effectively treating high volumes of wastewater by retaining active microorganisms within the system. The retention can be achieved by the immobilisation of the microbes on a support material, physical separation by membrane, or in the form of microbial granules, allowing the systems to have shorter hydraulic retention times (HRTs) and higher organic loads (van Lier 2008). High-rate anaerobic reactors have been widely employed for wastewater treatment, utilising various designs such as the anaerobic filter (AF), anaerobic moving bed biofilm reactor (AMBBR), upflow anaerobic sludge blanket (UASB) reactor, expanded granular sludge bed (EGSB) reactor, anaerobic membrane (AnMBR), and anaerobic hybrid reactors (van Lier 2008). Among these, granular sludge-based systems (UASB and EGSB) are particularly prevalent due to their cost-efficiency relative to other designs (Goffi *et al.* 2018), which have demonstrated successful application in treating a range of wastewater types including those from meat processing, dairy production, sugar processing, starch production, and palm oil milling (Bokhary *et al.* 2023).

The anaerobic wastewater treatment is usually operated under mesophilic (30–35 °C) or thermophilic (45–60 °C) conditions to meet the criteria for effluent quality (Lettinga *et al.* 2001). However, industrial wastewaters are often discharged at lower temperatures (<20 °C), requiring huge input of energy in heating the wastewater and maintaining the operating temperature at the mesophilic or thermophilic range, entailing a considerable operational cost (Smith *et al.* 2014). Therefore, low-temperature AD (LtAD) is an attractive cost-effective alternative to the conventional mesophilic/thermophilic treatment for industrial wastewater. LtAD has been demonstrated to treat wastewater for the last two decades. Its potential to treat sewage and industrial wastewater has been demonstrated both at the laboratory and pilot-scale (McHugh *et al.* 2006; Akila & Chandra 2007; McKeown *et al.* 2009b; Keating *et al.* 2018; Paulo *et al.* 2020; Liu *et al.* 2023). Recently, this technology has been successfully treating municipal sewage at full-scale (Trego *et al.* 2021), showing that LtAD is a promising technology for real-world scenarios. Yet, full-scale applications of LtAD in industrial wastewater treatment have not been demonstrated so far.

Therefore, the main objective of this study is to demonstrate the feasibility of LtAD treating industrial effluents at full-scale. We monitored the performance of two full-scale high-rate LtAD reactors working at ambient/low temperatures, treating meat processing wastewater and dairy wastewater, respectively. Furthermore, the active microbial community profiles of both reactors were examined by high-throughput amplicon sequencing.

2. MATERIALS AND METHODS

2.1. Reactor design and operation

The reactor design (Figure 1) was a direct scale-up of a pilot unit described previously (Paulo *et al.* 2020) and a similar design was used at full-scale to treat municipal wastewater (Trego *et al.* 2021). For each reactor, the wastewater is first diverted from the dissolved air flotation (DAF, remove excess suspended solids in the wastewater to prevent clogging in pipelines) tank to a transfer tank, where pH is continuously monitored and adjusted with sodium hydroxide to an acceptable pH range for the AD process (Figure 1). Both reactors were inoculated with commercial anaerobic granular sludge (sludge loading 20 g VSS/L) from a mesophilic AD plant treating starch wastewater. The upflow velocity of the reactors was maintained at 1.5–2.0 m/h.

Reactor A has 100 m^3 (diameter: 3.5 m, height: 11 m, active volume 88 m^3) and treats meat processing wastewater with a HRT 8 h, which was monitored for 12 months (May 2016–May 2017). The total chemical oxygen demand (tCOD) of the influent to the LtAD plant (after DAF process) ranged from 890 to 2,950 mg/L. The operating temperature during the investigation ranged between 0 and 31 °C (Table 1, Figure 2).

Reactor B has 190 m^3 (diameter: 4.5 m, height: 12 m, active volume 163.2 m^3) and treats dairy wastewater, which was monitored for 6 months (June–Dec 2017). The reactor started with an HRT of 32 h, and it was reduced to 16 h in step-wise (Table 1), within the first 2 months of operation. The tCOD of the influent was ranged from 460 to 2,888 mg/L. The operating temperature during the investigation was between 0 and 32 °C (Table 1, Figure 3).

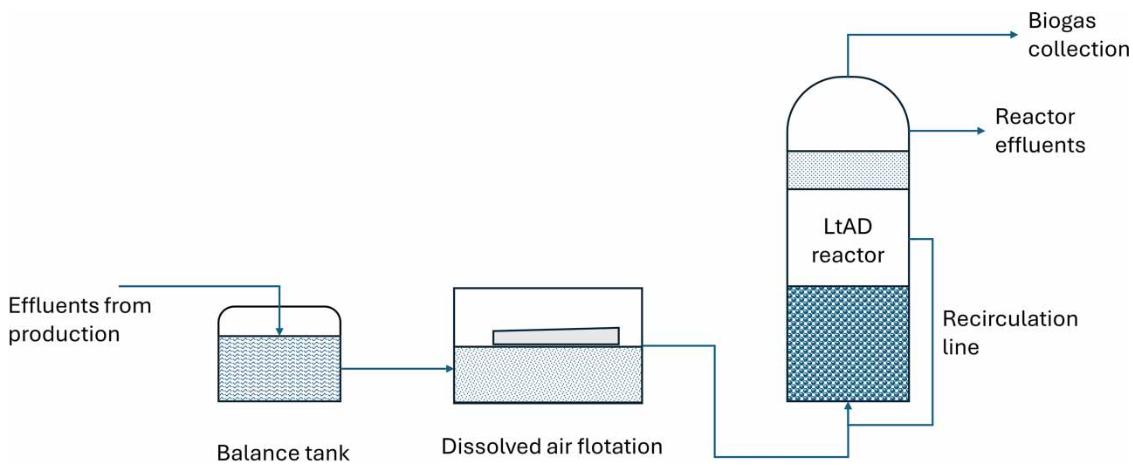


Figure 1 | Schematic diagram of the low-temperature anaerobic wastewater treatment process.

Table 1 | Summary of the operational and performance characteristics during the first year of operation for Reactors A and B

Operational parameters	Reactor A	Reactor B
HRT (h)	8	32, 28, 24, 20, 16
Temperature (°C)	0–31	0–32
Average temperature (°C)	12.5 ± 5.0	12.3 ± 5.2
Influent COD range (mg/L)	434–2,950	602–2,272
Average influent COD (mg/L)	2,029 ± 525	1,027 ± 412
OLR (kg/L/day)	1.3–8.9	0.6–4.3
Average OLR (kg/m^3/day)	6.1 ± 1.6	1.4 ± 0.7
Average tCOD removal (%)	86 ± 6	84 ± 6
Average sCOD removal (%)	–	86 ± 5
pH	6.4–8.5	6.3–7.0
Average methane content (%)	80 ± 8	74[a] ± 20
Average biogas flow (m^3/h)	3.4 ± 1.5	2.9 ± 1.7

[a]Gas data were collected inconsistently due to technical issues ± standard deviations.
– indicates data unavailable.

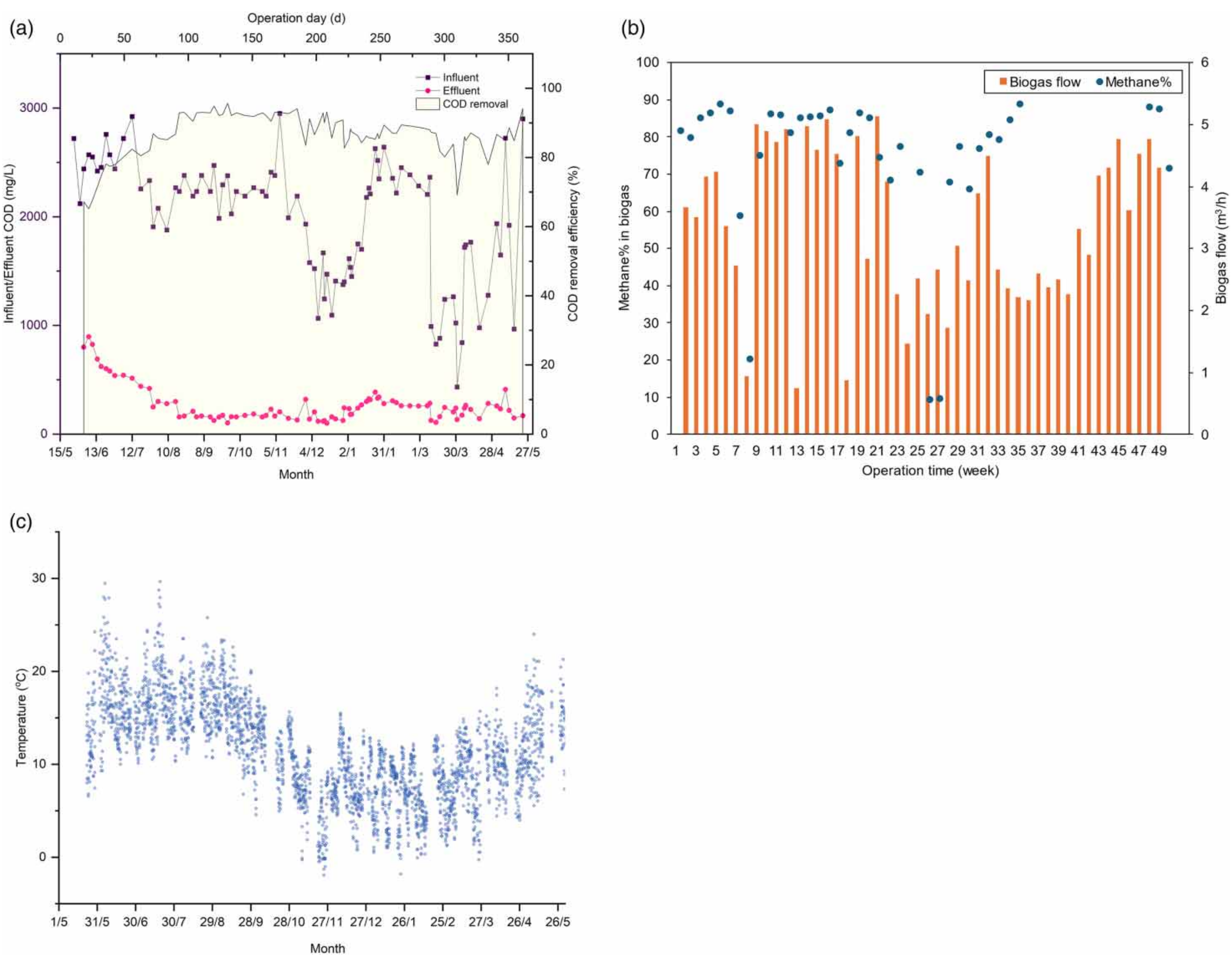


Figure 2 | Reactor A performance over the trial: (a) COD removal efficiency; (b) biogas production and methane percentage per week; and (C) reactor temperature daily dynamics.

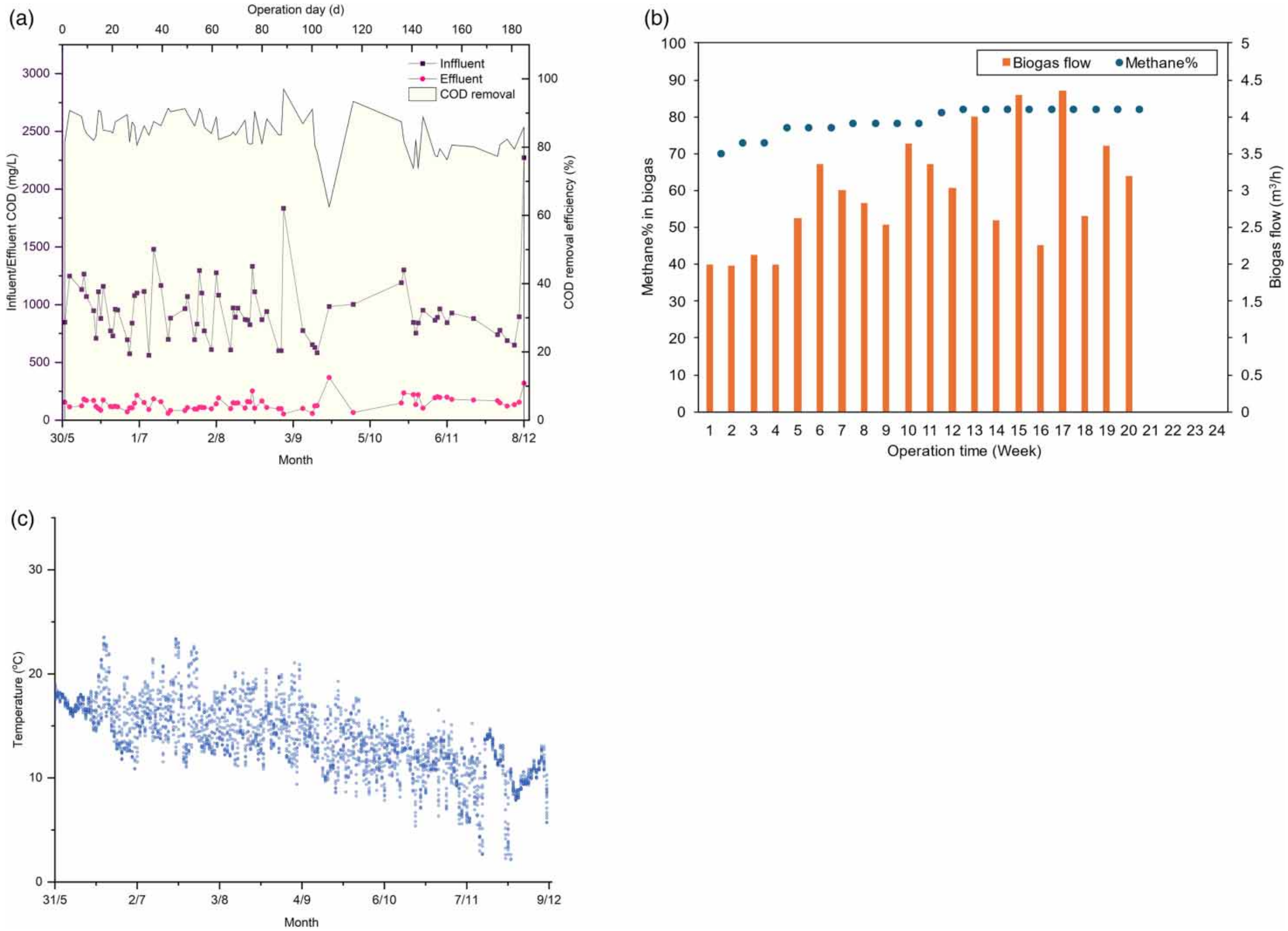


Figure 3 | Reactor B performance over the trial: (a) COD removal efficiency; (b) biogas production and methane percentage per week (no available data during last month of the trial due to technical issues); and (C) reactor temperature daily dynamics.

2.2. Analytical analysis

The quality of the reactor effluents was monitored every 2–3 days. The tCOD and soluble chemical oxygen demand (sCOD) of the effluents were analysed by using a kit from Reagecon (Shannon, Ireland) following the manufacturers' method. The biogas production is measured *in situ* with a gas meter (model ST75V; Fluid Components International, San Marcos, CA, USA). The gas content (CH_4, CO_2, O_2, and H_2) is determined in a gas analyser, model SWG100 Biocompact (Eurotron Instruments Ltd, Daventry, UK). The temperature of the reactor and the pH of the influents and effluents were monitored by the built-in sensor.

2.3. Microbial community analysis

Biomass samples were collected for both reactors at the beginning and the end of the investigation period. Collected samples were flash-frozen by liquid nitrogen and stored at −80 °C for later RNA extraction. RNA was extracted in triplicates from each sample. RNA extraction, cDNA synthesis and library preparation were performed as described elsewhere (Paulo *et al.* 2020). The PCR-purified products were mixed together in equimolar amounts to create a library pool and sent for sequencing on the Illumina Hiseq 2000 platform (GATC Biotech AG, Konstanz, Germany). The raw sequencing data were processed by NG-Tax under default parameters (Ramiro-Garcia *et al.* 2018), with the SILVA 138.1 (Quast *et al.* 2013) as a taxonomy reference database.

3. RESULTS AND DISCUSSION

3.1. Fast reactor start-up

Both Reactor A and Reactor B had fast start-up (within 3 months) with high COD removal efficiencies via different strategies (Figure 2 and Figure 3). Reactor A was commissioned at 8 h HRT immediately (day 1) with a high organic loading rate

(OLR > 6.0 kg COD/m^3/d). The tCOD removal efficiency increased from 65 to 80% gradually within 30 days of operation (Figure 2). In contrast, Reactor B adopted a step-wise HRT reduction strategy to initiate the system according to the previous pilot-scale study (Paulo *et al.* 2020), decreasing from 32 to 16 h (Table 1, OLR 0.8–1.5 kg COD/ m^3/d) within the first 60 days. The tCOD removal efficiencies were over 85% during the start-up phase (Figure 3). These results showed that the full-scale reactors can quickly adapt to different influent wastewater with a wide range of OLR (0.77–6.0 kg COD/ m^3/d), suggesting a robust performance in treating agro-food wastewaters at ambient temperature (<20 °C). The different performances during the start-up phase between the two reactors suggest that reducing OLR is beneficial to the start-up of the LtAD reactor with efficient and stable performance. High OLR may overload the microorganisms, hindering the decomposition of the substrates, resulting in acidification, and inhibiting methanogenic activity (Wang & Zhou 2023). For instance, Bialek *et al.* (2014) reported that applying OLR over 2 kg COD/ m^3/d to LtAD reactor treating dilute dairy wastewater promoted acidification in the system. Thus, the step-wise increment of OLR allows the biomass to acclimate to the substrates and improve the robustness of the system for long-term operations.

3.2. Long-term reactor performances

We monitored both reactors for 12 months (Reactor A) and 6 months (Reactor B) to investigate their long-term performances in treating the wastewater. Both reactors maintained high levels of COD removal during the majority of the operational period (Table 1, Figure 2, Figure 3).

The average COD removal of Reactor A was 86 ± 6% and the average methane content in the biogas produced was 80 ± 8%. Notably, the reactor performance was stable (COD removal > 80%) even during the winter period (Figure 2), when the average operation temperature was below 15 °C. Moreover, the influent COD fluctuation has no significant impact on the reactor as well (Figure 2). Compared with other full-scale reactors treating meat processing/slaughterhouse wastewater, Reactor A showed better performance. Del Nery *et al.* (2007) studied two UASB reactors treating poultry slaughterhouse wastewater, which displayed a total COD removal efficiency of 67 ± 9% at an OLR of 1.6 ± 0.4 kg COD/m^3/d. Another UASB reactor treating pig and cattle slaughterhouse wastewater after a coagulation–flocculation pre-treatment at HRT 18–27 h, exhibited a COD removal efficiency of 70–92% (Miranda *et al.* 2005). Moreover, Qamar *et al.* (2022) assessed the performance of a full-scale treatment plant for slaughterhouse wastewater, showing that the UASB reactor performed poorly in COD removal (26 ± 3%) due to sludge floatation. During the operation, we noticed that the overdosing of inorganic coagulant chemicals (ferric sulphates) during the DAF process, resulted in occasional high levels (800–1,000 mg/L) of sulphate in the wastewater, which further led to increases in the content of H_2S in the biogas produced from reactor A. This issue was later solved by replacing ferric sulphate with ferric chloride as a coagulant chemical in the DAF process, which should be noted for the operation of AD reactors. Future improvements can be made by using biological coagulants such as chitosan and plant-based coagulants to further reduce chemical usage during wastewater treatments (Ang & Mohammad 2020).

Reactor B maintained its high performance until the end of the investigation period (tCOD removal >80%, methane content in biogas > 74%, Table 1, Figure 3), despite the cold climate during winter (Oct-Dec, average temperature 10 ± 3 °C, Figure 3). The performance of Reactor B is complementary with the previous pilot-scale trial (Paulo *et al.* 2020) treating dairy wastewater with similar design, achieving tCOD removal at 41–79% under ambient temperature (<20 °C), and laboratory-scale trials (Bialek *et al.* 2013; McAteer *et al.* 2020; Liu *et al.* 2023) operating below 15 °C, which obtained COD removal efficiency ranged 65–85%. The performance is also comparable to full-scale mesophilic anaerobic systems, even when operating at low HRT. For instance, a full-scale AF reactor treating complex dairy wastewater at 37 °C achieved COD removal efficiency of around 90% at HRT 44 h (Omil *et al.* 2003), and a full-scale multiplate anaerobic reactor treating cheese whey wastewater obtained tCOD removal above 87% at HRT 60 h (Guiot *et al.* 1995).

Overall, the performance of the two full-scale reactors showcased the efficacy of the LtAD reactor in treating wastewater from meat processing and dairy production, achieving remarkable COD removal even in cold climates (<10 °C) and relatively high OLR (up to 8.9 kg COD/m^3/day) while producing high-quality biogas suitable for heating or power generation. The single-stage design system is less costly (both operational and capital costs) and easier to operate than multi-stage systems for diluting wastewater (Cremonez *et al.* 2021). Furthermore, the reduced energy demand of the LtAD system contributes to lowering the carbon footprint of the wastewater treatment process and overall production operations. This technology is particularly attractive to low-strength wastewaters with low biomethane potential as LtAD can significantly improve the net energy output from the anaerobic treatment compared to mesophilic setups.

3.3. Microbial community

The active microbial community of each reactor at the beginning and end of the investigation period were analysed by high-throughput amplicon sequencing of the 16S rRNA (cDNA) (Figure 4). In general, the microbial community in both reactors were stable after long-term operation.

Active methanogenic archaea (relative abundance > 43%) were observed, represented by the genus *Methanothrix* (previously known as *Methanosaeta*), suggesting active acetoclastic methanogens existed in the microbial communities of both reactors. These results are compatible with the results observed in a similar pilot-scale reactor, where *Methanothrix* represented almost 50% of the active community (Paulo *et al.* 2020). Comparable results were also observed during laboratory-scale reactors at 15 °C where *Methanothrix* represented up to 50% of the active community (McAteer *et al.* 2020). Furthermore, a high abundance of *Methanothrix* in low-temperature AD systems at laboratory-scale reactors was reported by several other studies treating different types of wastewater (McKeown *et al.* 2009a; Smith *et al.* 2015; Seib *et al.* 2016; Keating *et al.* 2018; Sukma Safitri *et al.* 2022; Singh *et al.* 2023). The active presence of *Methanothrix* in the LTAD system is essential, as they can quickly convert acetate into methane, preventing acetate accumulation and subsequent inhibition of microorganisms (Nozhevnikova *et al.* 2007). Additionally, *Methanothrix* is the keystone for the formation and maintenance of strong, healthy granules (Hulshoff Pol *et al.* 2004; Gagliano *et al.* 2020), which is essential for effective sludge retention and treatment efficiency in high-rate AD bioreactors (van Lier *et al.* 2015). *Methanothrix* was also found to be in the core microbiome of the full-scale reactor treating municipal wastewater at ambient temperature (Trego *et al.* 2021). These findings reinforce the importance of *Methanothrix* for the healthy and stable performance of anaerobic reactors and highlight the relevance of them for LtAD reactors. The hydrogenotrophic methanogens in both reactors were represented by *Methanolinea* and *Methanobacterium* (Figure 4), which were observed by previous LtAD studies (Bialek *et al.* 2012; McAteer *et al.* 2020). Interestingly, a considerable relative abundance of *Methanomethylovorans* (2–4%) presented in Reactor A (Figure 4), indicating that specific methyl compounds such as methylamines, dimethyl sulphide, or methanethiol derived from protein-rich substrates in the meat processing wastewater are converted to methane directly (Ziganshin *et al.* 2013).

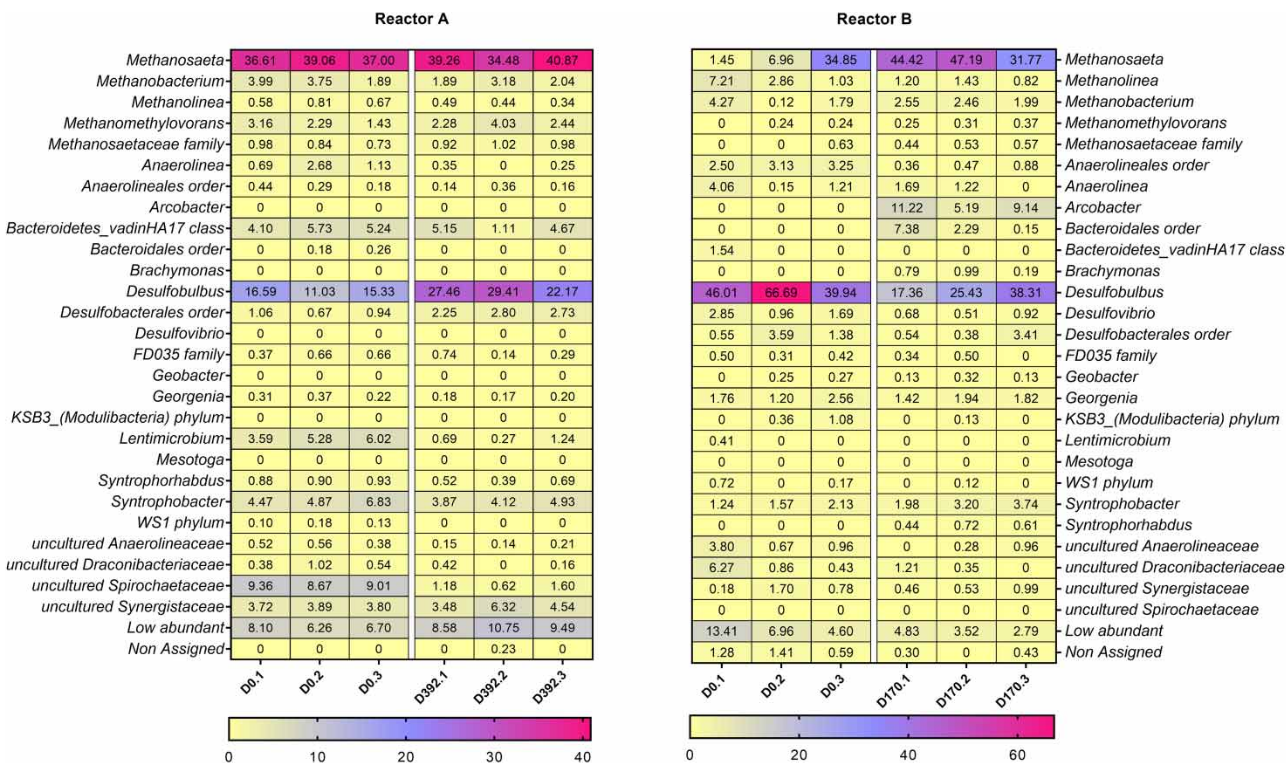


Figure 4 | Active microbial communities of Reactor A (day 0 and day 392) and Reactor B (day 0 and day 170) according to the relative abundance of 16S rRNA ($n = 3$). Low abundant refers to the taxa has the relative abundance low than 0.1%.

The active bacterial communities were distinguished between the two reactors at the start of the operation (Figure 4). In reactor A, abundant carbohydrate-protein fermentative bacteria, such as *Desulfobulbus* (11.0–16.6%), *Bacteroidetes_vadinHA17 class* (4.1–5.7%) *Syntrophobacter* (4.5–6.8%), and *Lentimicrobium* (3.6–6.0%), were detected (Figure 4), suggesting that the microbial community possess a good capability in fermentative metabolism (Paulo *et al.* 2020; Liu *et al.* 2023). Similarly, high abundances of syntrophic bacteria including *Spirochaetaceae* (8.6–9.4%) and *Synergistaceae* (3.7–3.9%) existed in the community indicating active metabolism related fatty acids assimilations (Singh *et al.* 2023). After a year of operation, the microbial community was stable which underscores the stable performance of the system (Carballa *et al.* 2015). AD of meat processing wastewater is challenging due to its high organic load including protein and lipids, which could inhibit microbes leading to process failure (Harris & McCabe 2015). In conjunction with the active methanogenic populations, the microbial community in Reactor A was able to efficiently convert the organic matter into biogas since the beginning of the operation. Thus, it is important to examine the microbial community and evaluate its activity to ensure its capability to treat specific wastewater before its application, which will be beneficial to reduce the time for start-up.

In reactor B, we observed active hydrolytic bacteria *Draconibacteriaceae* (0.4–6.3%) and carbohydrate-fermentative bacteria *Desulfobulbus* (39.9–66.7%), *Anaerolineales* (0.2–4.1%) and *Georgenia* (1.2–2.6%) at day 0 (Figure 4), suggesting that the microbial community possessed high hydrolytic and fermentative ability (Samain *et al.* 1984; Yamada *et al.* 2005; Woo *et al.* 2012; Puig-Castellví *et al.* 2022). Hydrolysis is considered as rate-limiting step of AD (Carballa *et al.* 2015), and this limitation is more prominent at low temperatures. Hydrolytic rates of soluble and particulate organic matter significantly decreased upon the reduction of temperature (Regueiro *et al.* 2014), and protein hydrolysis was poor during AD of dairy wastewater at 10 °C (Bialek *et al.* 2013). Therefore, the appearance of these active hydrolytic-fermentative bacteria in the microbial community underpinned the efficient hydrolytic activity of protein and carbohydrates in Reactor B even under cold conditions. Interestingly, a significant abundance of *Arcobacter* (5.2–11.2%) was detected in the microbial community after 6 months, implying its important role in the process (Figure 4). *Arcobacter* possesses a wide metabolic range including denitrification, sulphide oxidation, fermentation, and acetate oxidation (Roalkvam *et al.* 2015; Callbeck *et al.* 2019), which can be involved in variable processes in the system.

Notably, we observed abundant populations of *Desulfobulbus* in both reactors (Figure 4), which were known as desulfurising bacteria responsible for sulphide production in AD systems (El Houari *et al.* 2017). However, this group of microorganisms also holds fermentative metabolism with a wide range of substrates, such as lactate, propionate, pyruvate, and acetate (Samain *et al.* 1984), which are likely to be abundant during the AD of meat processing and dairy wastewater. Thus, it is possible that the function of *Desulfobulbus* in the reactors was fermentation instead of desulfurisation, especially low amount of H_2S was detected in the biogas produced from the reactors. Further investigations using the multi-omics method are necessary to unravel their metabolic functions during AD.

4. CONCLUSIONS

The findings presented in this paper represent the inaugural report on the performance of two full-scale LtAD reactors over a year-long operational period for the treatment of industrial wastewater. Both reactors exhibited commendable efficiency levels, comparable to those observed in other high-rate full-scale reactors operating under mesophilic conditions. These results align consistently with trends noted in laboratory and pilot-scale studies employing similar reactor configurations. Moreover, both reactors showcased a robustly active methanogenic community, akin to observations in other LtAD reactors, alongside a flourishing bacterial community. These factors collectively contributed to the stable operational performance of both reactors. In summary, the study underscores the feasibility and robust efficacy of full-scale LtAD for the anaerobic treatment of agro-food industrial wastewater, offering valuable insights into the potential for widespread implementation in such contexts.

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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