



Exploring nanobubble technology for enhanced anaerobic digestion of thermal-hydrolysis pre-treated sewage sludge

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ABSTRACT

Nanobubble technology was used to enhance anaerobic digestion (AD) of thermal-hydrolysis pre-treated sewage sludge for bioenergy recovery. The prepared air, CO₂, and H₂ nanobubble solutions, with concentrations of 9.88–10.2 × 10⁷ bubbles/mL, remained stable for at least 7 days. After adding them into AD reactors, significantly higher CH₄ production (37.1 %) was observed for the CO₂ nanobubble treatment, followed by air (25.6 %) and H₂ (14.5 %) nanobubble treatments, compared to the control group. CO₂ nanobubble treatment performed the best in improving acidogenesis/acetogenesis, resulting in significantly higher volatile fatty acid generation during the initial 3–4 days. A comparison of reactors supersaturated and non-saturated with oxygen has demonstrated most of the biogas uplift observed to result from the nanobubbles rather than from initial oxygen soluble levels, demonstrating the crucial role of nanobubbles in upgrading AD. This study demonstrates, for the first time, that nanobubbles can provide additional benefits when combined with established sludge pre-treatment technologies.

1. Introduction

Anaerobic digestion (AD) is widely used for treating waste biomass while recovering valuable products like biogas and organic fertiliser (Kizito et al., 2017; Rocamora et al., 2020). In the water sector, the generation of renewable energy from AD-sourced biogas facilitates achieving energy self-sustained wastewater treatment plants and aligns with the sector-wide Net-Zero commitment (Fargò et al., 2022). Consequently, numerous efforts have focused on optimising AD processes to achieve better CH₄ yields (Li et al., 2019). Among the strategies investigated, the introduction of a small amount of air or O₂ into the system, a process known as microaeration, has been reported to be as a cost-effective strategy for promoting the growth of facultative hydrolytic and acidogenic microorganisms by providing a favourable environment that enhances their metabolic activities and enzyme production, consequently enhancing CH₄ yield (Chen et al., 2020). Al-mashhadani et al. (2016) further demonstrated that smaller bubbles, i.e. N₂ microbubbles (Ø in µm), could reduce the Gibbs free energy and benefit the shifting of the thermodynamic equilibrium for increased CH₄ production. The existence of even smaller bubbles (Ø < 1 µm), referred to as nanobubbles or ultra-fine bubbles, was first experimentally confirmed

through atomic force microscopy imaging in 2000 (Lyu et al., 2019). Attributed to their unique properties, including high gas transfer efficiency, remarkable longevity, and the ability to generate reactive oxygen species (ROS) (Atkinson et al., 2019), nanobubble technology has been attempted to improve AD performance since 2019.

The addition of pre-prepared nanobubble solutions to ADs treating lignocellulosic feedstocks (lignin and cellulose) has been reported to increase CH₄ yield by 7.7 to 29.9 % (Ho et al., 2020; Wang et al., 2020c) (Table 1). Similar enhancements were found in ADs processing waste activated sludge (11–29.1 %), livestock waste (–5.2–39.3 %), food waste (14–43 %), lignocellulosic crop waste (–1.23–46.8 %), and co-digesting waste activated sludge and lignocellulosic biomass (0.6–30.3 %). Moreover, nanobubbles generated with six types of gases have been assessed to increase CH₄ yield from AD, including air (7.7–43 %), O₂ (–1.23–46.8 %), H₂ (6.7–39.3 %), CO₂ (2.1–25 %), N₂ (–5.2–30.3 %), and He (–4.2–4.6 %).

Regardless of the gas type, the potential generation of ROS from nanobubble collapse is hypothesised as contributing to C–C or C–O bond cleavage of organic matter, resulting increased feedstock digestibility (Chuenchart et al., 2021). Attributed to the high Brownian motion and hydrophobic surface, nanobubbles can attract substrates and

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provide favourable attachment sites for enzymes, allowing high mass transfer for enzymatic reactions which are critical in AD (Wang et al., 2021). Moreover, the high zeta potential of nanobubbles could also induce higher water mobility and benefit the hydrolysis rates during AD (Chuenchart et al., 2021). For air and O₂ nanobubbles, gas dissolution can create microaerobic environments in AD, promoting higher adenosine triphosphate (ATP) synthesis and metabolite transport, leading to improved hydrolysis and acidification resulting in higher volatile fatty

acids production and CH₄ formation (Zhang et al., 2018). Additionally, CO₂ and H₂ nanobubbles can serve as additional substrates for hydrogentrophic methanogenesis (Chuenchart et al., 2021). Beyond direct addition to AD, Hou et al. (2022) and Wang et al. (2020a) used air, N₂, and O₂ nanobubbles for pre-augmentation of inoculum during 24–214 days prior to AD, achieving CH₄ production improvements of 9.8–25 %. Microbial community analyses indicated an increased abundance of hydrolytic and acidogenic bacteria communities. However, direct pre-

Table 1

Summary of previous studies on nanobubble technology enhanced anaerobic digestion for biomass treatment and CH₄ production.

Feedstock		Intervention	Filled gas	Nanobubbles concentration (bubbles/ mL)	CH ₄ yield with nanobubble treatment (mL/g VS)	CH ₄ yield increment (%) ^a	Reference	
Waste activated sludge	WAS	In-situ Treatment	N ₂	1.4 × 10 ⁸	402.1	29.1	(Yang et al., 2019)	
			N ₂	1.3 × 10 ⁸	211.6	11	(Wang et al., 2019)	
			Air	2.3 × 10 ⁸	218.2	14		
			CO ₂	1.4 × 10 ⁸	222.4	16		
			H ₂	2 × 10 ⁸	223.9	17	(Fan et al., 2020a)	
	Air	2.3 × 10 ⁸	192	19.1				
	CO ₂	1.4 × 10 ⁸	195	20.8				
	H ₂	2 × 10 ⁸	225	39.3				
	Livestock waste	Swine manure	In-situ treatment	CO ₂	1.4 × 10 ⁸	201–230		20.3–25
				Air	NA	162.4	9.7	(Zhang et al., 2024)
CO ₂				NA	151.2	2.1		
He				NA	154.9	4.6		
N ₂				NA	140.4	−5.2		
Air		NA	227.1	17.7	(Hou et al., 2022)			
CO ₂		NA	226.1	17.2				
He		NA	184.7	−4.2				
N ₂		NA	199	3.2				
Food waste		Mixture of food waste ^c	In-situ treatment	Pre-treatment (inoculum)		Air	3 × 10 ⁸	200–300
	Air			>10 ⁸	225–300	14–43		
	N ₂			>10 ⁸	346.6	15.2		
	Air			>10 ⁸	375.3	24.7		
	Lignin			In-situ treatment	N ₂	NA	728	21.5
		CO ₂	NA		703	17.2		
		Air	2.3 × 10 ⁸		193.3	7.7		
		N ₂	1.3 × 10 ⁸		196.1	9.3		
		Cellulose	In-situ treatment		O ₂	10 ⁶ –10 ⁸	233	29.9
	CO ₂			1.4 × 10 ⁸	246	10.8		
air	2.3 × 10 ⁸			263.3	18			
N ₂	1.4 × 10 ^{8 d}			127.3	9.8			
Corn straw	Pre-treatment (inoculum)			O ₂	NA	141.7	22.3	
		N ₂	10 ^{8 d}	144.2	18.1			
		O ₂	10 ^{8 d}	179.2	46.8			
		N ₂	1.43 × 10 ⁸	289.1	−1.16			
		Lignocellulosic biomass	Rice straw	Pre-treatment (feedstock)	O ₂	3.8 × 10 ⁸	288.9	−1.23
CO ₂	1.1 × 10 ⁸				295.1	0.89		
H ₂	NA				224.5–263.5	11.5–25.3		
CO ₂	NA				117.6–132.2	4–17		
Corn straw	In-situ treatment				CO ₂	NA	~140	16.84
			CO ₂ + Fe ⁰ NPs	NA	~128	26.16		
			N ₂	1.43 × 10 ⁸	~300	7.8		
			H ₂	5.46 × 10 ⁷	~290	6.7		
			CO ₂	4.04 × 10 ⁷	~280	4.2		
Horseweed (<i>Erigeron canadensis</i>)	In-situ treatment		O ₂	2.6 × 10 ⁸	343.2	10.7–16.2		
		WAS+ lignin (50 mg/L)	1.4 × 10 ⁸	389.7–443.1	3–17.1			
		WAS+ lignin (100 mg/L)	1.4 × 10 ⁸	441.3–475.1	21–30.3			
		WAS+ cowpea hull	In-situ Treatment	N ₂	NA	129	11.4	
				Air	NA	116.8	0.6	

^a Differences were compared against a system with deionised water treatment without nanobubbles.

^b Experiment operated under thermophilic condition (55 ± 1 °C), all other studies were under mesophilic condition (35–38 °C).

^c Mixture of rice (25 %), noodle (22 %), pork (16 %), cabbage (29 %), and tofu (8 %). ^dNanobubble concentrations were not tested in the study but referred to from other publications.

treatment of rice straw using N_2 , O_2 , and CO_2 nanobubble solution did not result in significant enhancements in CH_4 yield (Wang et al., 2023a, 2023b). This may have been attributed to the shorter pre-acclimation period (5 days) and the inherent recalcitrance of lignocellulosic biomass posing challenges for nanobubble enhanced degradation under ambient environment during the pre-treatment.

Despite promising results, several knowledge gaps remain in the wider application and mechanistic understanding of nanobubble technology enhanced AD. At large sewage treatment plants, thermal-hydrolysis pretreatment has become a common step to breakdown organic compounds and improve the biodegradability of sludge prior to sludge digestion (Higgins et al., 2011). There is still a lack of evidence regarding the adaptability and efficiency of nanobubble technology to existing AD plants equipped with such sludge pre-treatment. Moreover, nanobubble solutions can result in a gas supersaturated conditions (Fan et al., 2021). Considering the known enhancement of AD performance through microaeration, wherein a small amount of air or O_2 is introduced, it is unknown how the supersaturation of the system contributes to CH_4 yield enhancements as opposed to the action of the nanobubbles themselves.

This study aims to evaluate and advance the application of nanobubble technology in AD, particularly in a deployable sludge digestion scenario. Prepared solutions of air, H_2 , and CO_2 nanobubbles were monitored to assess their stability in terms of bubble size distribution and concentrations. The impacts of these three types of nanobubbles on biogas and CH_4 generation in ADs preceded by thermal hydrolysis pre-treatment were compared. Moreover, the effects of supersaturated and non-supersaturated nanobubble solutions were compared to distinguish the contributions of dissolved gas and nanobubbles. The first-order kinetic model and the modified Gompertz model were used to assess the dynamics on CH_4 production in different treatment groups and explore the potential mechanisms. Additionally, the dynamics of volatile fatty acids in all reactors were determined to support the understanding of the enhancement mechanisms. With the results, this study could facilitate the deployment of novel nanobubble technology in existing ADs, contributing towards sustainable sewage sludge treatment and renewable energy generation.

2. Materials and methods

2.1. Nanobubble generation and characterisation

Various methods, including hydrodynamic cavitation, ultrasonic fragmentation, electrolysis, and porous membranes, have been developed to generate nanobubbles. Among these, the hydrodynamic method is widely recognised as an effective approach, capable of rapidly

producing a large quantity of nanobubbles ($>10^7$ bubbles/mL) within relatively short time. In this study, three gases (air, H_2 and CO_2) were selected to produce targeted nanobubble solutions using a commercially available hydrodynamic cavitation based nanobubble generator (Nikuni KTM20N, Aeration & Mixing Ltd., UK) connected via hoses to a cylindrical tank filled with 50 L of deionised water (Fig. 1a). The nanobubble generator operated with an inlet pressure of -0.03 MPa and an outlet pressure of 0.4 MPa. Air, H_2 , and CO_2 gases (99.99 %, BOC Gases, Guildford, UK) were separately introduced into the pump at a gas flow rate of 1.5 L/min and blended with the deionised water. To determine the optimal operational duration, triplicate 50 mL samples were taken using pipette at 5, 10, 15 and 20 mins after starting the device. The samples were stored in clean 50 mL centrifuge tubes for analysis of nanobubble size distribution and concentration by a nanoparticle tracking instrument (NanoSight LM20, Malvern Instruments Ltd., UK). Nanoparticle tracking and analysis technology is a non-contact measurement method based on optical principle, which irradiates the nanoparticles by laser beam to make the particles scatter light, the scattered light is converted into electric signal by photoelectric converter, and then the information such as the size, concentration and motion state of the particles is analysed by signal processing system. The particle gradation (D10, D50, D90) were recorded and calculated the span value to evaluate the range of NB size. Tests for nanobubble stability were also conducted on aliquots collected after 15-min of operation after 7 days of storage in a sample bottle at ambient temperature. To prepare the non-supersaturated air bubble solutions, the freshly prepared air-nanobubble solution was left in the tank (Fig. 1a) for 72 h, until the dissolved oxygen (DO) level (HQ30d, Hach Co., USA) dropped to the theoretical oxygen solubility at room temperature (~ 9 mg/L).

2.2. Batch anaerobic digestion (AD) experiments

2.2.1. Feedstock sludge and inoculum

Municipal sewage sludge and digestate used as feedstock and inoculum for the AD trials were collected from a full-scale wastewater treatment plant treating a population equivalent of 2.57 million. The feedstock sludge comprised a blend of primary sludge and waste activated sludge from the site's activated sludge process. The blended sludge was subjected to thermal hydrolysis pre-treatment (THP) at 6–7 bars pressure and 160 – 165 °C. After the pre-treatment, the total solids (TS) and volatile solids (VS) of the substrate were 102.8 and 79.8 g/L, respectively, determined by standard methods (APHA, 1998). The inoculum was collected from one of the site's anaerobic digesters, with concentrations of TS and VS at 58.1 and 36.4 g/L, respectively. Both feedstock sludge and inoculum were stored in 5 L sealed sample box in the wastewater treatment plant. The materials were transported to the

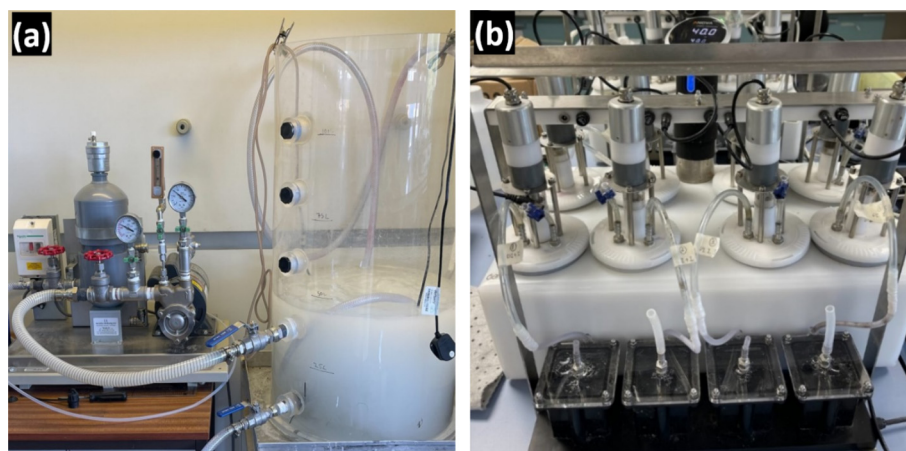


Fig. 1. Hydraulic cavitation nanobubble generation device (a), and biochemical methane potential (BMP) test setup (b).

lab within 1 h and stored in the 4 °C cold room prior the following tests within 1 week.

2.2.2. Biochemical methane potential (BMP) test

BMP tests were conducted in 1-L batch stirred reactors (CJC Labs Ltd., UK) immersed in a water bath to maintain a mesophilic temperature at 38 °C (Fig. 1b). Each reactor was fed with inoculum (395 mL) and feedstock sludge (90 mL), with an inoculum to substrate VS ratio of 2. For the control group, an additional 30 mL of deionised water was added to the reactor, while the same amount of nanobubble solution was added to the test reactors. Four types of nanobubble solution, i.e. air (super-saturated), air (non-supersaturated), H₂, and CO₂, were tested. The batch AD experiment lasted 30 days and the biogas production was continuously monitored using tipping bucket gas counters (CJC-048, CJC Labs Ltd., UK). The gas tightness of the reactor was tested with a nitrogen charge prior to start-up of each reactor. CH₄ concentration in the biogas was determined via Gas Chromatography (Valco Instruments Company Inc., USA) every two days. A blank control group with equivalent volume of inoculum and deionised water was operated. Biogas and CH₄ yields (mL/g VS_{added}) were calculated from their accumulated production in mL subtracted by the accumulated biogas production from the blank and normalising to the mass of VS from the sewage sludge fed to the reactor.

Besides experimental CH₄ production (EMP), theoretical CH₄ production (TMP) was calculated using Eqs. (1)–(2) (Wang et al., 2020c). Feedstock carbon (C), hydrogen (H), and nitrogen (N) were tested by an element analyzer (Vario EL cube, Elementar, Germany), while ash content was measured gravimetrically by burning samples in a muffle furnace. Oxygen (O) content was calculated by subtracting C, H, N, and ash contents from 100 wt% (Lakshman et al., 2021). The EMP/TMP ratio was calculated to represent the anaerobic biodegradability (%) of the feedstock. All reactors were operated in duplicate, and average results are reported.

$$C_a H_b O_c + \left(a - \frac{1}{4}b - \frac{1}{2}c\right) H_2 O \rightarrow \left(\frac{1}{2}a + \frac{1}{8}b - \frac{1}{4}c\right) CH_4 + \left(\frac{1}{2}a - \frac{1}{8}b + \frac{1}{4}c\right) CO_2 \quad (1)$$

$$TMP = 22.4 \times \left(\frac{1}{2}a + \frac{1}{8}b - \frac{1}{4}c\right) / (12a + b + 16c) \quad (2)$$

where a, b and c represent the molar fractions of C, H and O, respectively.

2.2.3. Volatile fatty acids (VFAs) dynamics test

Sacrificial AD consisting of 175-mL plastic bottles were operated to monitor the dynamics of VFA formation and consumption. Experimental conditions and the ratio of feedstock, inoculum, water/nanobubble solution, and headspace were the same as for the BMP test. Bottles were manually swirled 2–3 times a day, and gas produced was vented every 1–2 days using a needle to penetrate the rubber septum. After thorough mixing, three sacrificial bottles were removed for sampling every day for the first 10 days. Subsequently, samples were taken on days 10, 11, 14, and 30 from different bottles. Samples were centrifuged at 5000 rpm and 20 °C for 15 mins and the supernatant was filtered through 0.45 µm membrane filters before analysis. The concentrations of VFAs, including acetic acid, propionic acid, isobutyric acid, n-butyric acid, isovaleric acid, and n-valeric acid, were measured by an ultra-fast liquid chromatograph (LC-30 CE, UFLC XR system, Shimadzu Ltd., Japan). An equivalent methodology to that reported by (Bajón Fernández et al., 2015) was used with the exception of the HPLC run time, which was set at 60 min. In Brief, the columns Biorad (cat. 125–0115) (105 mm × 7.8 mm) and the guard column (Biorad cat. 125–0131) were maintained at 65 °C. The injection volume was 15 µL and each sample was run for 0.5 h using 1 mM sulphuric acid pumped at a flow rate of 0.8 mL/min. A mixture of VFAs (acetic acid, propionic acid, butyric acid and valeric

acid analytical grade) at concentrations between 0.05 and 1 g/L was used as internal standard.

2.2.4. Calculations and kinetic models

Average and standard deviation of each parameter monitored from each group were presented in this paper. The statistical difference of the same parameters from different AD tests was analysed through Kruskal-Wallis test using SPSS 22 ($p < 0.05$). The first-order kinetic model (Eq. (3)) and modified Gompertz model (Eq. (4)) were used to simulate the CH₄ production dynamics.

$$C = C_{\max} \times (1 - \exp.(-k \times t)) \quad (3)$$

$$C = P \times \exp.[-\exp.((R_{\max} \times e)/P \times (\lambda - t) + 1)] \quad (4)$$

where C is the cumulative CH₄ production (mL CH₄/g VS), P_{max} is the maximum CH₄ production potential (mL CH₄/g VS), R_{max} is the maximum CH₄ production rate (mL CH₄/g VS/d), λ is the lag phase (d). k is the first-order hydrolysis rate constant (d⁻¹) and t is the duration of the AD process (d).

3. Results and discussion

3.1. Nanobubble characterisations and stability

The generation of air nanobubbles was used to determine the optimal pump operation duration for nanobubble solution preparation. After operating the generator for 5 mins, the nanobubble concentration reached 3.31×10^7 bubbles/mL, with a mean bubble diameter of 201 nm (Fig. 2a). Extending the pump operation time to 15 mins increased the nanobubble concentration to 1.01×10^8 bubbles/mL (mean diameter of 351.2 nm). However, further increasing the operation time to 20 mins resulted in stable nanobubble concentration and mean diameter, maintaining values of 1.01×10^8 bubbles/mL and 354.3 nm, respectively. The nanobubble concentrations (10^7 – 10^8 bubbles/mL) aligned with findings from other studies using the same equipment (Lyu et al., 2023; Wu et al., 2019, 2022), and were comparable to studies using other hydraulic cavitation-based approaches (Table 1). The cavitation process could release heat and elevate the temperature, potentially acting as an external stimulator to induce nanobubble collapse and hindering nanobubble formation. This could result in similar nanobubble generation when extending the operation time from 15 to 20 mins. The most effective operation time, determined to be 15 mins, was used for preparing nanobubble solutions for the AD experiments.

The air nanobubble generation process can produce a supersaturated solution with a DO level of 11–14 mg/L (Baram et al., 2022; Fan et al., 2021), a phenomenon also confirmed in this study with an initial DO of 12.2 mg/L in the nanobubble solution. After leaving the solution at ambient conditions (20–22 °C) for 72 h, the DO level decreased to 9.1 mg/L, comparable to the DO level in deionised water and equivalent to the theoretical solubility of oxygen (8.9–9.2 mg/L). Both supersaturated and non-supersaturated air nanobubble solutions were used in the AD experiments to calculate the different contributions of dissolved gas and nanobubbles.

The freshly prepared air, H₂ and CO₂ nanobubble solutions showed similar nanobubble distribution, with a mean bubble size between 351.2 and 421.5 nm and concentrations of 9.88 – 10.2×10^7 bubbles/mL. The nanobubble concentrations showed no significant changes after resting at ambient conditions for 7 days (Fig. 2b). Some variations in D90 (90% tile of bubble size) were observed (Fig. 2b), potentially attributed to the nanobubble coalescence process (Chan et al., 2015). The results align with previous studies indicating that nanobubbles can persist in water from days to months (Zhang et al., 2018) due to the negative surface charge associated stable bubble surface and reduced buoyance. The exceptional stability of nanobubbles may contribute to long-term effects in AD studies with operation durations extending several weeks.

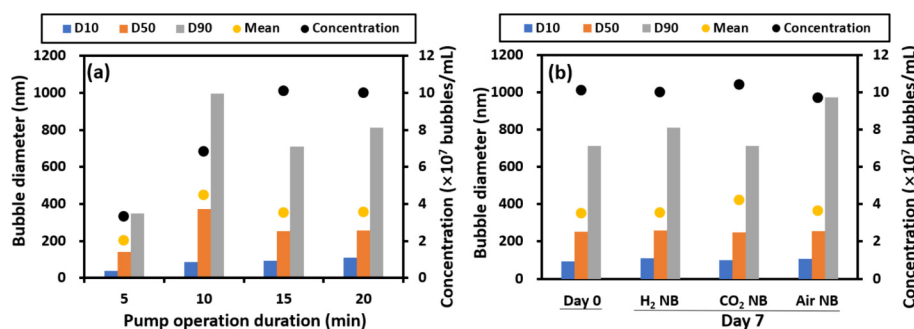


Fig. 2. Variability of air nanobubble (NB) diameter distributions and concentrations with operation time of the nanobubble generator (a), and changes after leaving different nanobubble solutions at ambient conditions for seven days (b). D10, D50, and D90 represent 10%tile, 50%tile and 90%tile of the bubbles size. Note, data of day 0 in (b) are the average values from different gas nanobubble solutions.

3.2. Nanobubbles impact on biogas production

The cumulative production of both biogas (Fig. 3a) and CH₄ (Fig. 3b) increased rapidly until day 7, reducing the rate of production thereafter until the reactors were stopped on day 30. In the CO₂, Air and H₂ nanobubble treatment groups, the cumulative biogas yield reached 643.3, 569.5, and 521.9 mL/g VS_{added}, respectively, showcasing improvements of 33.3 %, 18 %, and 8.1 % compared to the control group. Similar to previous studies (Wang et al., 2019; Yang et al., 2019), nanobubble treatment did not alter the CH₄ concentration (49 %–54 % after day 7) in any of the groups. Compared to the control group, the CH₄ production increments followed the same order: 37.1 % (372.4 mL/g VS), 25.6 % (340.9 mL/g VS), and 14.5 % (310.9 mL/g VS) in the CO₂, Air and H₂ nanobubble treatment groups, respectively.

The anaerobic biodegradability of the feedstock sludge, defined as EMP/TMP ratio, increased from 33.8 % in the control group to 38.7–46.4 % for all nanobubble treatments (Table 2). This was theorised to be attributed to the nanobubble-induced generated ROS, increased water mobility and improved microbial electron transport activity (Chuenchart et al., 2021; Zhu et al., 2024). ROS can theoretically increase water mobility by disrupting hydrogen bonds and enhancing solubilisation of organic matter, which improves microbial electron transport activity by stimulating enzyme functions and optimising the electron transport chain. These effects lead to an upregulation of the EMP pathway and an increase in the EMP/TMP ratio, indicating enhanced metabolic efficiency and methane production in the AD process. The more biodegradable sludge resulted in enhanced CH₄ production (14.5 %–37.1 %) compared to the control group, which aligns with reported values (0.59–39.3 %) from previous studies conducting in-situ nanobubble treatment (Table 1). The current study focused on sludge that had been pretreated on site with thermal-hydrolysis, which is expected to have reduced the contribution of nanobubbles to further

enhance hydrolysis (Wang et al., 2020c), explaining the higher biogas uplifts observed for CO₂ than air nanobubbles as CO₂ can act as a substrate in intermediate reactions of the AD process. Injection of CO₂ nanobubbles resulted in the greatest positive impact on biogas and CH₄ generation, which has been previously attributed to an increased homoacetogenic activity promoting acetoclastic methanogenesis (Bajón Fernández et al., 2019; Muntau et al., 2021) or to a boost on hydrogenotrophic methanogenesis (Fan et al., 2020a, 2020b). Interestingly, H₂ nanobubble treatment resulted in lower improvements in biogas and CH₄ generation than seen for CO₂ and air nanobubbles, although the increase (14.5 %) was within the range (6.7–39.3 %) reported in previous studies (Fan et al., 2020a; Wang et al., 2019; Zhu et al., 2024). It is postulated that the reduced benefit of H₂ injection compared with air or CO₂ could be linked to a lack of sufficient electron acceptors on the solution or to a mild inhibition resulting from hydrogen supersaturation exceeding thermodynamic limits for acetogenic bacteria (Wainaina et al., 2019; Rocamora et al., 2023).

Addition of air nanobubble solutions with and without supersaturated dissolved O₂ was conducted to quantify the relative contributions to biogas uplifts of increased O₂ dissolved levels and the nanobubbles themselves. The biogas and CH₄ productions were only slightly higher (569.5 and 340.9 mL/g VS_{added}, respectively) in the supersaturated air nanobubble solution treatment group compared to the non-supersaturated nanobubble solution treatment group (562 and 336.4 mL/g VS_{added}). The very limited CH₄ yield increment (~1.3 %) achieved with the supersaturated trials provide evidence of the higher contribution towards biogas uplifts of the nanobubbles themselves rather than dissolved oxygen. Therefore, the unique characteristics of nanobubbles themselves, through processes such as ROS generation and enhanced biofilm/substrate contact time, along with improvements in associated functions, including increased enzyme activity and altered microbial communities, were postulated to be the key drivers for NBs benefiting

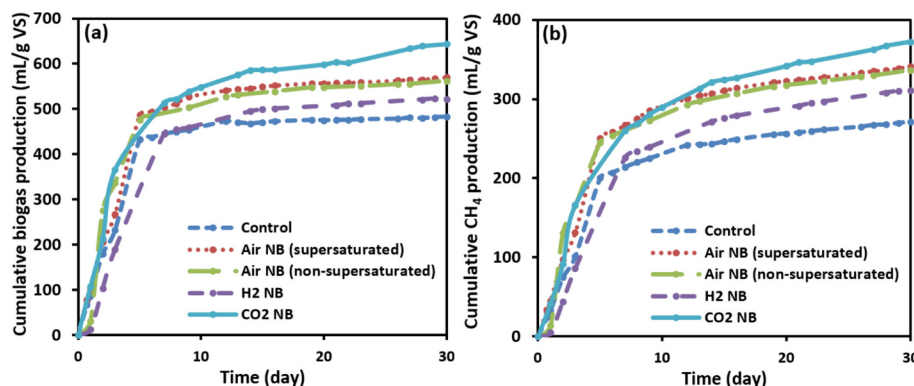


Fig. 3. Cumulative biogas (a) and CH₄ (b) production in anaerobic digestion of sewage sludge with on-site thermal-hydrolysis pre-treatment under in-situ treatments of air nanobubble (NB), H₂ NB, CO₂ NB, and control groups.

Table 2

The theoretical CH₄ production (TMP), experimental CH₄ production (EMP), and anaerobic biodegradability of the feedstock sewage sludge, as well as the kinetics model simulation results of CH₄ production in different groups.

Experimental groups		Control	Air NB (supersaturated)	Air NB (non- supersaturated)	H ₂ NB	CO ₂ NB
TMP (mL/g VS)				802.5		
EMP (mL/g VS)		271.5	340.9	336.4	310.9	372.4
Anaerobic Biodegradability (%)		33.8	42.5	41.9	38.7	46.4
Kinetics model simulation						
First order kinetics model	C _{max} (mL/g VS)	246.86	332.21	323.76	313.99	360.89
	k (d ⁻¹)	0.21	0.21	0.23	0.14	0.17
	R ²	0.98	0.99	0.98	0.98	0.99
Modified Gompertz model	R _{max} (mL/g VS/d)	40.3	49.23	58.23	37.94	35.62
	P (mL/g VS)	257.1	323.1	313.17	295.6	349.35
	λ (d)	0.31	0.19	0.29	0.97	0.12
	R ²	0.99	0.99	0.97	0.99	0.98

AD performance. Moreover, Zhang et al. (2006) confirmed a high-gas-density state in nanobubbles, where the inner gas may exist as an aggregation, rather than following the Ideal Gas law or Boyle's law for gas content calculation in bubbles (Ferraro et al., 2020). Therefore, the diffusion of the gas inside nanobubbles is likely to be slow and to take place over an extended period, longer than initial dissolved gas, contributing to biological reactions in AD over a long-term.

3.3. CH₄ production kinetics

The Modified Gompertz model and the first-order kinetics model are commonly used to describe CH₄ production kinetics in AD (Ponzelli et al., 2022). In this study, both models effectively simulated ($R^2 > 0.97$) the CH₄ production dynamics from all groups (Table 2), supporting their applicability in predicting CH₄ production from AD of sewage sludge under nanobubble technology intensification. The k value of the first-order model is an important parameter directly reflecting the rate of hydrolysis, with a higher value indicating faster substrate hydrolysis. As the feedstock sludge in this study underwent thermal-hydrolysis pre-treatment, the k values (0.14–0.23 d⁻¹) from nanobubble treatment groups were not higher than for the control group (0.21 d⁻¹). The results suggest that nanobubble technology is able to stimulate CH₄ production by contributing to acidogenic and methanogenic processes.

Similar to the results of cumulative CH₄ production (Fig. 3b), the maximum CH₄ production rate (R_{max}) obtained from the Modified Gompertz model was highest in CO₂ nanobubble treatment group (349.35 mL/gVS/d) with a 35.9 % increase compared to the control group (257.1 mL/gVS/d). The lag time (λ) is a parameter that indicates the initial adaptability of microbes to the AD system, where a lower λ value is desirable for a rapid biogas formation. The λ values in all groups were relatively low ($\lambda < 0.97$ day), which may be attributed to the feedstock and inoculum used for the laboratory trials being sourced from the same site, so the microbial communities would have been adapted to the substrate material. For large-scale AD operations, lower lag time offers increased efficiency, consistent biogas supply, and optimised reactor design.

3.4. VFAs dynamics

VFAs are intermediates produced during the acidogenesis and acetogenesis stages of AD through the transformation of easily biodegradable organic matter after the hydrolysis phase (Chuenchart et al., 2021). Attributed to thermal-hydrolysis pretreatment, the initial VFA concentrations (1000–1700 mg/L) in this study were significantly higher than those (150–750 mg/L) observed in other AD studies involving waste activated sludge (WAS) and its co-digestion with lignocellulosic biomass (Yang et al., 2019, 2020).

The higher accumulation of VFAs recorded in all nanobubble injected ADs over the first week of the trial, when compared with the control

reactors, suggests that nanobubbles had a role in further enhancing hydrolysis, potentially attributed to the generated ROS, even if the material was subjected to thermal treatment on-site. There is previous evidence of thermal pre-treatment hydrolysing macromolecules but also increasing solubility of others that then remain solubilised but unhydrolyzed (Wilson and Novak, 2009). The increased VFA levels on the test reactors (Fig. 4) could result from nanobubbles supporting hydrolysis of those previously solubilised macromolecules, a hypothesis that merits further investigation. The changes of VFA generally followed CH₄ production (Fig. 2b), with the highest accumulation observed in the CO₂ nanobubble treatment group, followed by air (supersaturated), H₂, and air (non-supersaturated) nanobubble treatment groups. The results were distinct to previous AD studies focused on other non-pre-treated feedstock, as air/O₂ nanobubble additions are normally deemed as better treatment to induce higher biodegradability of sludge and VFA production due to higher oxygenation capacity of the organic substrates. In this study, therefore, CO₂ nanobubble addition improved acidification during digestion, leading to the highest enhanced VFA production (Chuenchart et al., 2021). It is postulated that the reduced benefit of H₂ injection compared with air or CO₂ could be linked to a lack of sufficient electron acceptors on the solution or to a mild inhibition resulting from hydrogen supersaturation exceeding thermodynamic limits for acetogenic bacteria (Wainaina et al., 2019; Rocamora et al., 2023).

Further analysis of VFA species revealed a higher increment in acetic acid and propionic acid, with no obvious differences in the proportions of isobutyric, n-butyric and isovaleric acids. VFA concentrations in all groups showed a sharp reduction after the initial acidification (Fig. 4), as expected on batch trials where feedstock supply is limited. In summary, the results support that nanobubble treatment may stimulate microbial communities and enzyme activities in thermally pre-treated AD, offering a tailored approach for AD intensification.

4. Conclusions and future perspectives

This study demonstrated that the specific properties of nanobubbles, rather than supersaturated gas, serves as the primary driver in enhancing AD performance with nanobubble technology. Nanobubbles played a crucial role in increasing feedstock biodegradability and CH₄ production. Moreover, CO₂ nanobubbles, compared to air and H₂ nanobubbles, were most effective in enhancing acidogenesis/acetogenesis for VFA production and methanogenesis for CH₄ yield. The following efforts are recommended towards technology development and implementation:

- 1) All previous and current studies were conducted in batch AD systems, further efforts are urged to carry out a demonstration study in a long-term continuous reactor.
- 2) Different gases have shown specific functions in AD intensification, thus, tailored operational strategies, such as multiple stages of

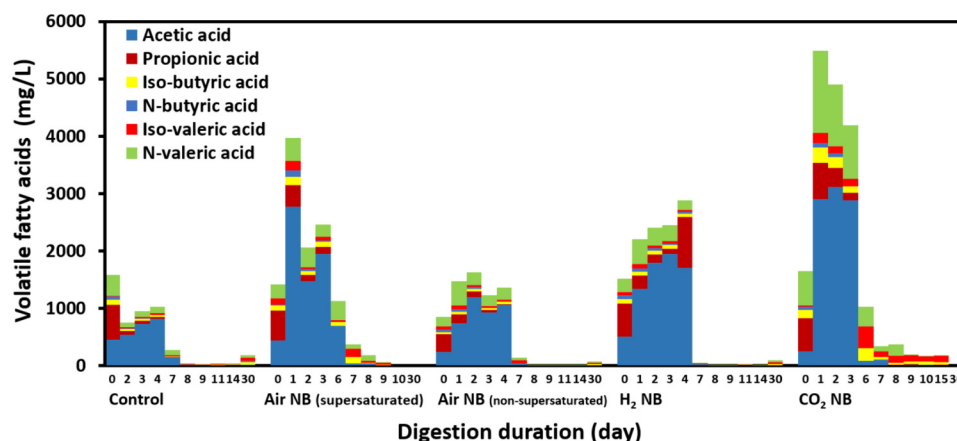


Fig. 4. The dynamics of different volatile fatty acids (VFA) in anaerobic digestion of thermally pre-treated sewage sludge under in-situ treatment of air nanobubble (NB), H₂ NB, CO₂ NB, and control groups.

different types of nanobubble solutions additions and the additions of different nanobubble concentrations and sizes, need to be evaluated.

- 3) Instead of using deionised water, the application of digestate recirculation as the carrier solution for nanobubbles is hypothesised to make the process more meaningful from an engineering perspective.
- 4) The hydraulic cavitation nanobubble generation method is recognised as an effective approach to prepare nanobubble solutions. Other methods, such as the modified membrane filtration method, may be more suitable for directly delivering nanobubbles into AD systems. However, concerns about membrane fouling, clogging, and the energy required to overcome the pressure in AD reactors needs to be investigated.

CRediT authorship contribution statement

Tao Lyu: Writing – original draft, Visualization, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Fei Wu:** Writing – original draft, Methodology, Investigation, Data curation. **Nasreen Nasar:** Investigation, Data curation. **Xin Li:** Writing – review & editing. **Peter Jarvis:** Writing – review & editing. **Yadira Bajón-Fernández:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

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

Data availability

Data available within the article.

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Exploring nanobubble technology for enhanced anaerobic digestion of thermal-hydrolysis pre-treated sewage sludge

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