

Process mechanisms of nanobubble technology enhanced hydrolytic acidification of anaerobic digestion of lignocellulosic biomass

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ABSTRACT

This study explored the efficiency of CO₂-, N₂-, and H₂- nanobubble treatment in anaerobic digestion (AD) of rice straw, with a focus on the processes and metabolic pathways of hydrolytic acidification, and revealed the underlying mechanisms. Mechanistic investigations revealed that nanobubbles, particularly CO₂ nanobubbles, significantly increased the degradation of amorphous cellulose, resulting in higher levels of soluble carbohydrates (6.27% – 11.13%), VFAs (4.39% – 24.50%), and a remarkable cumulative H₂ yield (74 – 94 times). Microbial community analysis indicated that the CO₂ nanobubble promoted the growth of acidifying bacterial communities, such as *Mobilitalea*, *unclassified_f_Lachnospiraceae*, and *Bacteroides*. This indicates that the introduction of CO₂ nanobubbles improved the total abundance of predicted functional enzymes were increased by 14%, resulting in the production of more easily degradable intermediates. Based on the analysis of total methane production and kinetic analysis, it can be concluded that nanobubble addition enhanced methane production levels of 4.22% - 7.79% with lower lag time (λ) (0.88–1.06 day) compared to the control group (1.09 day). The results also elucidated changes in relative enzymatic activities involved in the bioconversion of cellulose and hemicellulose during the hydrolysis stage with nanobubble treatment. This work is more beneficial for understanding the promoting effect and mechanism of nanobubbles on AD, facilitating the more precise application of nanobubble technology in the field of renewable energy.

Keywords: bioresources recovery, hydrolytic acidification, micro-nanobubble technology, renewable energy, rice straw, sustainable waste management

1. Introduction

The escalating global demand for sustainable and renewable resources has attracted significant interest in utilising biomass waste as a valuable raw material for bioresource

recovery [1]. Rice cultivation sustains a significant portion of the global population and generates approximately 1062 million tons of agricultural residues annually in the form of rice straw, which is a typical lignocellulosic biomass [2,3]. Conventional disposal methods for the lignocellulosic biomass, such as open field burning or landfills, contribute to air pollution and result in the loss of valuable organic matter and nutrients [4]. Anaerobic digestion (AD) of rice straw has been developed and widely recognized as a promising approach for producing biogas and nutrient-rich fertilizer [5]. Moreover, AD can promote climate change mitigation, sustain a circular economy on farms, and contribute to the attainment of the global net zero target[6].

Rice straw, a lignocellulosic biomass, exhibits a complex fibre structure resulting from the intricate cross-linking of lignocellulose into highly polymerized crystals [7]. Lignocellulosic biomass exhibits lower biodegradability compared with other organics such as carbohydrates. Therefore, the CH₄ yield from the AD of lignocellulosic biomass accounts for less 60% of its theoretical potential [8]. As known, the hydrolysis process, which is the initial step in the AD process, imposes constraints on the conversion of lignocellulosic materials into desirable end products [8]. Researchers have made several efforts to enhance the AD performance of rice straw, including pre-treating the materials to disrupt the lignocellulosic structure [9], microbial augmentation by adding specific bacterial cultures during the AD process [10], and co-digestion of rice straw with manure feedstocks [11]. However, the shortcomings of these approaches become evident when they are applied. For example, potentially hazardous byproducts, including 5-hydroxymethylfurfural, furfural, and phenolic compounds, may arise following acid pretreatment, particularly under elevated temperatures [12]. In order to avoid secondary pollution, certain adsorption methods or adsorbents are also needed [13]. Improper handling of adsorbents can also cause pollution and increase treatment costs. Hence, developing a novel intensification approach that requires fewer chemical additives and proves to be more cost-effective and efficient is vital.

Nanobubbles refer to bubbles with a diameter of less than 1 μm , and their existence was experimentally confirmed via atomic force microscopy imaging in 2000 [14]. In addition to their small size and large surface area, nanobubbles exhibit other unique characteristics such as remarkable longevity, low buoyancy, a negative surface charge, and the ability to generate reactive oxygen species (ROSs, e.g., $\bullet\text{OH}$, H_2O_2 , and O_2^-) [15]. As an emerging technology, nanobubbles have been used to address environmental issues since 2004, with the first attempt to enhance AD performance using nanobubbles occurring in 2019. Wang and Yang [16] added a solution of air, N₂, CO₂, and H₂-filled nanobubbles to the AD of waste activated sludge and achieved a 14% – 21% improvement in CH₄ production.

For AD of lignocellulosic biomass ([Table 1](#)), several studies have been demonstrated that using nanobubble solutions as the additives can significantly increase CH₄ production by up to 29% in AD of variable lignocellulosic feedstocks ([Table 1](#)). The direct pre-treatment of the feedstock

rice straw with different nanobubbles (N₂, O₂ and CO₂) led to a significant enhancement in volatile fatty acids (VFAs) production from 1873.3 (control) to 5889.6 mg·L⁻¹ (CO₂-nanobubble) [17]. A previous study demonstrated that the addition of nanobubble solution during the AD of food waste could enhance the enzymatic activity and increase the VFAs yields by 6-18% in hydrolytic acidification process [18]. Wang and Yuan [19] reported that the introduction of N₂-nanobubbles enhanced cellulase activities by 12.6% during the AD of corn straw, thereby improving the degradation of crystalline cellulose by microorganisms. Song and Hou [20] found that the addition of CO₂-nanobubble enhanced β-glucosidase and dehydrogenase activities and improved corn straw hydrolysis, which could further promote a 17% increase in the maximum methane yield. Furthermore, CO₂ and air nanobubbles enriched the microbial community of hydrolysis and acidification bacteria, such as *Aminicenantes* and *Spirochaetae* [21]. Wang and Yuan [19] showed that the nanobubble treatment groups exhibited a higher relative abundance of *Thermotoga*, *Chloroflexi*, *Bacteroidetes*, *Spirochaetae*, *Synergistetes*, and *Firmicutes* than the control group. However, there have been no reports on systematic microscopic studies of the mechanism of nanobubbles as additives in the AD process of lignocellulosic materials.

Table 1. Nanobubble technology integrated anaerobic digestion (AD) of lignocellulosic biomass for methane (CH₄) production.

Intervention	Feedstock	Filled gas in NB	CH ₄ yield (mL/g·VS)			Reference
			Control	NB	% Improve	
Pre-treatment	Corn straw (pre-augment inoculum)	N ₂	115.9	127.3	9.8	[22]
		O ₂		141.7	22.3	
		N ₂	122.1	144.2	18.1	[23]
		O ₂		179.2	46.8	
	Rice straw	N ₂	292.5	289.1	/	[17]
		O ₂		288.9	/	
		CO ₂		295.1	/	
In situ treatment	Lignin	N ₂	599	728	21.5	[24]
		CO ₂		703	17.2	
	Cellulose	Air	179.4	193.3	7.7	[19]
		N ₂		196.1	9.3	
		O ₂		233.0	29.9	
	Cellulose	CO ₂	223.3	246.0	10.2	[21]
		air		263.6	18.0	
	Corn straw	20%-100% H ₂	234.7	224.5-263.5	11.5 - 25.3	[25]
	Corn straw	25%-100% CO ₂	113.1	117.6-132.2	4 - 17	[20]

To address the knowledge gaps, this study investigated the effects and mechanisms of nanobubble technology in enhancing the hydrolysis-acidification stage of AD of rice straw. First, the mechanisms and consequences of N₂, H₂, and CO₂ nanobubble treatments on specific hydrolysis-acidification process were evaluated, and the final products in the solid, liquid, and gas forms the hydrolysis and acidification phases were quantified. Additionally, the relative

abundance of bacteria and the activity of key enzymes during the hydrolysis-acidification of rice straw by treatment of CO₂-nanobubble were analysed to elucidate the mechanism of CO₂-nanobubble on hydrolysis and acidogenesis process. Furthermore, different kinetic models were used to describe the basic mechanisms. This study aims to elucidate the mechanism of nanobubble technology to facilitate the AD approach for sustainable bioresource recovery and renewable energy generation.

2. Materials and methods

2.1. Materials

Rice straw was gathered from the Lianshui County, Jiangsu Province, China, and then dried at 105 °C before crushed for 2 minutes. The main characteristics of the rice straw were as follows: cellulose ($36.03 \pm 0.6\%$), hemicellulose ($29.60 \pm 0.15\%$), and lignin ($4.78 \pm 0.38\%$) based on dry weight, respectively (Table S1). The inoculum used in this study was sampled from a local sewage treatment plant in Beijing, China. Before used, the inoculum was centrifuged at 5000 rpm for 10 min to concentrate, and then digested at 37 ± 1 °C for one week to exhaust the residual biodegradable substances. The total solid (TS) and volatile solid (VS) of inoculum were $5.87 \pm 0.17\%$ and $2.77 \pm 0.08\%$, respectively (Table S1).

2.2. Nanobubble preparation and characterization

Three gases, i.e. N₂, H₂, and CO₂, were selected in this study to produce target nanobubble solutions using a nanobubble generator purchased from Zhongnong Tianlu Company Ltd, China. All gases (purity >99.9995%) were purchased from Beijing Haipu Gas CO., Ltd. During the nanobubble generation process, gas and deionized water were pumped into the nanobubble generator with inlet flow rates of 100 and 1000 L·h⁻¹, respectively. The operation time for the nanobubble generation was 15 mins, except for H₂ (5 mins). The concentration and average size of nanobubbles in the final solution were measured using the NanoSight system (Nanoparticle tracking analysis, NTA NS500, Malvern Instrument Ltd. UK).

2.3. The effect of nanobubble treatment on AD of rice straw

2.3.1. Alterations in hydrolysis-acidification phases

The hydrolysis-acidification tests were conducted in AD reactors with a working volume of 200 mL for each. During the test, each reactor was loaded with 100 mL inoculum mixed with deionised water (control group) or nanobubble (treatment groups), and rice straw with the ratio of VS_{rice straw}: VS_{inoculum}=3:1 and adding 10 mM sodium 2-bromoethane sulfonate (BES) (C₂H₄BrNaO₃S) to inhibit methanogens [26]. As a result, the initial total VS in each reactor was 5.54 g·L⁻¹. After well mixed, the sealed AD reactors were flushed by N₂ for 5 mins to create anaerobic conditions. Each control/treatment group was conducted with 3 parallel for 10 days, and 3 bottles were removed every two days for sampling. Notably, although the methanogenesis was inhibited in this test, some gases (such as H₂) could still be produced. The

volume of generated biogas was measured using a 50 mL air-tight glass syringe connected to the headspace of each reactor. Then, 1 mL of biogas was collected for analysis the gas components of H₂, N₂, CO₂ and CH₄ by a gas chromatograph (GC-8A, Shimadzu, Japan).

TS and VS were measured based on the standard methods [27]. pH was measured using a pH meter (FE28, Mettler-Toledo, Switzerland). The original rice straw and solid residues were subjected to morphological analysis using scanning electron microscopy (SEM) (S-4800, Hitachi, Japan). Cellulose, hemicellulose, and acid detergent lignin contents were determined using a fibre analyzer (2000, Ankon Technology Corp., USA) according to Van Soest [28]. Fourier transform infrared spectroscopy (FTIR) were acquired using an FTIR spectrophotometer (ALPHA-II) with a resolution of 4cm⁻¹. Half amount of the samples was put into centrifuge at 10,000 rpm for 25 minutes, and the supernatant was filtered by 0.45 µm membrane filters before the analysis. Soluble carbohydrates were quantified using Phenol-sulfuric acid method [29]. SCOD was measured by Lianhua LH-DE500 reagent based on the fast digestion-spectrophotometric method using a wavelength of 610nm. The gas chromatography (GC-2010 plus, Shimadzu, Japan) was employed to determine the VFA concentrations. Samples were taken from the reactor on finished to analyze the microbial community. The extraction, sequencing, and analysis of the biological samples were meticulously carried out at Majorbio Bio-pharm Technology. A comprehensive account of the specific steps and primers employed can be found in the supplementary document. The operational taxonomic units (OTUs) and the relative abundance of Bacteria were accurately derived from the 16S rRNA sequencing data, ensuring precise taxonomic classification and quantification.

2.3.2. Biogas and CH₄ production

This biochemical methane potential (BMP) test carried out under the same conditions as the hydrolysis-acidification test, except changing of the inoculum to substrate ratios was 1.5:1. The experiments were performed in triplicate and conducted in an incubator at 37 ± 1 °C for 57 days. The similar gas sample collection and analysis were conducted. Utilizing the first-order kinetic model (Eq. 1), the estimation of theoretical methane yields and hydrolysis constants (*k*) was performed [30]. The modified Gompertz model (Eq. 2), frequently employed for predicting biogas evolution, is constructed upon the interplay among bacterial proliferation, substrate consumption, and methane generation [31].

$$M(t) = P(1 - e^{-kt}) \quad (1)$$

$$M(t) = P \times \exp\{-\exp[1 + R_{\max} e(\lambda - t) / P]\} \quad (2)$$

where *M(t)* (mL·gVS_{added}⁻¹) is the cumulative CH₄ production at time *t*; *P* (mL·gVS_{added}⁻¹) is the simulated maximum CH₄ yield (mL·gVS_{added}⁻¹); *k* is hydrolysis rate (d⁻¹); *t*(d) is the digestion duration; *R_{max}* (mL·gVS_{added}⁻¹·day⁻¹) is the maximum CH₄ production rate; *λ* (d) is the lag phase time.

The theoretical CH₄ production (TMP) was calculated based on the organics content of the rice

straw. The relevant contents of C, H and O from the material were measured by an organic element analyzer (Vario EL cube, Elementar, Germany) and the calculation follows Eq. (3) and (4) [19]. The anaerobic biodegradability index (BI) of rice straw can be represented by employing the ratio of experimental CH₄ production (EMP) to TMP. The BI reflects the conversion efficiency of organic compounds into methane during AD [32].

$$C_aH_bO_c + (a - \frac{1}{4}b - \frac{1}{2}c)H_2O \rightarrow (\frac{1}{2}a + \frac{1}{8}b - \frac{1}{4}c)CH_4 + (\frac{1}{2}a - \frac{1}{8}b + \frac{1}{4}c)CO_2 \quad (3)$$

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

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

2.4. Statistical analysis

The statistical difference of the same parameters from different AD tests was analysed through Kruskal-Wallis test using SPSS 22 (*p* < 0.05). Structural equation models were employed to quantify the direct and indirect effects of the type of nanobubbles, pH and SCOD on the production of H₂ using AOMS 26 software.

3. Results and discussion

3.1. Nanobubble characterizations

Various methods, including ethanol–water exchange, temperature change, electrochemical reaction, and hydraulic cavitation, have been developed to produce nanobubbles [33]. The hydraulic cavitation method is widely recognized as an effective approach, capable of rapidly producing numerous nanobubbles (>10⁷ bubbles·mL⁻¹) within minutes [14,34], which was used in this study. After operating the system for 15 mins, the system achieved concentrations of N₂ (Fig. 1a) and CO₂ (Fig. 1b) nanobubbles at 1.43 × 10⁸ and 5.46 × 10⁷ bubbles·mL⁻¹, respectively. Although the generator is different, the obtained nanobubble concentrations (10⁷–10⁸ bubbles·mL⁻¹) were consistent with those reported in other studies using hydraulic cavitation-based approaches [35]. As the operation time increased from 5 to 15 min, the generated H₂ nanobubble concentrations remained at the level of 10⁷ bubbles·mL⁻¹, which may be due to the low viscosity, small molecular size, and high solubility of H₂, which makes H₂ nanobubbles less stable. Therefore, a H₂ nanobubble solution containing a concentration of 4.04 × 10⁷ bubbles·mL⁻¹ (Fig. 1c) was used in this mechanistic exploration study. Additionally, more than 99% of the nanobubbles in the three solutions exhibited a size below 400 nm. Moreover, in addition to the formation of bubbles at the nanoscale, the process of generating nanobubbles could lead to the (super) saturation of gas in the liquid solution as the nanobubble slowly released the gas [36]. Therefore, the pH of the N₂, CO₂, and H₂ nanobubble solutions were 8.66 ± 0.23, 4.35 ± 0.08 and 5.50 ± 0.12, respectively.

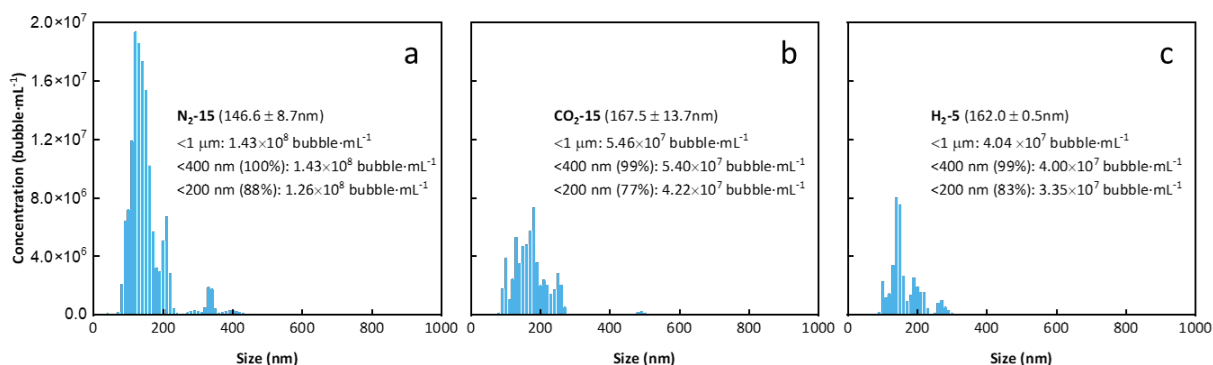


Fig. 1. The size distributions of N₂ (a), CO₂ (b) and H₂ (c) nanobubbles in solutions.

3.2. Effect of nanobubble technology on the hydrolysis-acidification stage of AD of rice straw

3.2.1. Solid phase – the structure of rice straw and lignocellulose contents

The changes of micro-structural arrangements occurred in rice straw after various gas nanobubble pretreatments (Fig. 2). The surface of the pretreated rice straw exhibited a rugged, rough texture with a high surface area, leading to changes in the stable agglomerated structure of lignocellulose, while the surface of the control was even and smooth. The scanned surface of untreated rice straw exhibited cracks to a certain extent and maintained a regular and complete network structure. After treatment with H₂ nanobubbles, the surface of rice straw exhibited signs of loosened cell walls, characterized by channelling and peeling. In contrast, the surfaces of rice straw treated with N₂ and CO₂ nanobubbles featured a higher porosity than both H₂ nanobubble-treated samples and the control. The destruction of the cell wall can be attributed to the removal of lignin and the disruption of the lignocellulose complex structure after nanobubble treatment. Thus, the surface areas accessible to enzymes increased, thereby enhancing the convertibility of lignocellulosic biomass [37]. Nanobubbles with high mobility can migrate from the bulk phase to the surface of cellulose particles, breaking through an oligosaccharide layer that creates mass transfer resistance during the hydrolysis – acidification of rice straw. These results indicated that all nanobubble treatments used in this study disrupted the compact structure of rice straw, suggesting that all of these treatments enhanced the biodegradability of the rice straw to varying degrees.

During the hydrolysis and acidification of rice straw, the cellulose and hemicellulose contents in the nanobubble treatment groups decreased to an average of 16.55% – 18.34% and 16.27% – 18.10%, respectively, compared with the control group (18.77%, and 17.19%). The percent lignin content was higher in nanobubble treatment groups than in control group mainly due to the lower degradation of lignin than other substances and the increased lignin resulting from its concentration effect on the treated samples.

The FTIR spectra of deionized water, N₂-, CO₂- and H₂-nanobubble treated rice straw were analyzed (Figs. 2e and f). The results demonstrated that the spectra of rice straw treated with deionized water and three types of nanobubbles exhibited similar profiles but with varying

intensities of the absorption bands. Cellulose can exist in two forms, namely cellulose type I, which is the typical crystalline form, and cellulose type II, which is the regenerated or amorphous form. The absorption band at 1430 cm^{-1} indicated the presence of cellulose I, which was highly resistant to biological digestibility. The absorption band at 896 cm^{-1} indicated the presence of cellulose II, which was less resistant to enzymatic and microbial hydrolysis. The lateral order index (LOI) was calculated using the absorbance ratio A_{1418}/A_{893} [38,39]. The LOI of control and N_2 -, CO_2 - and H_2 -nanobubble treatment groups were 0.76, 0.77, 0.85 and 0.74, respectively. This result indicates that N_2 - and CO_2 -nanobubbles could improve enzymatic and microbial hydrolysis, resulting in increase of LOI. This result further validates the conjecture of Wang and Sun [17], which suggested that nanobubbles could enhance the degradation of amorphous cellulose. Notably, the absorption band at 1510 cm^{-1} was attributable to the aromatic components in lignin. The nanobubble treatment group exhibited lower absorbance than the control group. Previous research has revealed that nanobubble generates reactive oxygen species (ROSs) when nanobubble collapses. The generation of $\bullet\text{OH}$ through the use of nanobubble can aid in breaking C-C or C-O bonds present in lignin molecules, resulting in the depolymerization of lignin. Additionally, this process can also target the active sites on the benzene ring, leading to the opening of the ring [40].

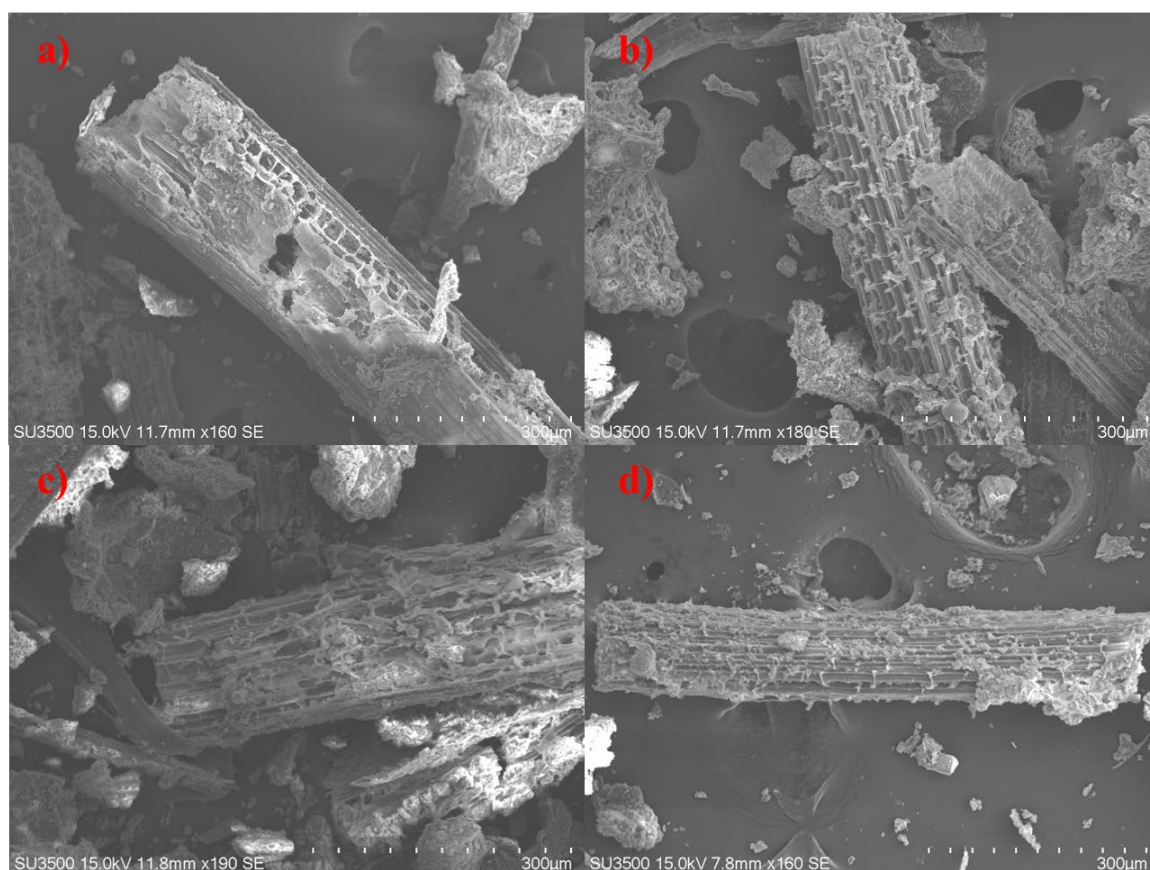
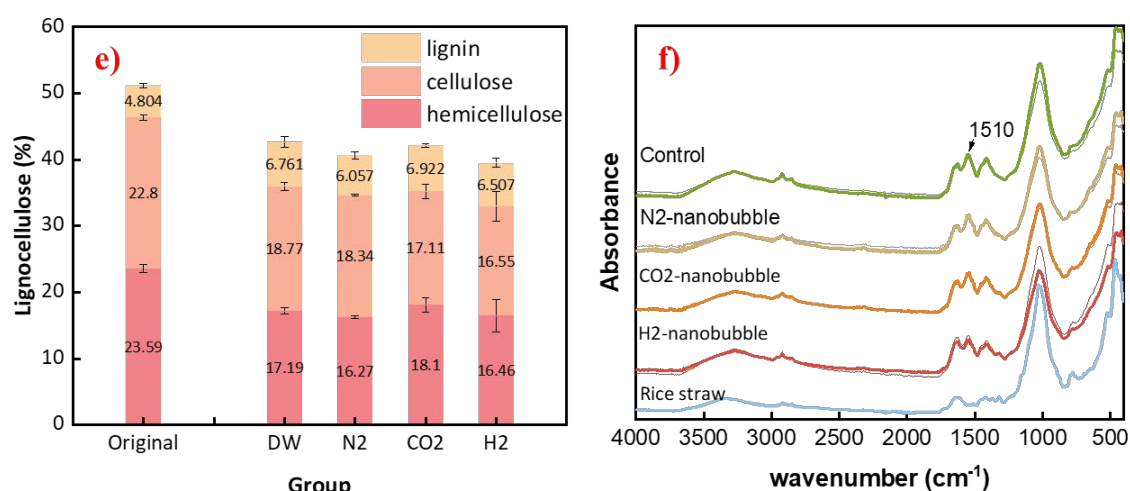


Fig.2. Changes of physical (a: control; and nanobubble treatment of b: N₂; c: CO₂; d: H₂) and chemical (e) and (f) structures of rice straw after hydrolysis and acidification.

3.2.2. Liquid phase – soluble organic matter

During the initial stage of AD, the solubilization hydrolysis reaction continuously hydrolyzes the insoluble organic matter in rice straw into soluble organic matter. Subsequently, hydrolytic enzymes degraded the complex organic substances, resulting in the production of soluble carbohydrates, such as glucose. As indicated in [Fig. 3a](#), SCOD concentration of CO₂ nanobubble treatment group increased faster compared to other treatments during the hydrolysis-acidification stage, reaching a peak of $14740 \pm 283 \text{ mg} \cdot \text{L}^{-1}$ on the 8th day. This indicated a 37.2%

increase in SCOD of the treatment group compared with the control group ($p < 0.05$). The increase in the SCOD concentration indicated the direct breakdown and efficient utilization of organic materials by microorganisms [41]. The concentrations of soluble carbohydrates in the nanobubble treatment groups were 6.27% (N_2 -nanobubble), 11.13% (CO_2 -nanobubble), 10.10% (H_2 -nanobubble) higher than those in the control group on the 10th day (Fig.3b). These results suggested that the addition of CO_2 and H_2 nanobubbles significantly enhanced the solubilization of polysaccharides, while the effect of N_2 nanobubbles on this enhancement was minimal.

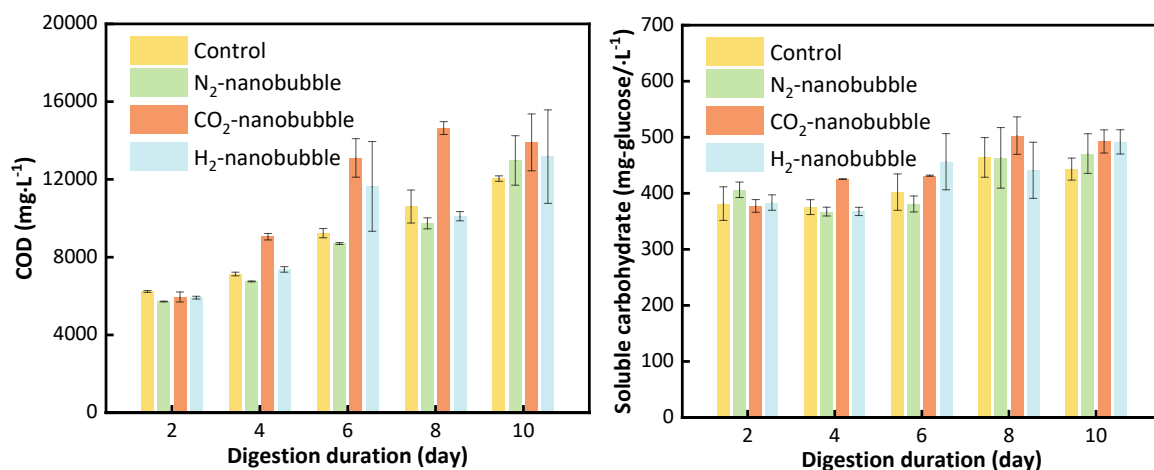


Fig. 3. Changes of SCOD (a), soluble carbohydrates (b) during hydrolysis-acidification stage.

After hydrolysis, soluble carbohydrates underwent acidification to produce VFA, including acetate (HAc), n-butyrate (n-HBu), iso-butyrate (iso-HBu), propionate (HPr), n-valerate (n-HVa), and iso-valerate (iso-HVa) (Fan et al., 2021). In this study, the increase in VFAs concentration of the nanobubble treatment groups mainly resulted from HPr and n-HBu (Fig. 4), which was increased by 23.14% (N_2 -nanobubble), 22.80% (CO_2 -nanobubble), 11.95% (H_2 -nanobubble) and 47.72% (N_2 -nanobubble), 352.42% (CO_2 -nanobubble), 379.42% (H_2 -nanobubble), respectively compared to the control. In addition, the highest HAc production was detected in N_2 -nanobubble treatment group (5714 mg·L⁻¹), which was 26.55% higher than that in the control group. While the H_2 nanobubble treatment group exhibited a lower HAc concentration of 12.38% than the control group. In addition to the VFAs yield, nanobubble significantly affected the VFAs ratio, such as the ratio of HBu to HAc (B/A ratio) and HPu to HAc (P/A ratio). These ratios served as an indicator to evaluate the efficiency of H_2 yields [43,44] (Table 2). A significant increase in B/A and P/A ratio were observed in the CO_2 and H_2 nanobubble treatment group. This result suggests that treatment of N_2 nanobubble did not modify the metabolic pathway for hydrogen production, consistent with the findings from a previous study [45]. Nevertheless, the CO_2 and H_2 nanobubble treatments could alter the metabolic pathway for hydrogen production, shifting from HAc fermentation to HPu and HBu, which was

286 responsible for difference in hydrogen production.

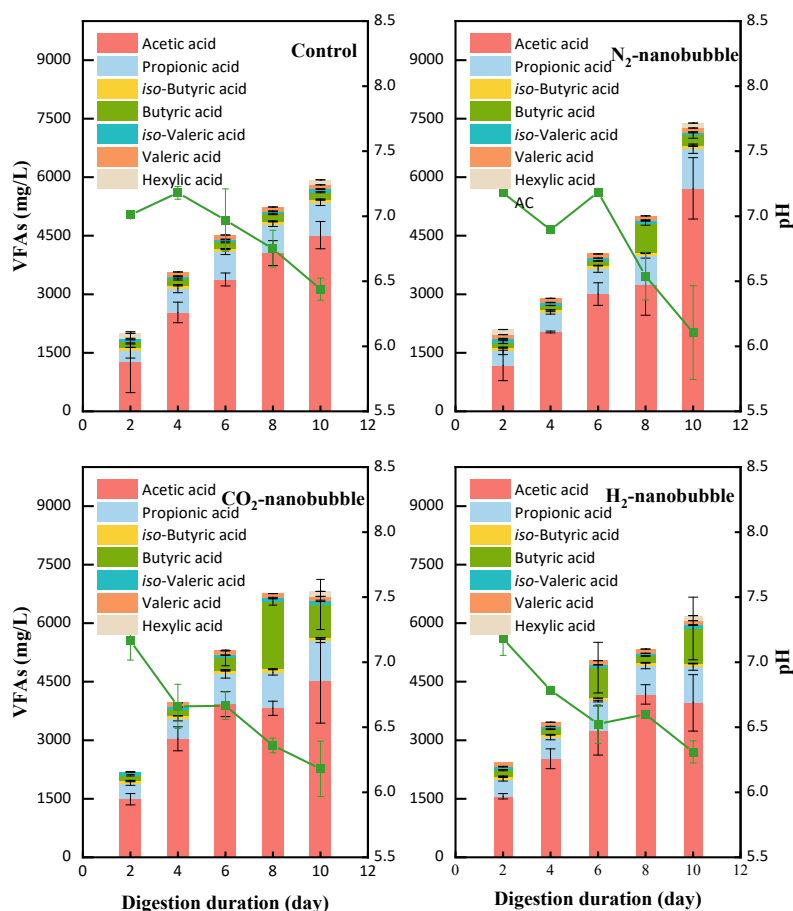


Fig. 4. Changes of VFAs and pH during hydrolysis-acidification stage

3.2.3. Gas phase - Hydrogen yield and kinetic analysis

The products, such as alcohols and VFAs, produced in the hydrolytic acidification stage were further converted into HAc and H₂ by acetic acid-producing and hydrogen-producing bacteria. Therefore, the H₂ production (Fig. S3) can indirectly indicate the degree of hydrolytic acidification of rice straw. The cumulative H₂ yield from the reactors with nanobubble treatment was significantly higher than control (Fig. S1). Notably, the CO₂ nanobubble treatment group exhibited the highest cumulative H₂ yield of 0.628 mL·gVS⁻¹, which was approximately 94 times that of the control group (0.007 mL·gVS⁻¹). Moreover, the N₂ and H₂ nanobubble treatment groups achieved cumulative H₂ yields of 0.497 and 0.512 mL·gVS⁻¹, respectively, which were approximately 74 and 76 times that of the control group. These results were consistent with those in previous studies, which indicated a 21% – 65% enhancement in H₂ production during the AD of food waste [18].

Table 2 presents the estimated kinetic parameters. In the control group, the R² value for the Gompertz model was only 0.6. The modified Gompertz model provided a better fit for the nanobubble treatment groups, with R² value exceeding 0.96. The maximum H₂ production

potential (P_{H_2}) of CO₂ nanobubble treatment group was the highest (0.711 mL·gVS⁻¹), while the maximum hydrogen production rate ($R_{H_2, max}$) of CO₂ and N₂ nanobubble treatment groups were higher than those of H₂-nanobubble and control. In addition, the CO₂ nanobubble treatment group featured a shorter lag time λ (2.65 day) than the N₂ and H₂ nanobubble treatment groups (2.75 and 3.21 day). In a study conducted by Hou [45], it was observed that the negatively charged surfaces of nanobubble have the ability to attract H⁺ ions. This attraction facilitated the reduction of H⁺ ions by NADH to produce H₂ through the reaction $HADH+H^+ \rightarrow NAD^++H_2$.

Table 2. Performance of hydrogen production and P/A and B/A ratio during hydrolysis-acidification stage

	EHP (mL·gVS ⁻¹)	Modified Gomertz model				P/A	B/A
		P_{H_2} (mL·gVS ⁻¹)	$R_{H_2, max}$ (mL·gVS ⁻¹ ·d ⁻¹)	λ (day)	R^2		
control	0.00758	0.007	0.003	0	0.922	0.183	0.042
N ₂ -nanobubble	0.57118	0.561	0.246	2.750	0.968	0.180	0.049
CO ₂ -nanobubble	0.72194	0.711	0.242	2.654	0.997	0.224	0.188
H ₂ -nanobubble	0.58811	0.609	0.139	3.206	0.994	0.234	0.228

The effects of different factors were quantified through the construction of structural equation models involving different types of nanobubbles, SCOD, pH, and H₂. This result revealed that the presence of nanobubbles was directly or indirectly related to the H₂ yield (Fig. 5). The type of nanobubble, SCOD, and pH directly exhibited direct and positive correlations with H₂ (λ = 0.319, 0.228, and 0.355). The type of nanobubble and SCOD were able to change the yield of H₂ by indirect ways. In terms of standardized total effects, nanobubble (0.309) and pH (0.355) played a great role in the yield of H₂. However, the production of H₂ negatively correlated with the SCOD content. Overall, this study indicated that nanobubbles could affect the yield of H₂ by influencing the SCOD and pH.

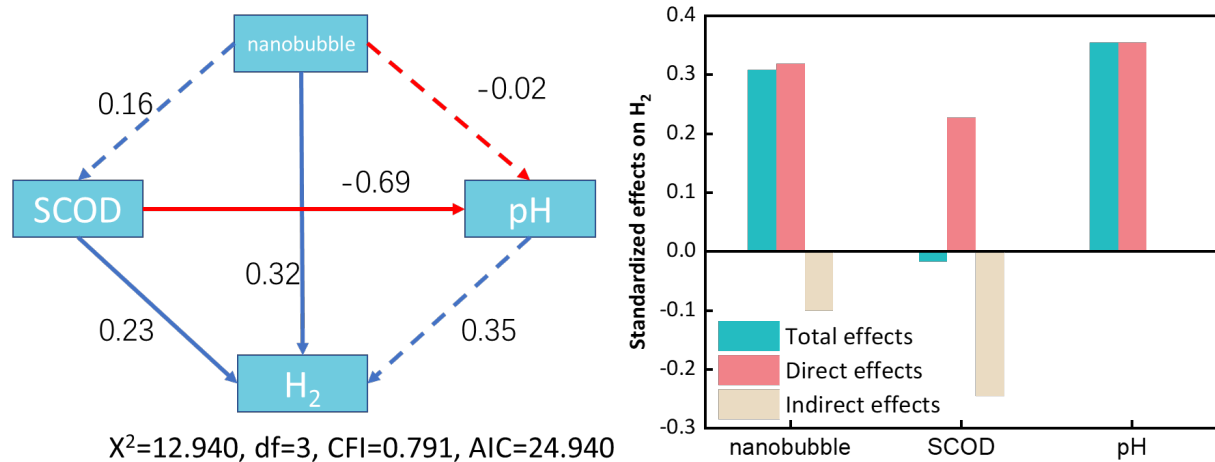


Fig. 5. Structural equation models show the direct and indirect impact of nanobubble on the abundance of H₂

3.2.4. Microbial community – bacteria function analysis and the key enzymatic activities

The relationship between the nanobubble addition and the functional genus species was separately analyzed for control and CO₂ nanobubble treatment groups (Fig.6). Results revealed that CO₂-nanobubbles could increase the abundance of bacterial communities, including *Mobilitalea*, *unclassified_f_Lachnospiraceae* and *Bacteroides*. After CO₂-nanobubble treatment, *Mobilitalea* became the dominant bacterial group, which were involved in VFA production during the acidogenic phase of AD. Members of genus *Mobilitalea* were able to ferment saccharides to produce acetate, ethanol, H₂, and CO₂ [46]. *Unclassified_f_Lachnospiraceae* can use monosaccharides and polysaccharides generated from the hydrolysis of cellulose and hemicellulose as carbon sources for growth and play a crucial role in the production of butyric acid [47]. *Bacteroides* plays a significant role in hydrolysing a broad array of polysaccharides and producing VFAs in AD [48]. The experimental results indicate that the CO₂ nanobubble can promote the hydrolysis-acidification process of AD by altering the bacterial community structure and increasing the abundance of functional bacteria. This phenomenon can elucidate the increased VFAs, particularly butyric acid, in the CO₂-nanobubble reactors.

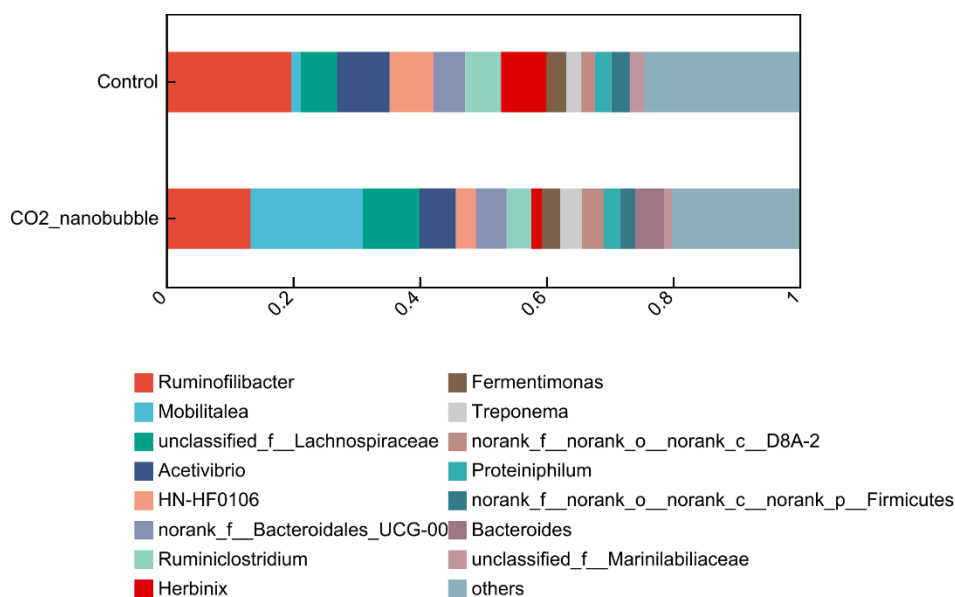


Fig.6. Relative abundance of bacteria during control and CO₂-nanobubble treatment groups

Cellulose and hemicellulose in rice straw were converted into acetate through the metabolic process of the hydrolysis-acidification stage of AD (Fig. 7). Hydrolysis depended on the combination of diverse enzymes to break down hemicellulose and cellulose. The names and corresponding numbers of enzymes are provided in Table S3.

Compared with the control group, the total abundance of predicted functional enzymes was improved by 14% in the CO₂-nanobubble added group. This result confirmed that an increase in the activity of debranching enzymes, particularly non-reducing end alpha-L-arabinofuranosidase (3.2.1.55), was responsible for removing the xylan side-chain units. CO₂-

nanobubble groups exhibited higher enzymatic activities (3.2.1.55 and 3.2.1.37) than the control. Xylan 1,4-beta-xylosidase (3.2.1.37) and non-reducing end alpha-L-arabinofuranosidase (3.2.1.55) jointly cleaved the bonds between individual xylose molecules and, the side chains of xylan, facilitating downstream processes [49]. The result indicates that the introduction of CO₂-nanobubbles enhanced the overall hemicellulose degradation in rice straw. Cellulases (3.2.1.4) were essential enzymes responsible for breaking the internal linkages at the amorphous sites for cellulose decomposition. The CO₂-nanobubble group displayed a slightly lower cellulase activity (3.2.1.4) than the control group. However, there was an observed increase in the activity of beta-glucosidases (3.2.1.21), which played a vital role in the depolymerizing of cellobiose by disrupting the glycosidic linkages to terminal non-reducing residues in beta-D-glucosides and oligosaccharides. This process led to the production of glucose, which can be used in subsequent processes, such as acidogenesis. This further confirmed previous research by Wang and Yuan [19], indicating that nanobubbles can attack the oligosaccharide layer through the actions of cellulolytic microorganisms and cellulase. The increased surface and contact areas facilitated by nanobubbles further aided this process. Additionally, the nanobubble-treated group featured a higher abundance of hexokinases (2.7.1.1) than control group. Hexokinases are intracellular enzymes that catalyze the phosphorylation of glucose, mannose, and fructose into corresponding hexose 6-phosphates. The resulting phosphate esters can subsequently be metabolized into pyruvate through glycolysis or used for various biosynthetic processes [50]. Therefore, the addition of CO₂-nanobubbles mainly enhanced the activities of 1,4-beta-xylosidase and non-reducing end alpha-L-arabinofuranosidase but featured a minimal effect on the cellulases activity. The research results indicate that CO₂ nanobubbles can increase the production of easily degradable intermediates for subsequent acidogenesis, acetogenesis, and methanogenesis processes by promoting enzymatic activities.

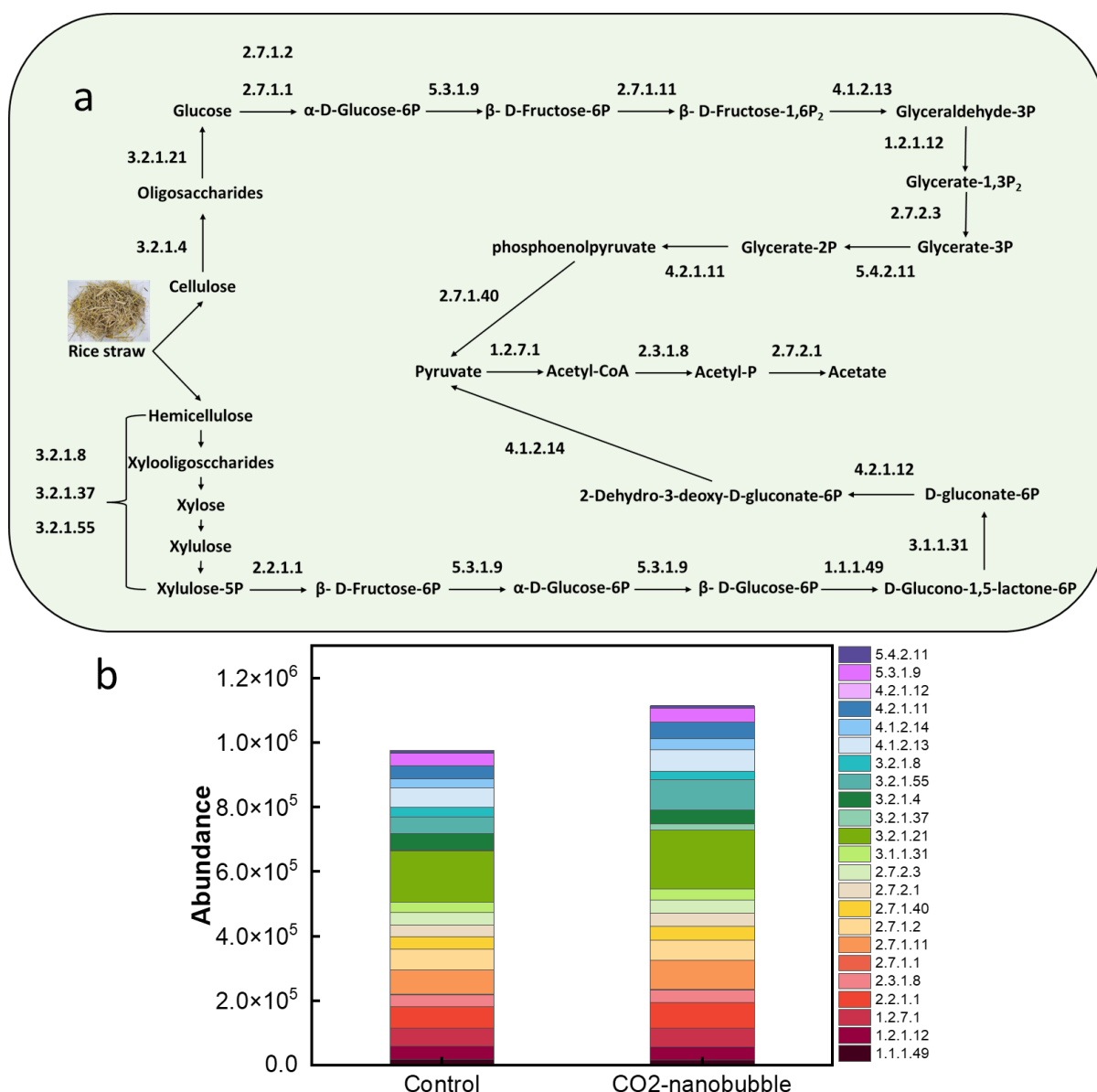


Fig.7. Metabolic pathway construction of the CO₂-nanobubble involved in the conversion of lignocellulose to acetate.

3.3. Nanobubble technology enhanced AD for biogas/CH₄ production

3.3.1. Biogas and CH₄ production

To further investigate the effect of nanobubbles on AD performance in terms of methanogenesis, BMP tests were conducted. The daily production of biogas (Fig. 8a) and CH₄ (Fig. 8c) from all groups followed the similar trend, where CO₂ nanobubble treatment group exhibited the highest daily production of biogas and CH₄ ($20.71 \pm 0.55 \text{ mL} \cdot \text{gVS}^{-1} \cdot \text{d}^{-1}$) and reached its peak on day 8 compared with other groups. Additionally, the N₂ nanobubble treatment group featured significantly higher CH₄ production ($20.65 \pm 6.00 \text{ mL} \cdot \text{gVS}^{-1} \cdot \text{d}^{-1}$) and reached its peak on day 9 compared with both the H₂ nanobubble treatment ($17.00 \pm 0.57 \text{ mL} \cdot \text{gVS}^{-1} \cdot \text{d}^{-1}$, day 11) and the control ($15.36 \pm 1.16 \text{ mL} \cdot \text{gVS}^{-1} \cdot \text{d}^{-1}$, day 11) groups. Moreover, all groups featured significant difference in daily biogas/CH₄ generation mainly during the fast generation period, which tended to be similar after 14 days. Despite their potential lifespan of

months [51] in clean water, nanobubbles tended to exhibit a faster gas diffusion rate and bubble collapse when used in AD processes. This resulted in a similar daily biogas/CH₄ production after 14 days [16]. Nevertheless, these findings demonstrated that nanobubble treatment can accelerate the bioenergy production speed and quantity during the fast biogas/CH₄ production stage.

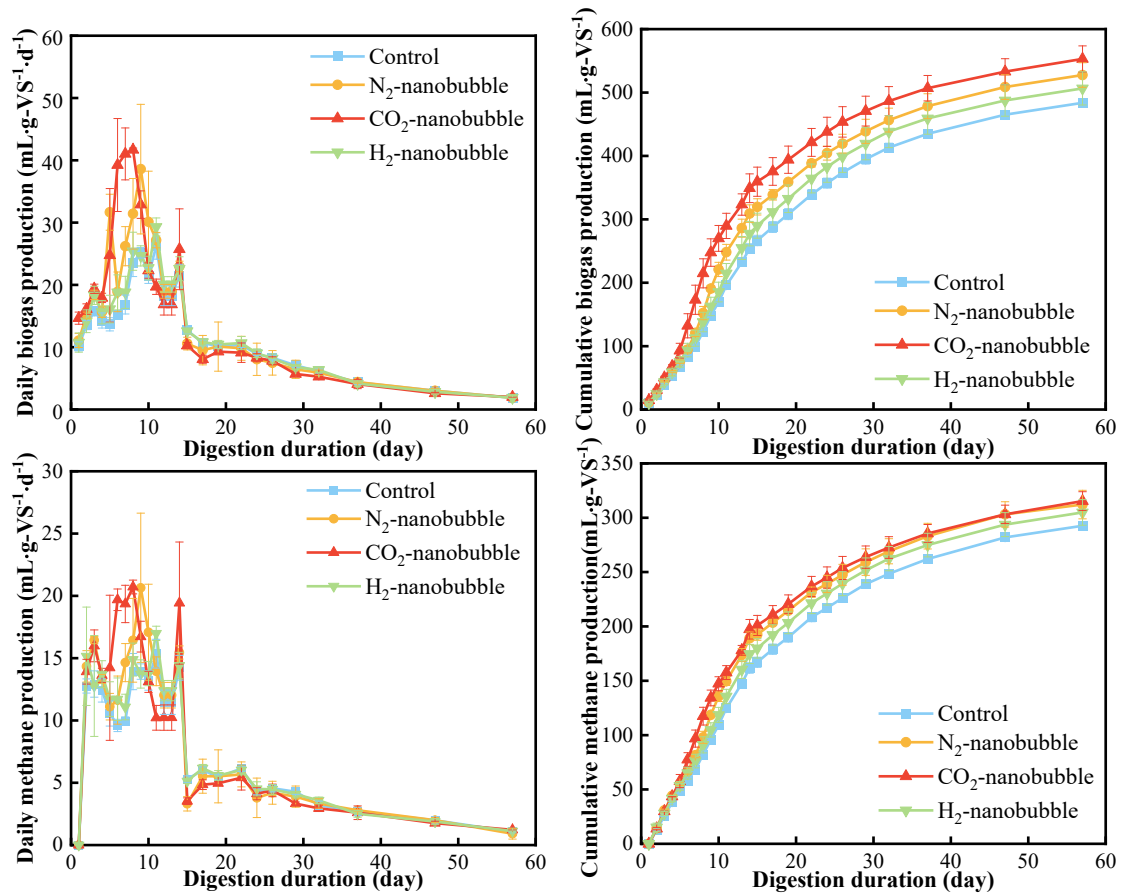


Fig. 8. Effects of different gas nanobubble treatment on cumulative methane yield during anaerobic digestion of corn straw: (a) daily biogas production, (b) cumulative biogas production, (c) daily methane production, (d) cumulative methane production.

In CO₂, N₂, and H₂ nanobubble treatment groups, the cumulative biogas production achieved 552.91 ± 20.59 , 527.51 ± 19.38 , and 506.51 ± 26.12 mL·gVS⁻¹, respectively (Fig. 8b). These values corresponded to a 14.29%, 9.04%, and 4.70% increase in the treatment groups, respectively, compared with the control group (483.79 ± 4.23 mL·gVS⁻¹). Similarly, the cumulative CH₄ production levels in CO₂, N₂, and H₂ nanobubble treatment groups were increased by 7.79%, 6.70%, and 4.22%, respectively, compared to the control group (Fig. 8d). These results agreed with the previous studies which showed 4-30% enhancement of biogas and CH₄ production during the AD of biomass (Table 1). The collapse of nanobubbles could generate free radicals [52] and was hypothesized to contribute to the breakdown of lignocellulosic material into easier biodegradable compounds for the AD process [53]. In this

study, this hypothesis was proved through the calculation of the anaerobic biodegradability of materials (Table S2) based on the ratio of EMP and TMP (Eqs. 3 – 4). The CO₂ nanobubbles treatment group exhibited the highest value (70.05%), followed by the N₂ nanobubbles treatment groups (69.34%), H₂ nanobubbles treatment groups (67.73%), and control groups (64.99%) (Table S2).

Furthermore, the lower pH of the CO₂ nanobubble solution can create an acidic condition during the AD of rice straw and enhance the hydrolysis-acidification process, resulting in the highest CH₄ yield [17]. Previous studies observed significantly higher (11.54-25.29%) biogas/CH₄ generation when using H₂ nanobubble solutions in AD processes [25]. However, the positive effect was not obtained in this study which may be due to the high concentrations of H₂ nanobubbles which could induce oxidative stress [54] and inhibit the AD process [25]. Additionally, the digestion time might account for differences in CH₄ generation. This study extended the digestion time to 57 days, which was longer than the 22 days duration in a study by He and Song [25]. The CH₄ production increased by 13.5% in CO₂ nanobubble treatment group in 22th day of this study. Overall, the results indicate that nanobubble technology could promote the AD process for CH₄ production. However, an appropriate gas source and nanobubble concentrations need to be applied towards best practice.

3.3.2. Kinetic analysis

The modified Gompertz model and the first-order kinetic model are commonly used to describe the CH₄ production kinetics in AD [55]. In this study, both models effectively simulated ($R^2 > 98\%$) the CH₄ production dynamics from all groups (Table 3). Consistent with the cumulative CH₄ production results (Fig. 8), the CO₂ nanobubble treatment group exhibited the highest maximum CH₄ production rate (R_{max}) at 14.95 mL·gVS⁻¹·d⁻¹ according to the Gompertz model, which was 22.90% higher than that in the control group (11.74 mL·gVS⁻¹·d⁻¹). Furthermore, the k value of the first-order model is another important parameter that directly reflects the rate of hydrolysis, with a higher value meaning faster substrate hydrolysis [56]. The fitting results revealed that k the control, CO₂, N₂, and H₂ nanobubble treatment groups featured k values of 0.042, 0.050, 0.055, and 0.045 d⁻¹, respectively. These results indicate that nanobubble technology can stimulate CH₄ production and improve rice straw hydrolysis.

The lag time (λ) is a parameter that indicates the initial adaptability of microbes to the AD system [55], and a lower λ value is more desirable as it improves the economic benefits of the process. The nanobubble treatment resulted in a decrease in λ values, ranging from 0.88 to 1.06 days, compared with 1.09 days in the control group. This decrease indicated a quicker start-up period for the rice straw AD process.

Table 3. Performance of methane production and VS reductions during rice straw digestion with addition of deionized water or nanobubble.

Group	Modified Gomertz model				First order kinetics model			VS reduction (%)
	P(mL·g VS ⁻¹)	R _{max} (mL·gVS ⁻¹ ·d ⁻¹)	λ (d)	R ²	P(mL·gVS ⁻¹)	k (d ⁻¹)	R ²	
Control	275.92	11.74	1.09	0.991	333.22	0.042	0.987	24.85±0.98
N ₂	299.46	14.02	1.06	0.988	340.13	0.050	0.984	26.56±0.89
nanobubble								
CO ₂	288.34	14.95	0.88	0.985	332.60	0.055	0.984	26.08±2.53
nanobubble								
H ₂	286.41	12.72	1.00	0.991	340.36	0.045	0.987	24.33±2.62
nanobubble								

3.4. Implementation potential and research needs

The application of nanobubble technology in AD is an area of active research, but scientific and technical challenges still need to be addressed. Nanobubbles featured a negatively charged zeta potential, which led to increased charge repulsion between particles, preventing particle agglomeration [57]. Additionally, nanobubbles exhibited a longer spin–spin relaxation time (T_2) than normal water molecules, which represents a higher water molecular mobility[20]. This increased mobility can promote full contact between microorganisms and organic matter, thereby facilitating nutrients transportation. Higher water mobility may improve the fluidity of digested solutions and enhance mass transfer efficiency. In this study, the effect of nanobubble on hydrogen production from rice straw hydrolysis was better than that on methane production, indicating that nanobubbles altered the anaerobic digestion pathway of rice straw, shifting it toward alcohol or other substances.

In terms of engineering applications, the use of reflux biogas slurry instead of water as the carrier of nanobubbles will enhance the practicality of the process. This study shows that nanobubble has a more obvious promotion effect on the hydrolytic acidification stage compared with the methanogenesis stage. Therefore, nanobubbles should be used in the hydrolytic acidification tank of two-phase anaerobic fermentation systems. In addition, nanobubble technology can significantly promote H₂ production during anaerobic fermentation. In the future, this technology can be used to improve the dark fermentation process. Certainly, further research can investigate the feasibility and efficiency of implementing this strategy in a full-scale, long-term anaerobic digestion system.

4. Conclusions

This study elucidated, the influence and mechanism of nanobubble treatment on the hydrolysis-acidification and methane production processes during the AD of rice straw. The result revealed that nanobubbles could enhance the hydrolysis and acidification stages of AD of rice straw by disrupting the lignocellulosic material structure to produce higher levels of SCOD, soluble carbohydrates, VFAs, and H₂. Among the nanobubbles, the CO₂-nanobubble exhibited the highest significant effect on the acidification process. The microbial community

analysis revealed that the CO₂ nanobubble significantly increased the acidification-related bacterial communities, such as *Mobilitalea*, *unclassified_f_Lachnospiraceae*, and *Bacteroides*. Moreover, the introduction of CO₂ nanobubbles improved the activity of hemicellulolytic enzymes. Overall, the addition of nanobubbles facilitated the hydrolysis and acid production process in the AD of rice straw and could serve as a green and effective method for rice straw pretreatment to achieve more renewable energy during AD. The work of this article provides support for the application of nanobubble technology in the field of renewable energy, contributing to towards sustainable development goals.

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CRediT author statement

Yali Zhu: Conceptualization, Methodology, Investigation, Writing – original draft. **Tao Lyu:** Conceptualization, supervision, Writing - Review & Editing. **Daoyu Li:** Investigation, Resources. **Zongqin Zhang:** Investigation. **Jianbin Guo:** Conceptualization. **Xin Li:** Supervision, Writing – review & editing, Funding acquisition. **Wei Xiong:** Conceptualization. **Renjie Dong:** Conceptualization, Supervision. **Siqi Wang:** Visualization.

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Supplementary Material

**Process mechanisms of nanobubble technology enhanced hydrolytic
acidification of anaerobic digestion of lignocellulosic biomass**

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The supplementary material contains 4 tables and 3 figures.

Materials and methods

Table S1. Characteristics of rice straw and anaerobically digested sludge used in this study.

Characteristics	Unit	Rice straw	Inoculum
pH	-	ND	7.30 ± 0.01
Total solid (TS)	%, w.w.	86.48 ± 1.05	5.87 ± 0.17
Volatile solid (VS)	%, w.w.	75.23 ± 1.55	2.77 ± 0.08
Total volatile fatty acids (TVFAs)	mg/L	ND	1510.75 ± 41.34
C	%, d.w.	37.91	ND
H	%, d.w.	4.81	ND
O	%, d.w.	37.01	ND
N	%, d.w.	0.94	ND
C/N		40.33	ND
Cellulose	%, d.w.	36.03 ± 0.6	ND
Hemicellulose	%, d.w.	29.60 ± 0.15	ND
Lignin	%, d.w.	4.78 ± 0.38	ND

ND-not determined

Gas composition and other chemical compounds measurement

N₂, CH₄ and CO₂ were measured daily with a gas chromatograph (Shimadzu GC-8A, Japan). A biogas sample of 0.5 mL was extracted from a biogas bag using a 1 mL gas-tight syringe and subsequently introduced into the gas chromatograph. The gas chromatograph operated at temperatures of 120 °C, 50 °C, and 120 °C for the inlet, column, and detector, respectively. A stainless-steel column with dimensions of $\Phi 10 \text{ m} \times 2 \text{ mm}$ was employed, and the carrier gas utilized was H₂, maintained at a partial pressure of 0.38 MPa and a flow rate of 10–30 mL·min⁻¹. H₂ was measured using a GC (Shimadzu GC-8A, Japan) with a 13X stainless steel molecular sieve packed column (10 m $\times$ 2 mm). The temperatures of the thermal conductivity detector and column oven were 120 °C and 60 °C, respectively. The volatile fatty acids (VFAs) were analyzed using a GC (Shimadzu GC-2010 Plus, Japan) with a flame ionization detector and capillary column of type Rtx-wax (30 m $\times$ 0.25 mm $\times$ 0.25 μm). The column had an initial temperature of 60 °C (5-min hold time), ramp 10°C/min to 140 °C (2-min hold time), and ramp 20 °C/min to 230 °C (2-min hold time). The temperature of injector and detector were 230 °C and 250 °C, respectively.

16rRNA gene sequencing and analysis

First, samples were thawed at room temperature. Subsequently, DNA was isolated using the E.Z.N.A.[®] soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). And the concentration and purity of DNA were determined by 1% agarose gels electrophoresis and NanoDrop2000 spectrophotometer (Thermo Scientific, United States) and kept at -80 °C prior to further use. The bacterial 16S rRNA gene were amplified with bacterial primer pair 338F (50-ACTCCTACGGGAGGCAGCAG-30) and 806R (50-GGACTACHVGGGTWTCTAAT-30). To distinguish between different DNA samples, a distinctive barcode was allocated to the primer that targeted each sample. For the amplification of bacterial DNA, the reaction mixtures were composed of 10 ng of template DNA, 5 μmol of forward primer, 5 μmol of reverse primer, 0.4 μL of FastPfu Polymerase, 4 μL of 5 $\times$ FastPfu buffer, and 2.5 mmol/L of dNTP. Furthermore, in order to ensure the stability and activity of the polymerase during the involvement of the archaeal primer, a concentration of 0.2 $\mu\text{g}/\mu\text{L}$ bovine serum albumin (BSA) was incorporated. The polymerase chain reaction (PCR) was initiated by subjecting the mixture to a denaturing step at 95 °C for a duration of 3 minutes. Subsequently, a total of 27 cycles (using the bacterial primer pair) were performed, consisting of 30 seconds at 95 °C, followed by 30 seconds at 55 °C, and finally, 45 seconds at 72 °C. To conclude the PCR process, an extension step at 72 °C for 10 minutes was carried out. Then, the generated amplicons were extracted from 2% agarose gels (AxyPrep DNA Gel Extraction, Axygen Biosciences, USA), and quantified using QuantiFluorTM-ST (Promega, USA). The upgraded amplicons were sequenced on the Illumina platform (Majorbio Tech, China).

The raw FastQ files were subjected to quality filtration using QIIME software (version 1.17) to ensure data integrity and reliability. Specifically, raw reads with a low quality score (<20) were truncated from the beginning. Additionally, reads with a short length (<50 bp) were deemed

inadequate and subsequently discarded. Furthermore, filtered sequences that exhibited an overlap of more than 10 bp were assembled based on this overlap. Any reads containing uncertain nucleotides or erroneous reads resulting from nucleotide mismatches in primer adaptation were also eliminated from the analysis.

To obtain the operational taxonomic units (OTUs), the OTUs were initially clustered with the software UPARSE. The chimeric sequences were identified and discarded using UCHIME. Then, the trimmed reads were subsampled either for Bacteria or for Archaea, together with a recalculation of the OTUs numbers. Subsequently, the taxonomy of each gene sequence was identified by the software RDP Classifier against the SILVA v138 database with the 70% confidence threshold. Identifications were ranked by tag abundance. Relative abundance was calculated from the number of sequences that affiliated with a taxon divided by the total number of sequences per sample.

Table S2. The theoretical CH₄ production (TMP), experimental CH₄ production (EMP), and the anaerobic biodegradability (BD) of rice straw after different nanobubble treatments.

Group	TMP (mL/g-VS)	EMP (mL/g-VS)	BD (%)	VS reduction (%)
Control	450.16	292.55 ± 2.88	64.99	24.85 ± 0.98
N ₂ nanobubble	450.16	312.15 ± 13.12	69.34	26.56 ± 0.89
CO ₂ nanobubble	450.16	315.34 ± 8.62	70.05	26.08 ± 2.53
H ₂ nanobubble	450.16	304.89 ± 11.75	67.73	24.33 ± 2.62

782 **Table S3.** Relative abundance of bacteria during Control and CO₂-nanobubble treatment groups

	Control	CO ₂ - nanobubble
<i>Ruminofilibacter</i>	19.76%	13.35%
<i>Mobilitalea</i>	1.50%	17.70%
<i>Unclassified_f_Lachnospiraceae</i>	5.75%	9.01%
<i>Acetiveibrio</i>	8.29%	5.70%
<i>HN-HF0106</i>	6.90%	3.16%
<i>norank_f_Bacteroidales_UCG-001</i>	5.00%	4.85%
<i>ruminiclostridium</i>	5.68%	3.91%
<i>Herbinix</i>	7.15%	1.66%
<i>Fementimonas</i>	3.15%	2.83%
<i>Treponema</i>	2.40%	3.44%
<i>Norank_f_norank_o_birabk_c_D8A-2</i>	2.12%	3.44%
<i>Proteiniphilum</i>	2.55%	2.69%
<i>Norank_f_norank_o_birabk_c_norank_p_Firmicutes</i>	2.94%	2.29%
<i>Bacteroides</i>	0.004%	4.62%
<i>Unclassified_f_Marinilabiliaceae</i>	2.24%	1.25%

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784 **Table S4.** Hydrolytic enzymes regarding carbohydrate

Enzyme_ID	Enzyme_Category	Control	CO2-nanobubble
1.1.1.49	Glucose-6-phosphate dehydrogenase (NADP(+))	0.00073752	0.00056508
1.2.1.12	Glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)	0.00150396	0.00146269
1.2.7.1	Pyruvate synthase	0.0022044	0.00198186
2.2.1.1	Transketolase	0.00257102	0.00281214
2.3.1.8	Phosphate acetyltransferase	0.00139384	0.00137225
2.7.1.1	Hexokinase	4.475E-05	6.9996E-05
2.7.1.11	6-phosphofructokinase	0.00290365	0.00316181
2.7.1.2	Glucokinase	0.00246474	0.0021767
2.7.1.40	Pyruvate kinase	0.00148063	0.00145878
2.7.13.3	Histidine kinase	0.00993846	0.01044391
2.7.2.1	Acetate kinase	0.0014656	0.00143676
2.7.2.3	Phosphoglycerate kinase	0.00148798	0.00145042
3.1.1.31	6-phosphogluconolactonase	0.00125457	0.00123428
3.2.1.21	Beta-glucosidase	0.00609257	0.00635445
3.2.1.37	Xylan 1,4-beta-xylosidase	7.5057E-05	0.00069958
3.2.1.4	Cellulase	0.00203203	0.0014913
3.2.1.55	Non-reducing end alpha-L-arabinofuranosidase	0.00195321	0.0033293
3.2.1.8	Endo-1,4-beta-xylanase	0.00118198	0.00092258
4.1.2.13	Fructose-bisphosphate aldolase	0.00226171	0.00230459
4.1.2.14	2-dehydro-3-deoxy-phosphogluconate aldolase	0.00115155	0.00120601
4.2.1.11	Phosphopyruvate hydratase	0.00152513	0.00179964
4.2.1.12	Phosphogluconate dehydratase	1.5038E-06	1.6829E-06
5.3.1.9	Glucose-6-phosphate isomerase	0.00152185	0.00147764
5.4.2.11	Phosphoglycerate mutase (2,3-diphosphoglycerate-dependent)	0.000298	0.00029908

785

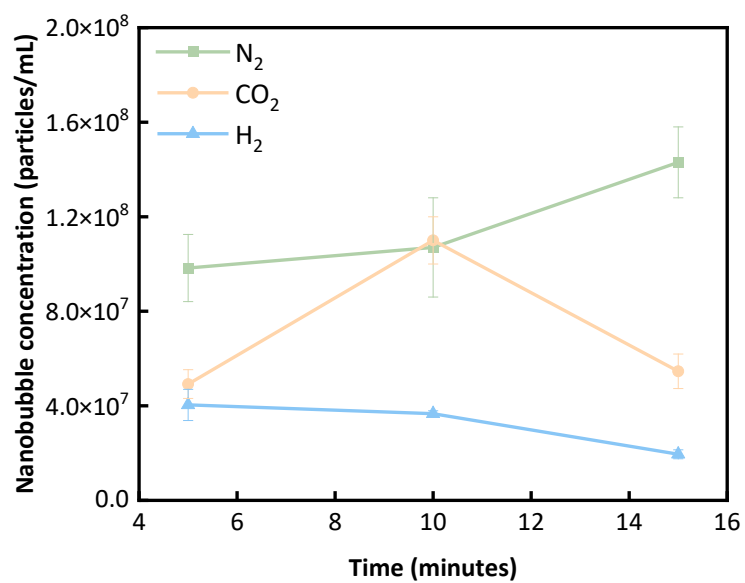


Figure S1. Nanobubble concentration during nanobubble generator operation at different times

789

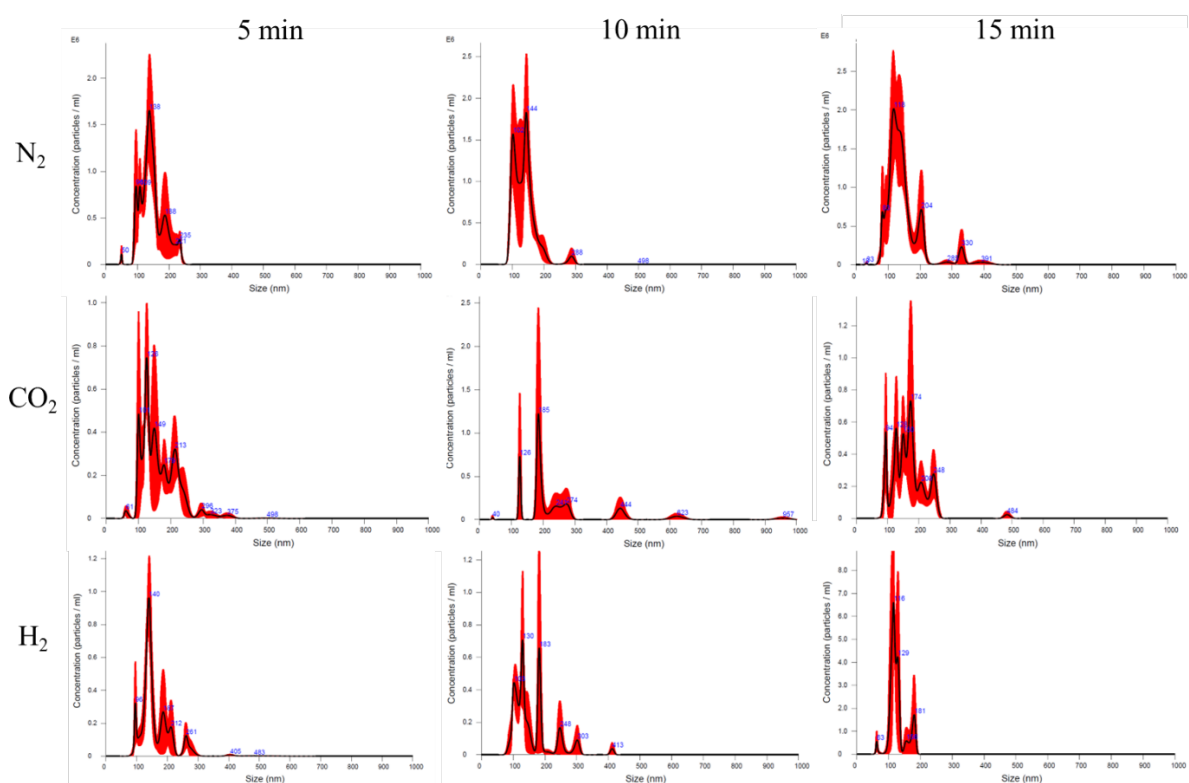


Figure S2. Nanobubble size distribution during nanobubble generator operation at different times

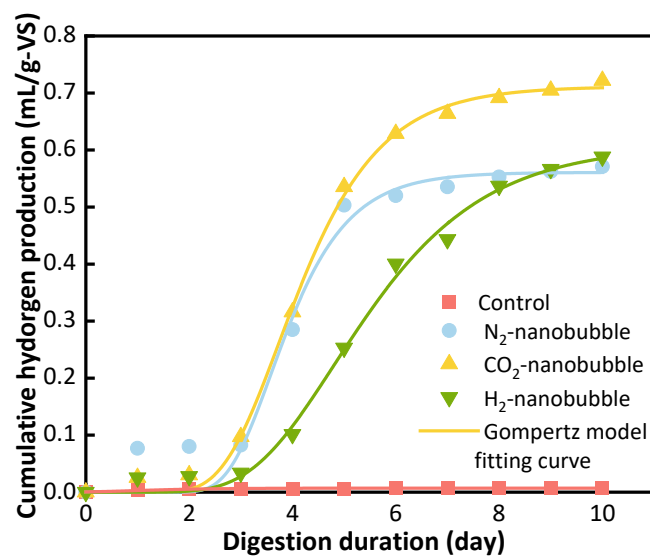


Figure S3. Cumulative hydrogen production during the hydrolysis-acidification stage of AD of rice straw



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