

Effects of nanobubble water on digestate soaking hydrolysis of rice straw

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Abstract

This study investigated the performance of combined nanobubble water (NW) and digestate in the soaking hydrolysis process. Two types of NW (CO₂NW and O₂NW) with digestate were used to soak rice straw for 1, 2, 3, 5, and 7 days. During soaking process, the volatile fatty acids (VFA) concentration in the treatment with O₂NW and digestate for 3 days (O₂NW-3 d) reached 7179.5 mg-HAc/L. Moreover, the highest specific methane yield (SMY) obtained in this treatment could reach 336.7 NmL/gVS. Although the addition of NW did not significantly increase SMY from digestate soaking, NW could accelerate the rate of methane production and reduce digestion time of T₈₀. The enrichment of *Enterobacter* in the soaking process was observed when using CO₂NW and O₂NW as soaking solutions which played important roles in VFA production. This study provides a new insight into environment-friendly enhanced crop straw pretreatment, combining NW and digestate soaking hydrolysis.

Keywords: Soaking pretreatment; Nanobubbles; Biogas production; Microbial community.

1. Introduction

Hydrolysis is usually the limit step of the anaerobic digestion (AD) process, especially for lignocellulosic materials (Wang et al., 2020). Hence, the pre-hydrolysis processes of crop straw, such as ensiling (Sun et al., 2019) or short-term digestate soaking in the tank (Wei et al., 2020), are commonly employed as biological pretreatment methods due to the technical feasibility and cost-effectiveness.

In recent years, nanobubble water (NW), with bubble sizes of less than 1000 nm in diameter (ISO 20480:2017), has attracted increasing interest in the AD process. Up to now, some studies have shown that NW could enhance the AD process by strengthening key microorganisms and enzyme activity (Chuenchart et al., 2021). This enhancement was attributed to the facilitation of efficient mass transfer of gases and nutrients due to small size and large surface area of nanobubbles (Wang et al., 2020). In addition, some studies reported that the addition of small amounts of O₂ into anaerobic reactors was beneficial for the growth of facultative hydrolytic and acidogenic microorganisms (Wu et al., 2021). Bajón Fernández et al. (2015) reported that CO₂ enrichment contributed to promoting the production of acetic acid via the Wood-Ljungdahl pathway.

However, these studies mainly focus on the effect of O₂NW and CO₂NW addition on the one-step AD process. Lack of study on the influence of NW on the pre-hydrolysis (soaking) process before AD. In addition, Wei et al. (2020) reported that digestate soaking, a biological pretreatment, could increase volatile fatty acid (VFA) production by providing abundant microorganisms. This study hypothesized that combining NW

(O₂NW or CO₂NW) and digestate might lead to a favorable hydrolysis effect.

Therefore, this study investigated the combined impact of NW with digestate on the hydrolysis process of rice straw. The main objectives include: (1) investigating the performance of O₂NW and CO₂NW on hydrolysis and acidification during soaking; (2) evaluating the kinetics of their effects on methane yield; (3) elucidating the change in the microbial community during soaking. This study aimed to provide new insights into the combining NW and digestate soaking pretreatment of rice straw.

2. Materials and methods

2.1. Rice straw, inoculum, and nanobubble water

The indica rice straw was collected from a field in Hubei, China, air-dried for several days, mechanically chopped, and then sieved through a 20-mesh screen. The digestate was taken from a stable mesophilic AD reactor at a sewage treatment facility in Beijing. The inoculum-1 for soaking pretreatment was prepared by centrifuging (1500 rpm for 50 min) the collected digestate to remove the supernatant by a pilot-scale centrifuge (JDR-80, Jiaderen Co., Ltd., China). The inoculum-2 for batch anaerobic digestion experiment was prepared by cultivating the digestate for one week until no biogas production.

CO₂NW and O₂NW were generated by mixing gas and deionized water (DW) in nanobubble rapid generation device (TL-MBG10-A, China). The preparation time for CO₂NW was 10 min and for O₂NW was 15 min (Wang et al., 2023).

2.2. Digestate and nanobubble soaking pretreatment

To address the problem of less epiphytic bacteria and low hydrolysis efficiency of the rice straw, this experiment aimed to improve soaking pretreatment by adding NW and digestate. The inoculum-1, which had a higher total solids (TS) content, could potentially address the issue of low reactor volume utilization efficiency in NW-based AD. The rice straw and inoculum-1 were loaded into 250-mL glass bottles as a ratio of 4:1 based on volatile solids (VS). Then the soaking solution (DW, CO₂NW, and O₂NW) was added at a ratio of soaking solution to rice straw 10:1 based on wet weight. Those treatments were labeled as DW, CO₂NW, and O₂NW, respectively. The soaking time was set at intervals of 1, 2, 3, 5, and 7 days. The bottles were blown for 1 min with N₂, sealed with a nitrile plug, and placed in a water bath at $37 \pm 1^\circ\text{C}$.

2.3. Batch anaerobic digestion

After the soaking pretreatment, the inoculum-2 was loaded to produce biogas as a ratio of 1:1.5 with rice straw, based on VS. Those treatments were also labeled as DW, CO₂NW, and O₂NW, respectively. The untreated rice straw was loaded with the same ratio (labeled as untreated). In addition, the biogas yield from the inoculum-2 alone was also measured. The bottles were blown and sealed again. Every bottle was placed in a water bath at $37 \pm 1^\circ\text{C}$ and manually shaken daily to ensure fermentation uniformity.

2.4. Analytical method

The standard methods were used to measure TS and VS (APHA, 2005). The cellulose, hemicellulose, and acid detergent lignin contents of the samples were

determined according to the method of Van Soest (1963). The VFA concentrations in the soaking liquid were filtered through a 0.22 μm microfiber filter and then subjected to gas chromatography (GC-2010 Plus, Shimadzu, Japan) analysis. The pH of the liquid was measured by a pH meter (FE28, Mettler-Toledo, Switzerland). The composition of biogas was determined using gas chromatography (GC8A, Shimadzu, Japan). The biogas volume was normalized to standard temperature and pressure conditions after being tested by a glass syringe.

2.5. Microbial community analysis

The samples were collected from treatments after 1, 3, and 5 days of soaking in different soaking solutions to analyze the microbial community. Following the manufacturer's instructions, DNA was extracted from samples using an E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). The DNA was amplified with primers 338F and 806R. The sequence was performed by an Illumina platform device (Illumina, San Diego, USA). The procedures for high-throughput sequencing of 16S rRNA were cited to an earlier study (Sun et al., 2019). The raw sequencing data were submitted to the Sequence Read Archive (SRA) of NCBI with project number PRJNA1056946.

3. Results and discussion

3.1. Effect of adding nanobubble water on soaking pretreatment

3.1.1 VFA production and pH

VFA served as essential intermediate metabolites in the AD process, and a greater concentration of VFA corresponds to an enhanced hydrolysis effect during soaking. The

VFA concentration of rice straw soaked in different types of soaking solution (DW, CO₂NW, and O₂NW) combined with digestate is shown in Fig. 1a. The VFA concentrations in the O₂NW treatments on days 1, 2, 3 were 3062.1 ± 174.8 , 5222.4 ± 864.7 , and 7179.5 ± 337.5 mg-HAc/L, which exhibited a significant increase of 22.2%, 8.5%, and 14.0%, respectively, compared with DW treatments at same soaking time ($p < 0.05$). Likewise, after adding CO₂NW for days 1, 2, and 3, it showed the increases of 16.9%, 7.6%, and 9.9%, respectively, compared with DW treatments ($p < 0.05$). Those results indicated that NW on digestate soaking pretreatment could promote VFA production. The pH changes of straw soaked with different water and digestate are shown in Fig. 1b. The pH values of DW, CO₂NW, and O₂NW treatments decreased rapidly from 7.67 ± 0.04 , 7.67 ± 0.05 and 7.59 ± 0.04 on the 1st day to 5.98 ± 0.20 , 5.81 ± 0.01 and 5.73 ± 0.08 on the 3rd day, respectively. However, after 3 days of soaking, the change of pH gradually slowed down.

The O₂NW treatment exhibited the most significant increase in VFA yield after soaking for 1 day. However, with prolonged soaking time, the rate of VFA increase in the NW-added treatment compared with the DW treatment gradually slowed down. This might be related to the high VFA concentration, causing a pH decrease, which might have a negative effect for acid-producing microorganisms (Fan et al., 2021).

Furthermore, due to the batch soaking used in this experiment, NW could only be added once during loading, potentially leading to limited effects on the pretreatment. However, if NW is generated in situ within the AD reactor, continuous supply with NW may hold

tremendous potential for lignocellulose pretreatment, especially by adding O₂NW.

3.1.2 Substrate degradation

The TS degradation rate of straw pretreated with soaking solution and digestate are shown in Fig. 1c. The TS degradation rates of CO₂NW and O₂NW treatments on day 1 (15.4% and 15.6%) and day 3 (18.5% and 19.4%) were higher than those of DW treatments (the TS degradation rates of DW on day 1 and day 3 were 15.1% and 17.6%, respectively). When the soaking time was extended for 5 days and 7 days, the TS degradation rate of the O₂NW treatments was still higher than that of the DW treatments. However, there was no significant difference in TS degradation rate between CO₂NW and DW treatments after soaking for 7 days.

3.2. Effect of methane yield after soaking pretreatment with nanobubble water

After soaking with NW and digestate for rice straw, the specific methane yield (SMY) of each treatment is shown in Fig. 1d. The highest SMY after soaking pretreatment was shown in the O₂NW-3 d treatment with 336.7 ± 3.1 NmL/gVS, which represented an increase of 11.6% compared with the untreated group (301.7 ± 1.5 NmL/gVS). However, when considering the impact of NW alone, the SMY from the O₂NW-3 d treatment was not significantly increased compared with the DW-3 d treatment (328.4 ± 3.7 NmL/gVS). Although the results exhibited that NW soaking with digestate could increase the methane production, the impact of sole NW was minimal.

When comparing treatments with different soaking times but using the same

solution (DW, CO₂NW, or O₂NW), the SMY showed a significant increase before day 3 ($p < 0.05$). As the soaking time was extended for 7 days, the methane production among different treatments showed a trend towards consistency. Although O₂NW had a higher TS degradation rate, there was no significant increase in methane production. As reported by Zhang et al. (2016), prolonged soaking time resulted in more organic matter loss, including the generation of CO₂, H₂, and CH₄. Therefore, soaking in O₂NW for 3 days was an optimal condition for pretreating rice straw to minimize soaking loss and maximize methane production.

Additionally, the SMY of the CO₂NW-1 d treatment was 330.8 ± 3.6 NmL/gVS, which was higher compared with the O₂NW-1 d treatment (Fig. 1d). This means that the soaking efficiency with CO₂NW was faster than with O₂NW after 1-day soaking. Due to its higher solubility compared with other gases, CO₂ nanobubbles dissolved gradually to form carbonic acid, which could partially dissociate to provide H⁺, creating an acidic environment for the soaking system (Guo et al., 2019). However, the organic acids production in other treatments also caused a decrease in pH of the soaking liquid. Therefore, as the soaking time was prolonged, the SMY of CO₂NW treatment did not significantly increase.

In this study, the modified Gompertz model was employed to fit the SMY of rice straw soaked with digestate and NW. For a 3-day soaking period, the lag phase reduced from 1.9 days in the untreated group to 0.2 to 0.5 days after soaking. This means that soaking pretreatment could shorten the lag period ($p < 0.05$). In addition, R_m (18.6–19.1

NmL/gVS·d) of each treatment after 3-day soaking was higher than that of the untreated group (16.5 NmL/gVS·d), particularly in treatments with added NW. This indicated that soaking with NW could accelerate the rate of methane production. The digestion time of T_{80} was decreased by 6.6%–16.6% after NW soaking compared with untreated. These results showed that soaking pretreatment with NW could accelerate biogas production and reduce digestion time.

3.3. Effect of bacterial community during soaking pretreatment with nanobubble water

The bacterial community after soaking for 1, 3, and 5 days with NW and digestate are shown in Fig. 2. It was obvious that *Enterobacter* and *Enterococcus* dominated in all treatments. *Enterococcus*, a common *Firmicutes* in acidic silage, dominated the first day of soaking; while *Enterobacter*, a *Proteobacteria* phylum, played a significant role in converting polysaccharides to acetic acid (Wang et al., 2019). With prolonged soaking, *Enterococcus* was gradually replaced by *Enterobacter*, which was better suitable for lower pH. The reason was that *Enterococcus* was a coccus, and its ability to adapt to harsh environments (low pH) was not as strong as *Enterobacter* (rod-shaped bacteria) (Wang et al., 2019). Interestingly, CO₂NW and O₂NW treatment exhibited a faster replaced trend, indicating that NW accelerated microbial activity. According to the comparison of the relative abundance of *norank_c_D8A-2*, it was observed that NW accelerated the elimination of original bacteria of inoculum. The relative abundance of *Acetomicrobium* gradually decreased which was related to the decrease in the pH of

soaking liquid after prolonged time. However, O₂NW treatment still had a higher abundance of *Acetomicrobium* after soaking 3 d. Fan et al. (2021) reported that airNW-based AD system had the ability to relieve acid inhibition. The higher relative abundance of *Clostridium_sensu_stricto_1* in the O₂NW-1d treatment compared with the other treatments. However, its relative abundance decreased after soaking 3 days, meaning that the pH dropped below 6 inhibiting butyric acid production (Lv et al., 2022).

Differences in soaking water type and duration resulted in differing pH among treatments, directly leading to changes in microbial communities. The VFA produced from the degradation of crop straw caused the pH to decrease. Higher VFA concentrations indicated better pretreatment effectiveness. But the decrease in pH could also inhibit or even cause death to acid-producing bacteria that were sensitive to harsh environments, which was unfavorable for the continuous production of VFA. Therefore, further research is needed to investigate the addition of buffering agents to maintain an appropriate pH, and enhance the activity of hydrolytic acidogenic bacteria.

Recent studies have reported that nanobubble could regulate key metabolic processes through interactions with biomolecular compounds of cells (Marcelino et al., 2023). On one hand, due to its small size and relative stability, nanobubble enhances the efficiency of transporting nutrients and gases (Wang et al., 2020). On the other hand, nanobubble could generate reactive oxygen species, which might be detrimental and beneficial for microorganisms (Chuenchart et al., 2021; Wu et al., 2021). Currently, the

detailed mechanisms of how nanobubble interacts with microorganisms remain unknown. Further investigation is needed to clarify whether nanobubble traverses into the cell, thus promoting or inhibiting the functions of microorganisms.

4. Conclusion

The effect of CO₂NW and O₂NW addition on improving soaking hydrolysis of rice straw was investigated. The SMY in digestate soaking with O₂NW for 3 d increased by 11.6% compared with the untreated group. But NW did not significantly increase SMY compared with DW soaking pretreatment. After 3-day of soaking, the VFA concentration of O₂NW treatment reached 7179.5 mg-HAc/L, and the TS degradation rate was 19.4%. Furthermore, the application of CO₂NW and O₂NW could accelerate the enrichment of *Enterobacter*. This study proposed that NW could enhance soaking hydrolysis effect and then accelerate subsequent biogas production by accelerating the microbial activity.

E-supplementary data for this work can be found in e-version of this paper online.

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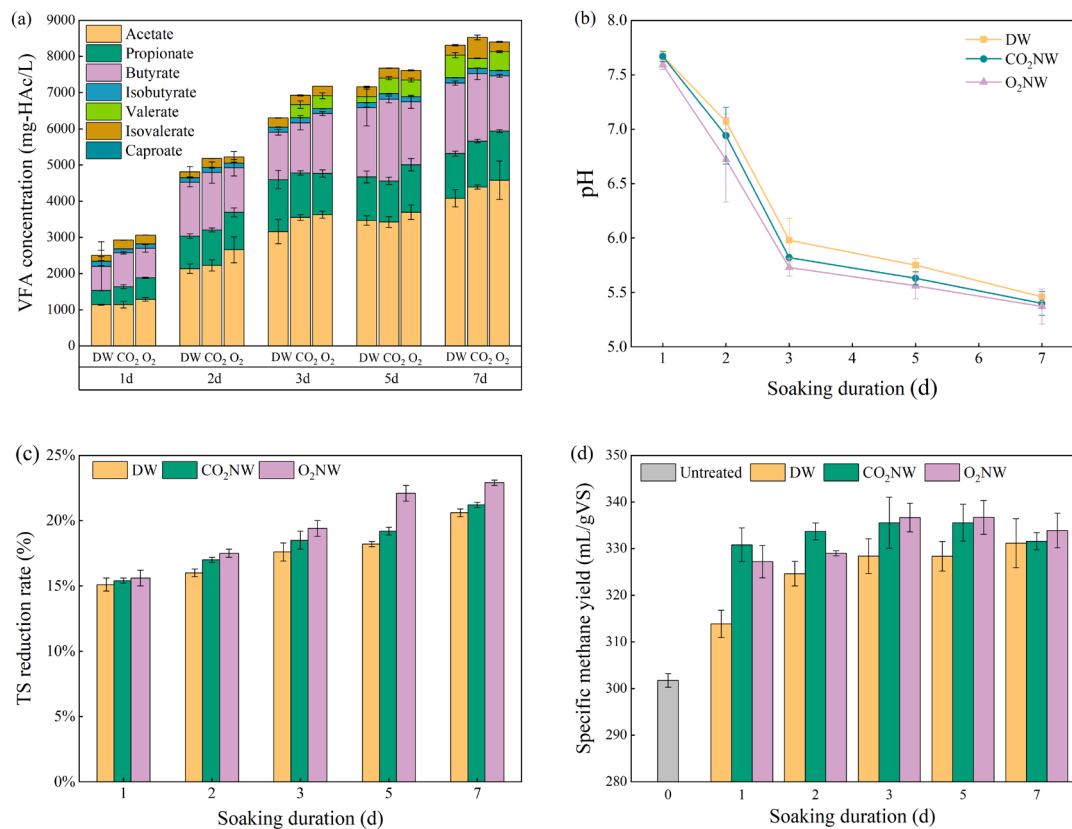


Fig. 1. The change of VFA concentration (a), pH (b), TS degradation rate (c), and specific methane yield (d) after soaking with different nanobubble water.

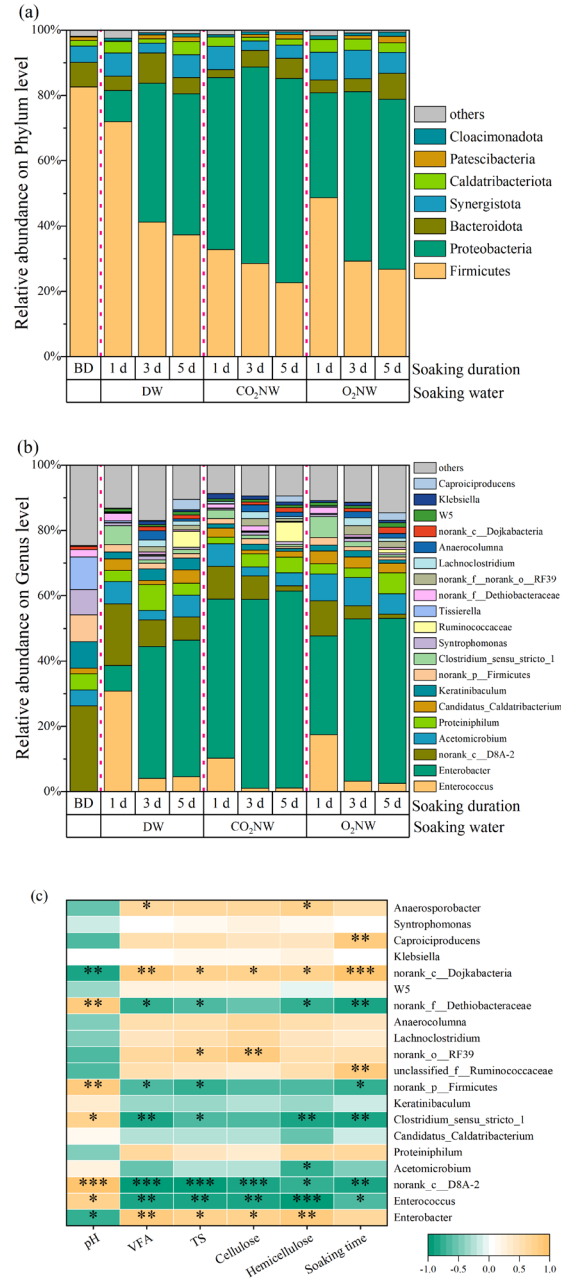


Fig. 2. Bacterial community after soaking at phylum level (a) and at genus level (b).

Correlation heatmap of environmental factors and bacteria in top 20 genera (c). (BD:

digestate; Intensity of color represents correlation coefficient; $0.01 < p < 0.05$, $0.001 < p$

< 0.001 and $p < 0.001$ are denoted as *, ** and ***, respectively.)



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