Improved methane production from corn straw using anaerobically digested sludge pre-augmented by nanobubble water

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Abstract

Nanobubble water (NBW) has been proven to efficiently improve methane production from organic solid wastes. However, the increase in reactor volume due to addition of NBW hinders its practical applications. In this study, anaerobically digested sludge was first pre-augmented by N_2-NBW and O_2-NBW using corn straw as sole substrate for methane production with electron transfer activity being monitored. 20%, 33% and 38% of cellulose and 29%, 35% and 35% of hemicellulose were reduced respectively from the control, N_2-NBW and O_2-NBW pre-augmented sludge reactors. N_2-NBW and O_2-NBW pre-augmented sludge reactors achieved methane yields of 127 and 142 mL/g-VS, about 10% and 22% higher than that from the control. Results show that use of NBW pre-augmented anaerobically digested sludge as inoculum can remarkably enhance methane yield from corn straw, providing a novel concept for NBW-based anaerobic digestion system with no increase of reactor volume and construction cost in practice.

Keywords: Corn straw; Anaerobic digestion; Methane production; Pre-augmentation; Nanobubble water

1. Introduction

The irregular climate change and energy crisis are considered to closely associate with excessive use of non-renewable fossil fuel, and biofuel may be a good solution for these issues (Robertson *et al.*, 2017). Biofuel generation from renewable resources can solve the energy shortage as well as the associated environmental problems. In recent years, agricultural wastes, such as crop straws have gained great attention due to their abundant distribution, carbon neutrality and ability to sidestep competition with food crops for land. They have also demonstrated significant potential to satisfy the feedstock targets for the advanced 2nd-generation biofuels (Cornejo *et al.*, 2019).

Anaerobic digestion (AD) of lignocellulosic biomass is a promising approach for bioenergy (mainly methane) production (Xu *et al.*, 2019). Corn straw is a typical agricultural waste that is produced in large quantities globally for food, feed and industrial applications. Corn productions in the US and China account for 40% and 20% of the world, respectively (Veljković *et al.*, 2018). In China, the annual production of corn straw is about 216 million tons, which shows high potential for methane production (Wei *et al.*, 2020). Although AD of lignocellulosic biomass has been widely applied to achieve the sustainable management of agricultural wastes, the low energy conversion efficiency limits its application mainly due to the following two aspects: 1) the low metabolic activity of microbes, and 2) the rigidity and recalcitrant structure of lignocellulose (Huang *et al.*, 2019).

To cope with the above problems, various pretreatments including physical, chemical and biological methods have been attempted to enhance biogas production from AD of lignocellulosic biomass. For physical methods, steam explosion pretreatment could decrease the hemicellulose content of corn straw by 28% and increase the neutral detergent solute by 24% (Shi *et al.*, 2019). Some chemical methods like phosphate addition could accelerate the biogasification process of rice straw particles and total solids or volatile solids reduction to a certain extent (Lei *et al.*, 2010). In addition, biological methods such as *Trichoderma reesei* addition could effectively remove lignin by 23% compared to the untreated rice straw (Mustafa *et al.*, 2016). However, the improvement of methane production is difficult to balance the additional energy consumption, high process cost and secondary pollution potentials due to the additives addition. Thus, environment-friendly methods are still urgently required to meet the sustainable development goals (SDGs).

Most recently, nanobubble water (NBW) has been proven to enhance methane production from AD of waste activated sludge and lignocellulosic biomass (Wang *et al.*, 2020; Yang *et al.,* 2019; Wang *et al.*, 2019a). NBW is defined as the water containing gas nanobubbles (NBs) with diameter less than 1000 nm that have some special physicochemical properties, such as negative surface charge, low buoyancy, high gas solubility (Lyu *et al*., 2019), rapid mass transfer rate (Agarwal *et al*., 2011) and better stability (dispersed in liquids or adhered to solid surfaces) (Ghadimkhani *et al.*, 2016). The NBs in NBW could improve the contact areas between the solid and liquid phases (Lyu *et al.,* 2019). The chemical reactions at the gas-liquid interface can be enhanced by injecting NBW rather than through normal aeration with macroscopic bubbles. The hydrophobic attractiveness force property of NBs enables them to adhere to solid surfaces, thus promoting the metabolism and chemical reactions (Wang *et al.*, 2020). In addition, the reactive oxygen species (ROS) produced by NBW are beneficial for vegetable seed germination, and the proper amount of ROS can increase the metabolism of cells (Liu *et al.*, 2016). The most advantageous aspect of NBW-based technology systems is that the additive, i.e. NBW addition does not bring about secondary pollution. NBW has been applied in many fields including food (Wu *et al.*, 2019), biomedicine (Hayakumo *et al.*, 2014) and agriculture (Ahmed *et al.*, 2018), due to its extremely high bioactivity (Agarwal *et al.*, 2011). The enhancement of NBW on biological activity can be mainly divided into two aspects, i.e. enzyme activity and microbial community. As Yang *et al.* (2019) reported, the activity of extracellular hydrolases (acid phosphatase, alkaline phosphatase, α-glucosidase and protease) was enhanced by 14-17% under NBW addition, promoting methane production from AD of waste activated sludge due to its enhanced hydrolysis. The higher water molecules mobility of NBW can also promote the hydrolysis of lignocellulosic biomass, providing abundant carbon and nutrient sources for the growth of microorganisms with resultant enriched microbial communities (Wang *et al.*, 2020).

Obviously, however, the shortcomings of the NBW-based AD technology are relating to its large reactor volume and then high construction cost. Therefore, the NBW-based AD systems need further improvements for its practical application. In this study, in order to increase the methane production from AD of lignocellulosic materials using NBW while at the same time with no increase in reactor volume, anaerobically digested sludge was firstly pre-augmented by N_2-NBW and O_2-NBW and then used as inoculum to produce methane from corn straw via AD process. Thus, a series of experiments were performed to identify the effects of NBW pre-augmentation on the activity of the lignocellulolytic microorganisms. The microbial activity during the pre-augmentation process and methane production efficiency were also explored. Comparative analysis was conducted between the NBW and deionized water (DIW) pre-augmented sludge as inoculum for methane production from AD of corn straw in addition to cellulose, hemicellulose and lignin reductions. Results from this study are expected to lay the foundation for practical application of NBW-based AD technology to effectively produce methane from organic solid wastes with a high lignocellulose content (corn straw in this study).

2. Materials and methods

2.1. Raw materials

Corn straw was collected from Japan Agricultural Co-operatives, Ibaraki, Japan. The harvested corn straw was washed with DIW and dried at 60±1^{o}C to constant weight, then passed through a cutting mill (WDL-1, Osaka Chemical Co., Ltd., Japan). The crushed biomass was sieved through a 0.6 mm pore size sieve and stored at room temperature in vacuum bags, which was used as the sole substrate for AD in this study.

The anaerobically digested sludge used in this study was sampled from Shimodate sewage treatment plant in Ibaraki, Japan. Before being used, the sludge was filtered through a 1 mm pore size sieve and centrifuged at 5000 rpm for 10 min to concentrate, and then digested at 35±1^{o}C for one week to remove the residual fibers and easily biodegradable substances. The sludge was stored anaerobically in a refrigerator at 4°C until further use. The characteristics of corn straw and anaerobically digested sludge are listed in Table 1. NBW was prepared as previously described (Yang *et al.*, 2019). O_2, N_2 and DIW were used for O_2-NBW and N_2-NBW production, respectively. O_2 (purity ⩾ 99.9995%) and N_2 (purity ⩾ 99.9995%) were purchased from Taiyo Nippon Sanso Co., Ltd., Japan. DIW was produced from a distillation unit (Elix, Merck Co., Ltd., Japan).

Table 1

2.2. Pre-augmentation of anaerobically digested sludge by DIW, N_2-NBW and O_2-NBW

The interaction between the digested sludge and NBW was explored during the long-term AD of corn straw. In this study, N_2-NBW and O_2-NBW were selected to pre-augment the anaerobically digested sludge. The total volume of the effective pre-augmentation reactors (600 mL serum bottles) was about 400 mL, which composed of 200 mL of anaerobically digested sludge and 200 mL of NBW or DIW. Corn straw was added into the bottles to achieve a feed-to-microorganisms (F/M) ratio of 1.5 (volatile solid (VS) basis) at the beginning of each pre-augmentation with DIW or NBW addition. The initial pH of the pre-augmentation reactor was adjusted to

7.0±0.05 by use of 2.0 M NaOH or HCl. Afterwards, all the pre-treatment bottles were sealed using rubber stoppers and purged with nitrogen gas for 5 min to create anaerobic condition and pressure balance with the atmosphere, which were then placed into a temperature-controlled incubator (35±1°C). The reaction residue was centrifuged at 5000 rpm for 10 min to remove the supernatant when methane production was less than 1% of the accumulated volume of methane during three consecutive days (Holliger *et al*., 2016), which was then used as the inoculum for the next step of pre-augmentation. Namely, the equal amounts of DIW or NBW and corn straw were added to repeat the above steps for 6 times, totally about 214 days for this pre-augmentation duration. All the experiments were conducted in triplicate. In the process of DIW or NBW pre-augmentation, 2 mL of reaction liquid was sampled from each bioreactor during each augmentation cycle (about 30 days) for the determination of electron transport system (ETS) activity.

2.3. Biochemical methane potential (BMP) tests

According to the protocol proposed by Angelidaki *et al*. (2009), BMP tests were conducted to assess the biodegradability of corn straw and methane production by using the above pre-augmented anaerobically digested sludge as inoculum. All the tests were performed in triplicate in closed Schott Duran serum bottles (100 mL). In these bottles, the added mass weight of pre-augmented anaerobically digested sludge as inoculum was 35 g, with an appropriate mass weight of corn straw to obtain an F/M of 1.5 (VS basis). In addition, to avoid detection error, the effective volume of each bottle was controlled at 60 mL by adding DIW. The BMP tests were performed under the same condition as the AD in section 2.2. Blank treatments were also carried out to eliminate the methane production from the inoculum only, which was anaerobically digested with DIW, N_2-NBW and O_2-NBW, respectively (Holliger *et al*., 2016). All the bottles were manually shaken twice at the fixed time points every day. Samples from every bottle were collected to analyze the total volatile fatty acids (TVFAs), pH, crystallinity degree, cellulose, hemicellulose and lignin contents, etc.

Net methane yield (mL/g-VS) from corn straw at all conditions was calculated as previously described (Wang *et al*., 2020). The BMP in this study was expressed as the volume of methane under standard conditions (1013 hPa, 273 K). A 20 mL graduated glass syringe directly connected to the headspace of each AD reactor was used to measure the biogas volume at lab condition (1013 hPa, 298 K). Then the methane volume (V) under standard condition was obtained by Eq. (1) (Lalak *et al*., 2016).

$$V=(273/T_{exp.})\cdot(P_{exp.}/1013)\cdot V_{exp.} \qquad (1)$$

where $T_{exp.}$ is lab temperature (298 K in this study); P_{exp} is the atmospheric pressure in the laboratory at lab temperature (1013 hPa in this study); $V_{exp.}$ is the methane volume obtained from the lab test, which was calculated by methane content and biogas volume from the experiment.

2.4. Analytical methods

2.4.1. Evaluation on AD performance

Total solids (TS) and volatile solids (VS) were measured according to the standard methods (APHA, 2012), and VS lost at 105±2°C was corrected by drying TS at 60±1°C to constant weight. Methane and hydrogen contents were measured by a gas chromatograph (GC-8A, Shimadzu, Japan) using nitrogen as carrier gas. The GC-8A was equipped with a thermal conductivity detector (80^{o}C) and a Porapak Q column (60^{o}C). For the analysis of volatile fatty acids (VFAs), 0.5 mL of reaction liquid was extracted using a 5 mL precision syringe with 0.55 mm diameter injection needle (24G NN-2425R, Terumo Co., Japan). The reaction liquid was centrifuged at 5000 rpm for 10 min and then filtered through a 0.22 μm microfiber filter. The pH of the filtrate was adjusted to 4 by 3% phosphoric acid solution before the VFAs analysis on a Shimadzu GC-14B/FID packed with Unisole F200 30/60 column. The injector and the column temperatures were set at 180 ^{o}C and 150 ^{o}C, respectively. The pressure of carrier gas (nitrogen) was maintained at 200 KPa.

2.4.2. Electron transport system (ETS) activity

The 2-(p-iodophenyl)-3(p-nitrophenyl)-5-phenyltetrazolium chloride-electron transport system (INT-ETS) method was used to analyze the microbial metabolic activity in NBW or DIW pre-augmented anaerobically digested sludge. ETS activity was measured according to the INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazole chloride) reduction method proposed by Zhang *et al.* (2018).

2.4.3. Major polymers quantification and structural characterization

All the samples from the reactors were respectively dried at 105±2^{o}C to constant weight followed by milling, and used for the determination of lignin, cellulose, hemicellulose contents and elemental content (C, H, O and N). After being hydrolyzed with 72% H_2SO_4 (w/w), lignin content was determined by gravimetric method. The liquid after hydrolysis was used to analyze glucose and reducing sugars, which were utilized for the estimation of cellulose and hemicellulose contents (Ververis *et al.*, 2007). Glucose was measured by a colorimetric micro-method (Mendel *et al.*, 1954), and reducing sugar was quantified with a dinitrosalicylic acid (DNS) colorimetric method (Miller, 1959). Elemental analysis of corn straw was performed on a CHN elemental analyzer (Perkin-Elmer 2400 II, USA).

The sample powders were also analyzed with a wide angle X-ray diffraction (D8 Advance, BrukerAXS, Germany) at 40 kV and 40 mA in the scanning angle of 10-40° (2θ) at a scanning speed of 1.0°/min, which can be used to estimate crystallinity. The crystallinity index (CrI) was calculated according to Eq. (2) (Park *et al.*, 2010).

$$\text{Crystallinity Index (CrI,\%)}=100 \times \{[I]_{crys}-[I]_{amor}\}/[I]_{crys} \qquad (2)$$

where $[I]_{crys}$ is the intensity for crystalline portion of biomass at 2θ=23°, and $[I]_{amor}$ is the intensity for amorphous portion of biomass at 2θ=19°, respectively.

2.5. Theoretical maximum methane yield from AD and biodegradability of corn straw

The theoretical methane production (TMP) can be estimated from the elemental composition (C, H, N and O) of corn straw by using the stoichiometric equations (Tarvin and Buswell, 1934), which was obtained according to Eqs. (3) and (4).

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

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

where a, b and c represent the mass fractions of organic carbon, hydrogen and oxygen in the dry matter of corn straw, respectively.

The anaerobic biodegradability (BD) of corn straw was calculated from the experimental methane production (EMP) divided by TMP (Raposo *et al.*, 2011), as shown in Eq. (5).

$$BD\ (\%) = 100 \times EMP/TMP \quad (5)$$

2.6. Model-based analysis

To further understand the effect of DIW or NBW pre-augmented anaerobically digested sludge as inoculum on the AD of corn straw, the Gompertz model (Eq. (6)) (Cossu *et al.*, 2016) was used to describe the methane yield data obtained from section 2.3.

$$M(t) = M \times \exp\{-\exp[1 + R_m e(\lambda - t)/M]\} \quad (6)$$

where, M (t) is the cumulative methane yield (mL/g-VS) at time *t*; M refers to the maximum methane yield (mL/g-VS); R_m is the maximum methane production rate (mL/(g-VS·d)); λ represents the lag time of methane production (d); *t* denotes the digestion duration (d); and *e* is the Euler's number, 2.718. M, R_m, and λ were simulated and estimated by the exponential equations using Origin 2018 software.

2.7. Statistical analysis

One-way analysis of variance was performed for statistical validation of the gained results. Origin 2018 software was used to estimate the significant difference among the treatments. Factors with a probability (*p* value) less than 0.05 were deemed as significant.

3. Results and discussion

3.1. Bioactivity of anaerobically digested sludge during pre-augmentation with DIW or NBW

The increase in reactor volume due to NBW addition hinders the applications of NBW-based AD technology for methane production from waste lignocellulosic biomass. Thus, in this study, NBW was firstly used to pre-augment anaerobically digested sludge with corn straw as the sole substrate in order to avoid the volume increase of the real AD reactors if being applied in practice. It is of the first importance to evaluate the activity of microorganisms during the pre-augmentation with NBW or DIW addition. Results show that N_2-NBW or O_2-NBW stored at 35±1^{o}C for 30 days can maintain its NBs concentration and zeta potential with no obvious difference. This phenomenon is similar with the result from Ghadimkhani *et al*. (2016). Thus, in order to utilize NBW to continuously enhance the biological activity of digested sludge during 214 days of augmentation, DIW, N_2-NBW and O_2-NBW were supplied to the reactors approximately every 30 days by taking NBs stability into consideration.

ETS is an effective indicator for the respiration of microorganisms and the potential performance of substrate removal in sludge (Wang *et al.*, 2016). As shown in Fig. 1, during the entire NBW or DIW pre-augmentation of anaerobically digested sludge, the ETS activities in all the reactors were enhanced along with the pre-augmentation times. Till the last pre-augmentation (the 6th pre-augmentation), the ETS activity in the reactors with DIW, N_2-NBW and O_2-NBW addition increased by 13.8%, 11.8% and 21.0% when compared to the 1st pre-augmentation. The highest

ETS activity was achieved in the O_2-NBW reactor (155.2±2.6 mg/(g·h)) after the 6th pre-augmentation, followed by N_2-NBW (149.1±3.5 mg/(g·h)) and DIW (134.8±3.1 mg/(g·h)) reactors. This observation suggests that the electron transport efficiency of microbial metabolisms was facilitated with the addition of NBW. O_2 can act as an electron acceptor to increase the EST activity (Gui *et al.*, 2017), which might be the reason for the highest ETS activity in the O_2-NBW pre-augmented anaerobically digested sludge. This observation indicates that NBW could increase the ETS activity of anaerobically digested sludge and enhance the microbial metabolism. In contrast, copper oxide nanoparticles treatment can greatly reduce ETS activity by 24-48% due to its toxic effects on microorganisms (Zhao *et al*., 2020). Silver nanoparticles may also decrease ETS activity, showing toxicity to microbial glucose metabolism (Liu *et al*., 2020). Results from this work suggest that the contaminant-free NBW is advantageous for the respiration of microorganisms.

Fig. 1

Under DIW or NBW pre-augmentation condition, the methane yield was also observed to increase along with the pre-augmentation times. In the 1st pre-augmentation, the methane yields from the DIW, N_2-NBW and O_2-NBW reactors showed no significant difference ($p > 0.60$), while the yields increased along with the pre-augmentation operation. During the last or 6th pre-augmentation, the O_2-NBW and N_2-NBW reactors produced more methane from corn straw, about 19.5% and 8.5%

respectively higher than the DIW reactor (the control group) after 214 days' pre-augmentation of anaerobically digested sludge. The changes in ETS activity were in agreement with the methane yields obtained in this study. Results from this work indicate that the microbial activity of anaerobically digested sludge was enhanced by NBW pre-augmentation, which can overcome the low metabolic activity of microbes. Still, future research is demanding on more direct evidence for changes in microbial community during the augmentation process by adding NBW. The enhanced metabolic activities of anaerobic microbes pre-augmented by NBW on corn straw degradation for methanogenesis were confirmed in the BMP tests by methane production and cellulose, hemicellulose and lignin reductions in the following sections 3.2.2 and 3.4.1, respectively.

3.2. Changes in crystallinity degree and digestibility of corn straw during BMP tests

3.2.1. Crystallinity degree

Crystallinity degree, an indicator for the digestibility of lignocellulosic biomass, can be used to reveal the relative extent of cellulose crystallinity of corn straw (Perrone *et al.*, 2016). Highly crystalline cellulose can be degraded by microorganisms during the hydrolysis stage, resulting in the decrease in crystallinity degree of biomass (Gupta *et al.*, 2016). It's necessary to investigate the effect of NBW pre-augmented anaerobically digested sludge on the decrease of corn straw crystallinity during BMP tests. In this study, the crystallinity degree of corn straw was determined by XRD (Table 2). After AD of corn straw, the largest reduction in cellulose crystallinity (21.1%) ($p < 0.01$) was detected in the O_2-NBW pre-augmented sludge reactors, followed by N_2-NBW (20.2%) (p =0.02) and DIW (17.4%) pre-augmented sludge reactors. There is no significant difference (p =0.14) in the reduction of cellulose crystallinity between the O_2-NBW and N_2-NBW reactors. A higher crystallinity reduction of corn straw reveals more crystalline cellulose was degraded during the AD process (Xu *et al.*, 2018). The decrease in crystallinity of corn straw could destroy the cellulosic crystalline areas and inner surface areas, subsequently improving the hydrolysis of corn straw. This observation might explain why more cellulose decomposition occurred in the reactors with NBW pre-augmented anaerobically digested sludge. Thus, use of NBW pre-augmented sludge as inoculum could enhance the degradation of corn straw.

Table 2

3.2.2. Reduction of cellulose, hemicellulose and lignin

In order to further understand the improvement of corn straw digestibility and the utilization of its major organic constituents, cellulose, hemicellulose and lignin contents were quantified during BMP tests with their reductions shown in Fig. 2. The DIW or NBW pre-augmented anaerobically sludge can achieve 20.4-38.4%, 28.8-35.4% and 8.4-9.4% of cellulose, hemicellulose, and lignin reductions, respectively during these tests. The observation also reflects that generally the NBW pre-augmented anaerobically sludge had a better performance on the degradation of corn straw. Specifically, all the BMP tests showed no significant difference in the reduction of lignin ($p > 0.05$), most probably attributable to the more recalcitrant nature of lignin than cellulose and hemicellulose (Li *et al.*, 2018). Compared to the DIW test group (control), the reductions in cellulose and hemicellulose were enhanced by 60.3-88.2% and 21.5-22.9%, respectively in the reactors with the NBW pre-augmented anaerobically digested sludge. As expected, the largest cellulose reduction was detected in the reactors with the O_2-NBW pre-augmented sludge ($p < 0.05$). These results imply that the anaerobically digested sludge augmented by O_2-NBW was enriched with microbial consortium that possess higher biodegradation capability of corn straw. The rigidity and recalcitrant structure of the lignocellulose can be destroyed by the NBW pre-augmented digested sludge. More corn straw can be decomposed and converted into soluble monomers. Thus the reductions of cellulose, hemicellulose and lignin can promote the degradation of lignocellulose and then improve intermediate products (VFAs and hydrogen) production for methanogenesis.

Fig. 2

3.3. Changes in *TVFAs and hydrogen during BMP tests*

3.3.1. TVFAs

During a typical AD process, conversion of macromolecule matters to soluble substrates is the first step, namely, the hydrolysis-acidification stage, which is also the limiting step of the AD of corn straw (Janke *et al.*, 2017). As the important intermediate products from the hydrolysis-acidification, TVFAs were detected as shown in Fig. 3a. Initially, the TVFAs increased rapidly in all the reactors, and the highest TVFAs were detected in the DIW reactors on day 6 (2337.3 mg/L), followed by N_2-NBW (1971.7 mg/L) and O_2-NBW (1747.2 mg/L) reactors on day 2. On day 18, almost no TVFAs production was detected in the O_2-NBW reactors, and a very small amount of TVFAs in the N_2-NBW and DIW reactors; from then onwards, very little TVFAs were detected in all the reactors. The trace amount of oxygen in AD might enhance the proliferation of hydrolytic bacteria, strengthening the hydrolysis and acidification to produce more TVFAs (Ruan *et al.*, 2019). Also, Wang *et al.* (2020) reported that micro-oxygen environment could enhance VFAs yield during the hydrolysis and acidification of cellulose under Air-NBW addition. It thus was expected that the trace amounts of oxygen in O_2-NBW could improve the production of VFAs during the hydrolysis of corn straw. However, lower TVFAs concentrations were detected in the O_2-NBW reactors, likely attributable to the higher consumption rate of TVFAs by the O_2-NBW pre-augmented sludge than TVFAs production rate from hydrolysis of corn straw. This observation implies that NBW pre-augmentation may enrich the methanogens in the anaerobically digested sludge, thus enhancing the methanogenic stage of AD. As a result, the O_2-NBW pre-augmented sludge reactors gained the highest methane yield, followed by the N_2-NBW and control groups. Further discussion could be found in section 3.4.

Besides, the oxidation of propionic acid and butyric acid can generate electrons that are transferred to protons to produce hydrogen (Tian *et al.*, 2019), providing a reasonable explanation for the reactors with O_2-NBW pre-augmented sludge to have a rapid consumption of VFAs. This is most probably due to the highest ETS activity gained by the O_2-NBW reactors that promoted the oxidation of VFAs to hydrogen. Results from this work show that anaerobically digested sludge pre-augmented by NBW could enhance VFAs production during the hydrolysis-acidification stage and then the consumption of VFAs to produce hydrogen for methanogenesis.

3.3.2. Hydrogen

Hydrogen as the major intermediate gaseous product during the BMP tests was monitored as shown in Fig. 3b. Hydrogen and carbon dioxide can be converted into methane by hydrogenotrophic methanogens (Amani *et al.*, 2010). As it can be seen, the hydrogen content was relatively high (2.7%, 4.1% and 4.8% in the DIW, N_2-NBW and O_2-NBW reactors, respectively) during the initial stage of AD; then its content gradually decreased to zero in all the reactors. NB has been claimed to generate reactive oxygen species (ROSs) when NB collapses, a cost-effective and non-chemical approach, which include singlet oxygen ($^1O_2$), hydroxyl radicals (•OH), and super-oxide anion radicals (O_2^-) (Lyu *et al.*, 2019). All of them have been proven to contribute the improved hydrogen yield from AD of organic compounds (Wang *et al.*, 2019b). In addition, O_2-NB favors the formation of free radicals compared to N_2-NB (Li *et al.*, 2009). These observations may explain why the highest hydrogen content was detected in the O_2-NBW reactors in this study. Generally, the activity of hydrogenotrophic methanogen can be enhanced under high hydrogen content condition, resulting in the accelerated consumption of hydrogen to produce methane. Therefore, a rapid consumption of hydrogen can be an indicative of a faster methane production during the subsequent methanogenesis (Ambuchi *et al.*, 2017).

Fig. 3

3.4. Methane production during BMP tests

3.4.1 Methane yield from AD of corn straw with anaerobically digested sludge pre-augmented by NBW or DIW

The cumulative methane yields from corn straw by using NBW or DIW pre-augmented sludge are shown in Fig. 4. Almost no gas was produced from the blank treatments (only the inoculum augmented by DIW, N_2-NBW and O_2-NBW), and there is no obvious difference in methane content in all the reactors. The methane yields from the NBW pre-augmented sludge reactors increased sharply before day 16 and reached a plateau afterwards. The final methane yield from the DIW pre-augmented sludge reactors was 115.9±3.5 mL/g-VS after 24 days' AD of corn straw. As expected, the highest methane yield, averagely 141.7±5.8 mL/g-VS ($p = 0.03 < 0.05$) was achieved in the O_2-NBW pre-augmented sludge reactors, followed by the N_2-NBW reactors (127.3±2.6 mL/g-VS) ($p = 0.03 < 0.05$), increasing by 22.3% and 9.8%, respectively when compared with the control reactors. Fu *et al*. (2016) claimed that microaeration could enhance methanogenesis of corn straw. When calcium peroxide was added into AD of lignocellulosic biomass, methane production could be enhanced by 11% (Eom *et al*., 2019). Xu *et al*. (2018) reported that *Bacillus Subtilis* micro-aerobic pretreatment of cellulosic material improved methane production and cellulose removal by 17.4% and 7%, respectively from AD of corn straw. Seen from this comparison analysis, it is certain that the NBW pre-augmented anaerobically digested sludge as inoculum could achieve the comparable enhancement effect on methane production from corn straw.

Fig. 4.

The mass fractions of organic C, H, O and N in the dry corn straw were determined as 39.5%, 5.7%, 35.9% and 1.9% (TS basis), respectively (Table 1). According to Eq. (4), the TMP of corn straw was calculated as 223.3 mL/g-VS. The BD value denotes the biodegradability of corn straw during the AD process according to Eq. (5). The highest BD was achieved in the O_2-NBW pre-augmented sludge reactors, about 63.5%, followed by N_2-NBW (57.0%) and DIW (51.9%) reactors (Table 2). These results show that the biodegradation of corn straw can be enhanced by the anaerobically digested sludge after NBW pre-augmentation, which has been further evidenced by the methane production from the BMP tests.

3.4.2 Gompertz model analysis

The methane yields from all the reactors were fitted to the Gompertz model, and the results are also summarized in Table 2. The Gompertz model parameters are usually used to better understand the methanogenesis process. The maximum methane yields from the model simulation were similar to the experimental data, indicating that the cumulative methane yield curve fitted well with the Gompertz equation. This is further supported by the high correlation coefficient (R^2=0.993-0.998). As for the O_2-NBW pre-augmented sludge reactors, the lag-phase time (λ) was the shortest among all the test reactors. As reported by Xu *et al*. (2018), a higher bacteria proportion is responsible for the shorter lag-phase time obtained. In this work it is hypothesized that O_2-NBW pre-augmentation may enrich the hydrolytic and methanogenetic bacteria in the anaerobically digested sludge, thus shortening the lag-phase time of AD, which is still under investigation. In addition, a shorter lag phase can improve the economic benefits of the AD system (Wang *et al.*, 2020). Results from kinetic modeling further indicate that use of NBW pre-augmented anaerobically digested sludge is promising for the enhancement of methane production from corn straw and its AD efficiency.

3.5 Implication of this study to practice

NBW has unique merits for the AD process, such as low cost, simple operation and strong pertinence. Compared with other pretreatment technologies, including acid, alkali and steam explosion, NBW-based AD system has low ecological risk with relatively low energy consumption. In this study, we first attempted NBW pre-augmentation of anaerobically digested sludge and then checked its effects on methane production from corn straw in lab-scale tests, which is also very important to better understand the fundamentals involved. NBW-based AD technology is still at its preliminary stage. Results from this study show that the NBW augmented anaerobically digested sludge could contribute to enhanced methane production from corn straw with no need of increasing reactor volume like previous studies. Future research should be directed to its great potentials for pilot- and/or full-scale AD systems, and their long-term practical application and commercial tests.

4. Conclusions

Anaerobically digestion sludge was subjected to NBW pre-augmentation to improve its bioactivity and methane production from corn straw via AD. The O_2-NBW pre-augmented sludge reflected the highest microbial bioactivity, achieving 22% increase in methane yield (141.7±5.8 mL/g-VS), and 88.2% and 22.9% increments in cellulose (38.4%) and hemicellulose (35.4%) reductions during AD of corn straw in comparison to the control (DIW reactors), respectively. This work demonstrates the concept and possibility of NBW pre-augmentation of anaerobically digested sludge and then the improvement of methane production from corn straw, targeting no increase of AD reactor volume in practice and sustainable management of lignocellulosic biomass.

CRediT authorship contribution statement

Xuezhi Wang: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Zhongfang Lei:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Writing - review & editing. **Kazuya Shimizu:** Formal analysis, Methodology, Supervision. **Zhenya Zhang:** Formal analysis, Methodology, Supervision. **Duu-Jong Lee:** Formal analysis, Writing - review & editing.

Supplementary materials

Supplementary data of this work can be found in online version of the paper.

Declarations of Interest

None

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References

1. Agarwal, A., Ng, W.J., Liu, Y., 2011. Principle and applications of microbubble and nanobubble technology for water treatment. Chemosphere 84 (9), 1175–1180.
2. Ahmed, A.K.A., Shi, X., Hua, L., Manzueta, L., Qing, W., Marhaba, T., Zhang, W., 2018. Influences of air, oxygen, nitrogen, and carbon dioxide nanobubbles on seed germination and plant growth. J. Agr. Food Chem. 66 (20), 5117–5124.
3. Amani, T., Nosrati, M., Sreekrishnan, T.R., 2010. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects-a review. Environ. Rev.

18, 255–278.

4. Ambuchi, J.J., Zhang, Z., Shan, L., Liang, D., Zhang, P., Feng, Y., 2017. Response of anaerobic granular sludge to iron oxide nanoparticles and multi-wall carbon nanotubes during beet sugar industrial waste water treatment. Water Res. 117, 89–94.
5. Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P., van Lier, J. B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59(5), 927-934.
6. APHA, 2012. Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association, Washington DC.
7. Cornejo, A., Alegria-Dallo, I., García-Yoldi, Í., Sarobe, Í., Sánchez, D., Otazu, E., Funcia, I., Gil, M.J., Martínez-Merino, V., 2019. Pretreatment and enzymatic hydrolysis for the efficient production of glucose and furfural from wheat straw, pine and poplar chips. Bioresour. Technol. 288, 121583.
8. Cossu, R., Morello, L., Raga, R., Cerminara, G., 2016. Biogas production enhancement using semi-aerobic pre-aeration in a hybrid bioreactor landfill. Waste Manage. 55, 83–92.
9. Eom, T., Chaiprapat, S., Charnnok, B., 2019. Enhanced enzymatic hydrolysis and methane production from rubber wood waste using steam explosion. J. Environ. Manage. 235, 231–239.
10. Fu, S.-F., Wang, F., Shi, X.-S., Guo, R.-B., 2016. Impacts of microaeration on the anaerobic digestion of corn straw and the microbial community structure. Chem. Eng. J. 287, 523–528.
11. Ghadimkhani, A., Zhang, W., Marhaba, T., 2016. Ceramic membrane defouling (cleaning) by air Nano Bubbles. Chemosphere 146, 379–384.
12. Gui, M., Chen, Q., Ni, J., 2017. Effect of sulfamethoxazole on aerobic denitrification by strain Pseudomonas stutzeri PCN-1. Bioresour. Technol. 235, 325–331.
13. Gupta, V.K., Kubicek, C.P., Berrin, J.-G., Wilson, D.W., Couturier, M., Berlin, A., Filho, E.X.F., Ezeji, T., 2016. Fungal enzymes for bio-products from sustainable and waste biomass. Trends Biochem. Sci. 41 (7), 633–645.
14. Hayakumo, S., Arakawa, S., Takahashi, M., Kondo, K., Mano, Y., Izumi, Y., 2014. Effects of ozone nano-bubble water on periodontopathic bacteria and oral cells-in vitro studies. Sci. Technol. Adv. Mat. 15 (5), 055003.
15. Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S., Baier, U., Bougrier, C., Buffiere, P., Carballa, M., de Wilde, V., Ebertseder, F., Fernandez, B., Ficara, E., Fotidis, I., Frigon, J.-C., de Laclos, H.F., Ghasimi, D.S.M., Hack, G., Hartel, M., Heerenklage, J., Horvath, I.S., Jenicek, P., Koch, K., Krautwald, J., Lizasoain, J., Liu, J., Mosberger, L.,

Nistor, M., Oechsner, H., Oliveira, J.V., Paterson, M., Pauss, A., Pommier, S., Porqueddu, I., Raposo, F., Ribeiro, T., Pfund, F.R., Stromberg, S., Torrijos, M., van Eekert, M., van Lier, J., Wedwitschka, H., Wierinck, I., 2016. Towards a standardization of biomethane potential tests. Water Sci. Technol. 74 (11), 2515–2522.

16. Huang, J., Zhu, Y., Liu, T., Sun, S., Ren, J., Wu, A., Li, H., 2019. A novel wet-mechanochemical pretreatment for the efficient enzymatic saccharification of lignocelluloses: Small dosage dilute alkali assisted ball milling. Energ. Convers. Manage. 194, 46–54.
17. Janke, L., Weinrich, S., Leite, A.F., Terzariol, F.K., Nikolausz, M., Nelles, M., Stinner, W., 2017. Improving anaerobic digestion of sugarcane straw for methane production: Combined benefits of mechanical and sodium hydroxide pretreatment for process designing. Energ. Convers. Manage. 141, 378–389.
18. Lalak, J., Kasprzycka, A., Martyniak, D., Tys, J., 2016. Effect of biological pretreatment of Agropyron elongatum 'BAMAR' on biogas production by anaerobic digestion. Bioresour. Technol. 200, 194–200.
19. Lei, Z., Chen, J., Zhang, Z., Sugiura, N., 2010. Methane production from rice straw with acclimated anaerobic sludge: Effect of phosphate supplementation. Bioresour. Technol. 101 (12), 4343–4348.
20. Li, P., Takahashi, M., Chiba, K., 2009. Degradation of phenol by the collapse of microbubbles. Chemosphere 75 (10), 1371–1375.
21. Li, W., Khalid, H., Zhu, Z., Zhang, R., Liu, G., Chen, C., Thorin, E., 2018. Methane production through anaerobic digestion: Participation and digestion characteristics of cellulose, hemicellulose and lignin. Appl. Energ. 226, 1219–1228.
22. Liu, S., Oshita, S., Kawabata, S., Makino, Y., Yoshimoto, T., 2016. Identification of ROS produced by nanobubbles and their positive and negative effects on vegetable seed germination. Langmuir 32 (43), 11295–11302.
23. Liu, S., Wang, C., Hou, J. Wang, P., Miao, L., 2020. Effects of Ag NPs on denitrification in suspended sediments via inhibiting microbial electron behaviors. Water Res. 171, 115436.
24. Lyu, T., Wu, S., Mortimer, R.J.G., Pan, G., 2019. Nanobubble technology in environmental engineering: revolutionization potential and challenges. Environ. Sci. Technol. 53 (13), 7175–7176.
25. Mendel, B., Kemp, A., Myers, D.K., 1954. A colorimetric micro-method for the determination of glucose. Biochem. J. 56 (4), 639–646.
26. Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31 (3), 426–428.

27. Mustafa, A.M., Poulsen, T.G., Sheng, K., 2016. Fungal pretreatment of rice straw with *Pleurotus ostreatus* and *Trichoderma reesei* to enhance methane production under solid-state anaerobic digestion. Appl. Energ. 180, 661–671.
28. Park, S., Baker, J.O., Himmel, M.E., Parilla, P.A., Johnson, D.K., 2010. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. Biotechnol. Biofuels 3, 10.
29. Perrone, O.M., Colombari, F.M., Rossi, J.S., Moretti, M.M.S., Bordignon, S.E., Carreira Nunes, C.D.C., Gomes, E., Boscolo, M., Da-Silva, R., 2016. Ozonolysis combined with ultrasound as a pretreatment of sugarcane bagasse: Effect on the enzymatic saccharification and the physical and chemical characteristics of the substrate. Bioresour. Technol. 218, 69–76.
30. Raposo, F., Fernandez-Cegrí, V., De la Rubia, M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M., Uellendahl, H., Wierinckm, I., De Wilde, V., 2011. Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. J. Chem. Technol. Biot. 86 (8), 1088–1098.
31. Robertson, G.P., Hamilton, S.K., Barham, B.L., Dale, B.E., Izaurralde, R.C., Jackson, R.D., Landis, D.A., Swinton, S.M., Thelen, K.D., Tiedje, J.M., 2017. Cellulosic biofuel contributions to a sustainable energy future: Choices and outcomes. Science 356 (6345), eaal2324.
32. Ruan, D., Zhou, Z., Pang, H., Yao, J., Chen, G., Qiu, Z., 2019. Enhancing methane production of anaerobic sludge digestion by microaeration: Enzyme activity stimulation, semi-continuous reactor validation and microbial community analysis. Bioresour. Technol. 289, 121643.
33. Shi, Q., Li, Y., Li, Y., Cheng, Y., Zhu, W., 2019. Effects of steam explosion on lignocellulosic degradation of, and methane production from, corn stover by a co-cultured anaerobic fungus and methanogen. Bioresour. Technol. 290, 121796.
34. Tarvin, D., Buswell, A.M., 1934. The methane fermentation of organic acids and carbohydrates. J. Am. Chem. Soc. 56 (7), 1751–1755.
35. Tian, H., Yan, M., Treu, L., Angelidaki, I., Fotidis, I.A., 2019. Hydrogenotrophic methanogens are the key for a successful bioaugmentation to alleviate ammonia inhibition in thermophilic anaerobic digesters. Bioresour. Technol. 293, 122070.
36. Veljković, V.B., Biberdžić, M.O., Banković-Ilić, I.B., Djalović, I.G., Tasić, M.B., Nježić, Z.B., Stamenković, O.S., 2018. Biodiesel production from corn oil: a review. Renew. Sustain. Energy Rev. 91, 531–548.
37. Ververis, C., Georghiou, K., Danielidis, D., Hatzinikolaou, D.G., Santas, P., Santas, R.,

Corleti, V., 2007. Cellulose, hemicellulose, lignin and ash content of some organic materials and their suitability for use as paper pulp supplements. Bioresour. Technol. 98 (2), 296–301.

38. Wang, D., Yang, X., Tian, C., Lei, Z., Kobayashi, N., Kobayashi, M., Adachi, Y., Shimizu, K., Zhang, Z., 2019a. Characteristics of ultra-fine bubble water and its trials on enhanced methane production from waste activated sludge. Bioresour. Technol. 273, 63–69.
39. Wang, D., Zhang, D., Xu, Q., Liu, Y., Wang, Q., Ni, B.-J, Yang, Q., Li, X., Yang, F., 2019b. Calcium peroxide promotes hydrogen production from dark fermentation of waste activated sludge. Chem. Eng. J. 355, 22–32.
40. Wang, W., Wang, S., Zhang, J., Hu, Z., Zhang, X., Sierra, J.M., 2016. Degradation kinetics of pentachlorophenol and changes in anaerobic microbial community with different dosing modes of co-substrate and zero-valent iron. Int. Biodeter. Biodegr. 113, 126–133.
41. Wang, X., Yuan, T., Guo, Z., Han, H., Lei, Z., Shimizu, K., Zhang, Z., Lee, D.-J., 2020. Enhanced hydrolysis and acidification of cellulose at high loading for methane production via anaerobic digestion supplemented with high mobility nanobubble water. Bioresour. Technol. 297, 122499.
42. Wei, Y.F., Yuan, H.R., Wachemo, A.C., Li, X.J., 2020. Anaerobic co-digestion of cattle manure and liquid fraction of digestate (LFD) pretreated corn stover: Pretreatment process optimization and evolution of microbial community structure. Bioresour. Technol. 296, 122282.
43. Wu, Y., Lyu, T., Yue, B., Tonoli, E., Verderio, E.A.M., Ma, Y., Pan, G., 2019. Enhancement of tomato plant growth and productivity in organic farming by agri-nanotechnology using nanobubble oxygation. J. Agr. Food Chem. 67 (39), 10823–10831.
44. Xu, H., Li, Y., Hua, D., Mu, H., Zhao, Y., Chen, G., 2019. Methane production from the anaerobic digestion of substrates from corn stover: Differences between the stem bark, stem pith, and leaves. Sci. Total Environ. 694, 133641.
45. Xu, W., Fu, S., Yang, Z., Lu, J., Guo, R., 2018. Improved methane production from corn straw by microaerobic pretreatment with a pure bacteria system. Bioresour. Technol. 259, 18–23.
46. Yang, X., Nie, J., Wang, D., Zhao, Z., Kobayashi, M., Adachi, Y., Shimizu, K., Lei, Z., Zhang, Z., 2019. Enhanced hydrolysis of waste activated sludge for methane production via anaerobic digestion under N_2-nanobubble water addition. Sci. Total Environ. 693, 133524.
47. Zhang, F., Hou, J., Miao, L., Chen, J., Xu, Y., You, G., Liu, S., Ma, J., 2018. Chlorpyrifos and 3,5,6-trichloro-2-pyridinol degradation in zero valent iron coupled anaerobic system:

Performances and mechanisms. Chem. Eng. J. 353, 254–263.

48. Zhao, S., Su, X., Wang, Y., Yang, X., Bi, M., He, Q., Chen, Y., 2020. Copper oxide nanoparticles inhibited denitrifying enzymes and electron transport system activities to influence soil denitrification and N_2O emission. Chemosphere 245, 125394.

Tables

Table 1 Characteristics of corn straw and anaerobically digested sludge used in this study.

Characteristics	Corn straw	Anaerobically digested sludge
pH	ND	7.27±0.01
Total solid (TS, %)	91.10±0.02	2.100±0.007
Volatile solid (VS, % of TS)	83.40±0.02	1.570±0.001
Organic C (% of TS)	39.5	ND
Organic H (% of TS)	5.7	ND
Organic O (% of TS)	35.9	ND
Organic N (% of TS)	1.9	ND
Crystallinity (%)	31.0±0.01	14.0±0.01
Cellulose (%)	32.5±0.8	14.3±1.7
Hemicellulose (%)	28.4±0.4	12.3±1.5
Lignin (%)	8.6±1.2	5.6±2.3

ND- not determined

Table 2

Methane production, crystallinity index, and parameters estimated from the modified Gompertz model by fitting to the experimental data.

Pre-augmented sludge reactors	TMP (mL/g-VS)	EMP (mL/g-VS)	BD (%)	Modified Gompertz model				Crystallinity reduction (%)
				M	R_m	λ	R^2	
DIW	223.3	115.9±3.5 c	51.9	122.5	8.4	1.0	0.998	17.4±0.08 [b]
N_2-NBW		127.3±2.6 b	57.0	136.2	9.9	1.4	0.993	20.2±0.54 [ab]
O_2-NBW		141.7±5.8 a	63.5	145.9	13.4	0.9	0.996	21.1±0.01 [a]

BD, biodegradability; DIW , deionized water; EMP, experimental methane production; M, simulated maximum methane yield (mL/g-VS); NBW, nanobubble water; R^2, correlation coefficient; R_m, maximum methane production rate (mL/g-VS/day); TMP, theoretical methane production; λ, lag phase time (day). Data are expressed as mean ± SD, and data with different superscript letters denote significant difference at $p < 0.05$.

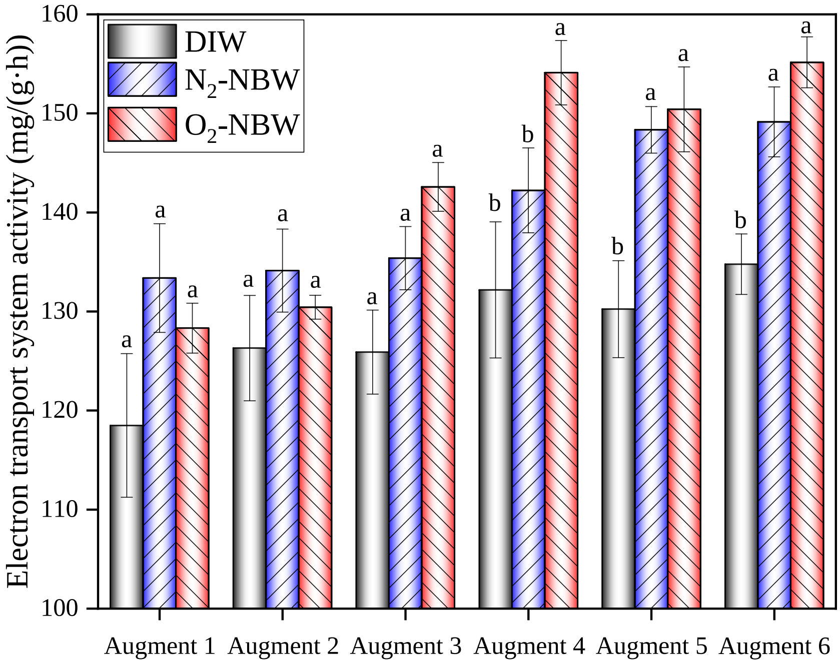

Fig.1. Changes in electron transport system activity during six times of pre-augmentation by DIW, N_2-NBW and O_2-NBW with corn straw as sole substrate. DIW, deionized water; NBW, nanobubble water. Data are expressed as mean ± SD, and the data with different superscript letters denote significant difference at $p < 0.05$.

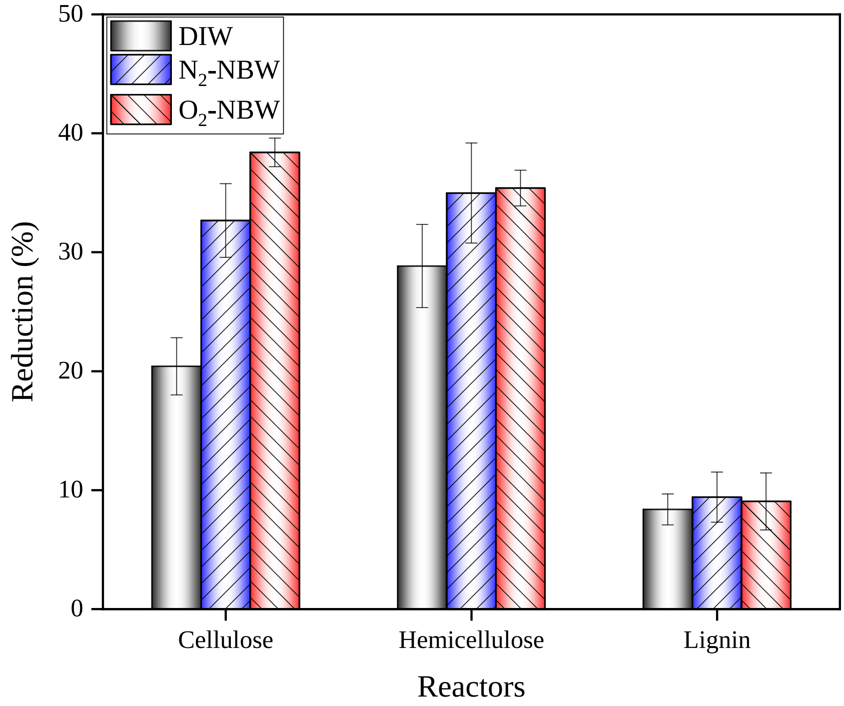

Fig. 2. Reductions of cellulose, hemicellulose and lignin by the DIW, N_2-NBW and O_2-NBW pre-augmented anaerobically digested sludge with corn straw as sole substrate. DIW, deionized water; NBW, nanobubble water.

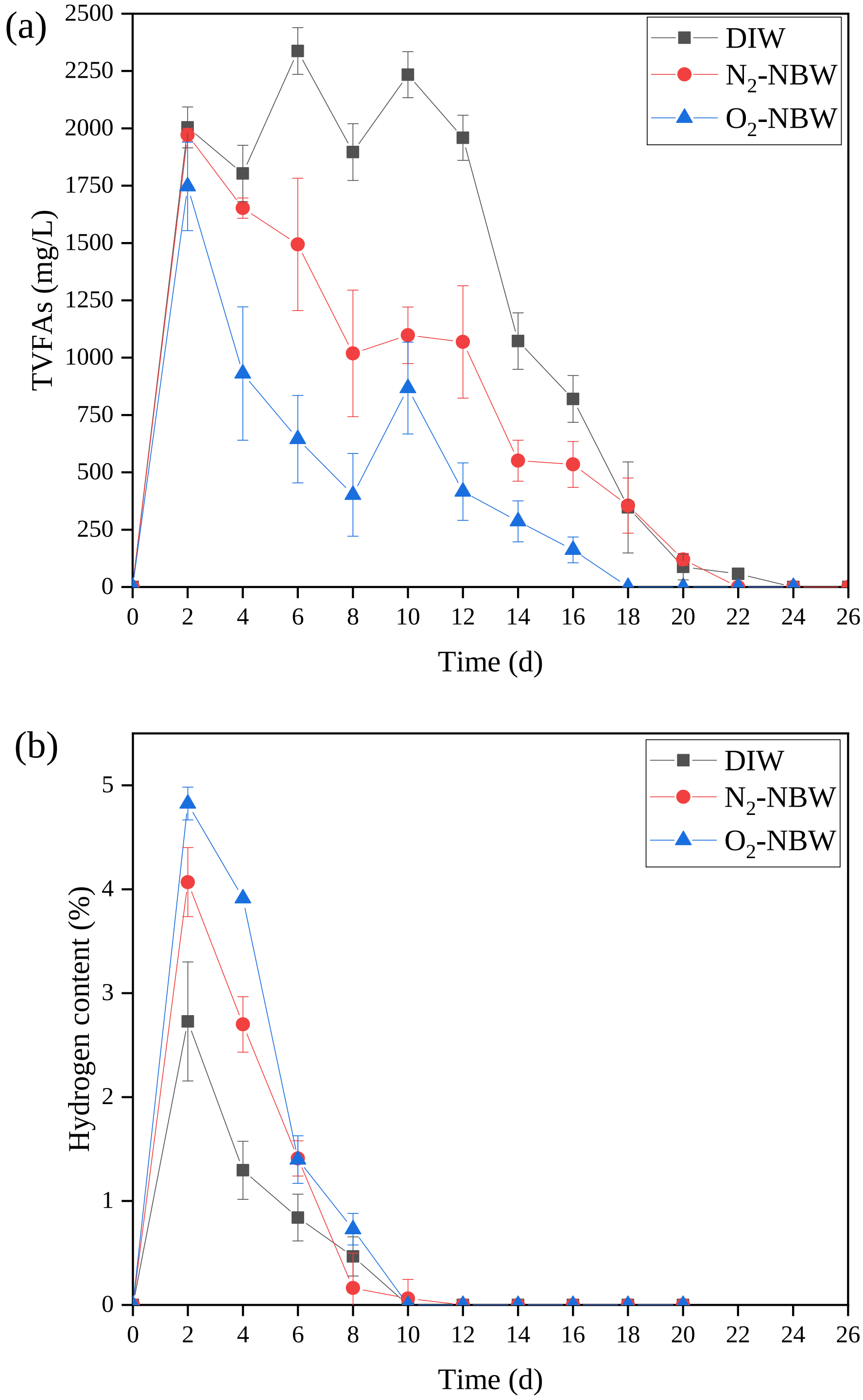

Fig. 3. Variations of total volatile fatty acids (TVFAs), a) and hydrogen content (b) in the DIW, N_2-NBW and O_2-NBW pre-augmented anaerobically digested sludge reactors during anaerobic digestion of corn straw. DIW, deionized water; NBW, nanobubble water.

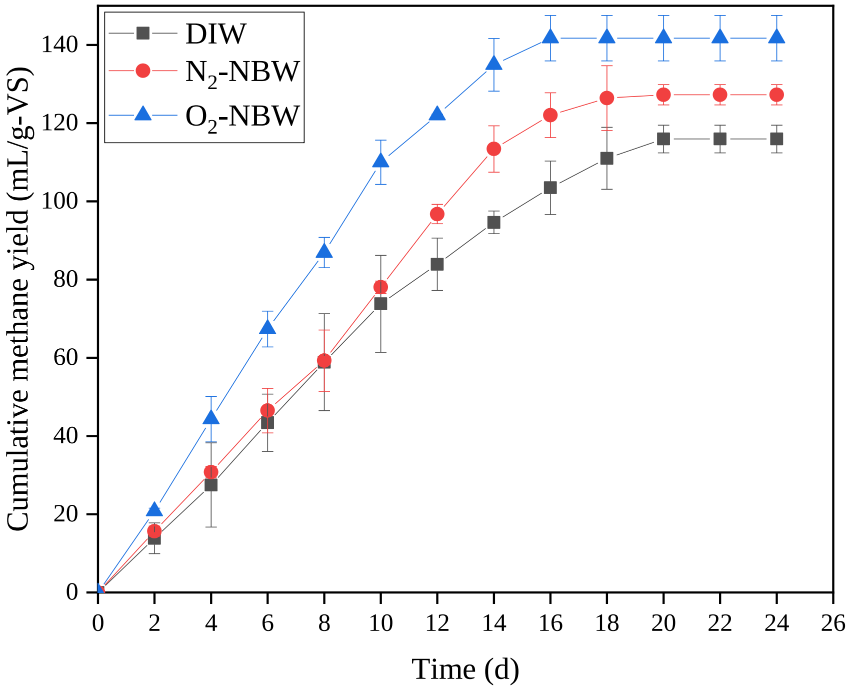

Fig. 4. Cumulative methane yields from the DIW, N_2-NBW and O_2-NBW pre-augmented anaerobically digested sludge reactors during the 24 days' anaerobic digestion of corn straw. DIW, deionized water; NBW, nanobubble water.