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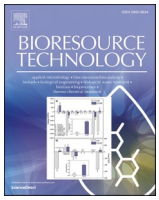

Impacts of free nitrous acid on stabilizing food waste and sewage sludge for anaerobic digestion

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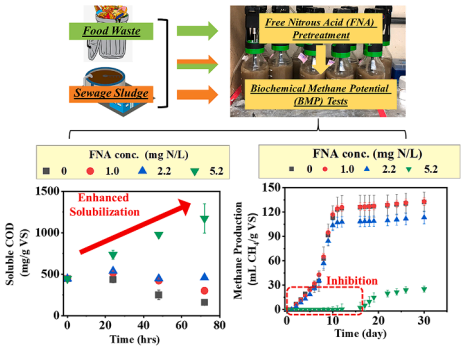
HIGHLIGHTS

- FNA pretreatment can significantly improve the solubilization of organic waste.
- pH change within the system can adversely impact the FNA pretreatment performance.
- Effective FNA concentration may decrease due to NO_2^- consumption by denitrifiers.
- FNA-pretreated organic waste does not always show a higher biogas production in AD.
- FNA at 1.0–5.2 mg FNA-N/L effectively controlled H_2S-odor in the organic wastes.

GRAPHICAL ABSTRACT



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ABSTRACT

This work investigated the effectiveness of free nitrous acid (FNA) in enhancing organic waste solubilization to improve biogas production in anaerobic digestion (AD). The results indicated that FNA pretreatment can enhance soluble organic content and control H_2S odor in tested organic wastes, including food waste, sewage sludge, and their combination. However, a significant decrease (>50 %) in FNA concentration was found in the reactors, possibly due to denitrifier-driven NO_2^- consumption. Biochemical methane potential (BMP) tests showed a 25 ± 8 % enhancement in CH_4 production in the reactors fed with mixed substrate pretreated with 2.9 mg FNA-N/L. However, the presence of NO_2^- (325.6–2368.0 mg N/L) in some BMP reactors, due to carryover from FNA pretreatment, adversely affected CH_4 production (>55 %) and prolonged lag time (>4.2 times). These findings are valuable for researchers and practitioners in waste management, offering insights for implementing FNA pretreatment to enhance the biodegradability of organic wastes in AD.

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1. Introduction

Population growth and urbanization have created critical challenge in managing solid waste (UN, 2022). Currently, approximately 60 % of food waste (FW) is disposed of in landfills, resulting in various problems, such as increased energy consumption for transportation, soil pollution, and greenhouse gas (GHG) emissions (EPA, 2023). As a sustainable alternative to landfill disposal, incorporating FW with sewage sludge (SS) in anaerobic digestion (AD) systems can offer benefits to both solid waste management and the wastewater sectors (Zhu et al., 2023). This includes maximizing the utilization of the AD capacity in existing water resource recovery facilities (WRRFs) and generating additional economic profits through tipping fees.

However, mixing FW with SS can increase the complexity of the organic wastes (Leitão et al., 2022). A considerable amount of research has been devoted to the influence of organic composition on the performance of AD, including issues such as undesirable C/N ratio, accumulation of long chain fatty acids, and elevated levels of ammonia (NH_3) (Li et al., 2017; Mirmohamadsadeghi et al., 2019). Therefore, pretreating organic substrates before AD has been used to enhance AD performance by improving substrate digestibility and facilitating mass transfer between different biological processes in AD. However, within these pretreatment methods, many have drawbacks concerning energy efficiency or economic feasibility. For instance, mechanical methods often entail high energy consumption, while chemical approaches require significant cost for adding large amounts of fossil fuel derived chemical agents (Tulun & Bilgin, 2019; Khanh Nguyen et al., 2021). Thermal hydrolysis pretreatment (THP), as a novel method for pretreating organic waste, has been used at full scale system with increased biodegradability, improved dewaterability, and higher digester organic loading rates (Yan et al., 2022). However, implementing THP often necessitates specialized reactors capable of handling high pressure and temperature, as well as skilled technicians. This can increase operational complexity and cost.

Recently, there has been a growing interest in the use of free nitrous acid (FNA) for sustainable wastewater management, driven by its bacteriostatic and bactericidal properties (Zuo et al., 2023). As FNA is the protonated form of nitrite (NO_2^--N), it can be sustainably produced from centrate ammonium (600–3300 mg NH_4^+-N/L) through partial nitrification (Si et al., 2018), making it a renewable resource recovered from onsite waste streams. The potential applications of FNA include controlling the odor and pipe corrosion in sewer systems, selectively eliminating undesirable biological processes in wastewater treatment, and facilitating algae harvesting in algae systems (Duan et al., 2020). Moreover, research has shown that implementation of FNA in SS management can improve sludge reduction, energy recovery, and pathogen removal (Zuo et al., 2023; Akaniro et al., 2024). Studies have shown that exposing waste activated sludge (WAS) to FNA (2–5 mg FNA-N/L) can enhance methane (CH_4) production by 13–48 %, reduce polymer use for dewatering, and lower the viscosity of sludge within AD (Meng et al., 2020; Szypulska et al., 2021). While the exact mechanisms of FNA in improving SS biodegradability remain unclear, researchers have proposed that FNA, along with reactive nitrogen and oxygen species (RNS and ROS) from the decomposition of FNA, can readily permeate cells and react with cellular components (Chislett et al., 2022). This interaction can result in the disintegration of the cell membrane and extracellular polymeric substrates (EPS), thereby releasing a significant portion of intracellular cellular organics into the liquid phase (Duan et al., 2020; Akaniro et al., 2024). These results demonstrate the promising potential of utilizing FNA to pretreat organic wastes with reduced energy and resource consumption.

However, current research mainly focuses on using SS as the sole substance in FNA pretreatment tests and evaluates effectiveness based on biogas yield using biochemical methane potential (BMP) tests (Duan et al., 2020; Zuo et al., 2023; Akaniro et al., 2024). The effectiveness of FNA in pretreating other organic wastes, such as FW, or mixed organic substances for co-digestion is unclear (Han et al., 2024). Additionally, understanding of the factors that could affect FNA concentration during pretreatment is still limited, which hampers the development of an effective strategy to optimize FNA pretreatment condition in large-scale system.

The objective of this study was to elucidate the relationship between FNA concentration, pretreatment durations, and organic wastes solubilization in both sole and co-substances systems. Three feedstocks, including FW, SS, and mixture of FW and SS, were tested, and the results were compared to examine if the performance of FNA pretreatment is influenced by the type and composition of organic wastes. The actual FNA concentration at different pretreatment times was determined by measuring the pH and NO_2^- concentration. The solubilization of substrates was evaluated by monitoring the soluble chemical oxygen demand (sCOD) following FNA pretreatment, and their digestibility in AD was assessed using BMP tests. In addition, the impacts of FNA on the production of H_2S were investigated using the mixture of FW and SS to examine the effectiveness of FNA pretreatment in controlling H_2S-related odor. The results obtained from this study are expected to provide insights into the optimal pretreatment condition required to maintain an effective FNA concentration. This will assist both researchers and practitioners in assessing the feasibility of using FNA to enhance stability and biogas production in AD, contributing to sustainable organic waste management practices.

2. Materials and methods

2.1. Organic wastes

Representative FW was prepared based on the compositions and ratios of FW determined by the U.S. Department of Agriculture (USDA) (Buzby et al., 2014), with the recipe provided in the supplementary material. The FW mixture was homogenized by blending using Xtreme 3.5hp Hi Powever Blender (Waring Commercial, Stamford, CT, USA), followed by sieving with a #10 mesh size (2 mm) to remove large particles. The homogenized FW was characterized and stored at −20°C until use. All the experiments conducted in this work used the FW prepared in the same batch to ensure the consistency of FW quality. The SS was collected from a returned activated sludge pipe in a local municipal WRRF and shipped to lab on ice on the same day of the experiment. The characteristics of FW and SS were summarized in the supplementary material.

2.2. Pretreatment experiments

Food waste (FW)-only pretreatment: The FW-only pretreatment experiments were conducted using three bench-scale reactors (named R1, R2, and R3) with 250 mL working volume. As shown in Table 1, 200 mL of FW was added to the reactor with MilliQ water added to meet the designed total volume of 250 mL. The HCl/NaOH (1 M) was used to adjust pH to 5.5 ± 0.1. An oxygen limiting condition was created by sealing the reactor and flushing the headspace of the reactor with He gas for five mins. A concentrated NO_2^- stock solution was prepared by using $NaNO_2$ to achieve the designed FNA concentrations, as specified in Table 1, in the reactors. The FNA concentration was calculated using the Eq. (1). The control reactor (R1) operated without NO_2^- addition, while the treatment reactors (R2 and R3) contained the same FNA concentration (5.3 mg FNA-N/L). A FNA concentration of 5.3 mg FNA-N/L was selected based on the literature that stated 5.0 mg FNA-N/L was an effective concentration for treating WAS (Meng et al., 2020; Romero-Güiza et al., 2019). The reactors were operated under two mixing conditions to investigate the role of mixing condition in the effectiveness of FNA pretreatment. The R1 (no FNA) and R3 (5.3 mg FNA-N/L) reactors were operated without mixing during the experiment to simulate the scenario of FW storage before AD. These reactors were manually mixed

Table 1
Setup conditions for experiments. FW, food waste; SS, sewage sludge; TS, total solids; VS, volatile solids; FNA, free nitrous acid; BMP, biochemical methane potential.

Experiment	Pretreatment Name	Pretreatment Duration	FW (mL)	SS (mL)	Total vol.** (mL)	TS (g/L)	VS (g/L)	Total Nitrite-N* (mg N/L)	FNA (mg N/L)	Selection for BMP	BMP Name
FW only	R1#	28 days	200	0	240	206.0 ± 0.7	204.8 ± 0.0	n.d.+	n.d.	✓	B1
	R2					193.0 ± 19.2	185.3 ± 27.5	941.0 ± 0.3	5.3 ± 0.4	✓	B2
	R3#					166.5 ± 18.2	156.3 ± 13.5	941.0 ± 0.2	5.3 ± 0.1	✓	B3
SS Only	R4	72 hrs	0	320	350	8.7 ± 0.1	6.8 ± 0.0	n.d.	n.d.	✓	B4
	R5							520.2 ± 87.3	2.9 ± 0.4	✓	B5
	R6							1150.0 ± 210.2	5.5 ± 1.1	✓	B6
	R7							2559.1 ± 540.4	17.6 ± 3.6	✓	B7
Mixture of SS and FW	R8	72 hrs	4	146	165	6.0 ± 0.1	5.6 ± 0.1	n.d.	n.d.	✓	B8
	R9							54.2 ± 0.1	0.3 ± 0.0	X	–-
	R10							188.0 ± 2.4	1.0 ± 0.0	✓	B10
	R11							321.7 ± 29.5	2.2 ± 0.9	✓	B11
	R12							1012.6 ± 26.5	5.2 ± 0.1	✓	B12

*, *Total nitrite-N = NO_2^--N + FNA-N (mg N/L)*
**, *MilliQ water was added to the reactor to meet the designed total volume.*
#, *Intermittent mixing: once per 7 days by flipping the reactor upside down for 2 mins.*
+, *n.d., not detected.*
The experiments used for obtaining the results were carried out in triplicate.

once every 7 days by flipping them upside down for 2 mins to obtain representative samples for analysis. Continuous mixing (110 rpm) was applied for R2 using a magnetic stir bar. The prepared reactors were incubated at 35 °C using heating blankets for 28 days. The 28-day pretreatment duration was chosen for this experiment based on previous studies which indicated that the time between FW collection and treatment in AD varied from days to over two months (Degueurce et al., 2020). Samples were collected every 7 days to track the changes in the FW in terms of pH, total solids (TS), volatile solids (VS), and soluble organic contents, including sCOD, soluble protein (sPN), and soluble polysaccharide (sPS).

$$FNA(mg\ \ N/L) = \frac{S_{NO_2^- - N}}{K_a \times 10^{pH}} \quad (1)$$

where the $S_{NO_2^- - N}$ is the dissolved NO_2^- concentration (mg NO_2^--N/L) and K_a is a function of temperature, T (°C), as described by the equation $Ka = e^{-2300/(273+T)}$ for a given temperature.

Sewage sludge (SS)-only pretreatment: The SS pretreatment experiments were conducted using four bench-scale reactors (named R4, R5, R6, and R7) with 350 mL working volume (Table 1). Similar reactor setup was used as the FW-only experiments described above except that SS collected from WRRF was used. The pH was controlled at 5.5 ± 0.1, and the oxygen condition was regulated using He flushing before adding NO_2^- stock solution. The control (R4) was operated without NO_2^- addition. Three FNA concentrations of 2.9, 5.5, and 17.6 mg FNA-N/L were used for testing in treatment reactors R5, R6, and R7, respectively. A continuous mixing condition was used by incubating the reactors on an orbital shaker at 120 rpm and 35 °C. Compared with the pretreatment duration of 28-day used in the FW-only pretreatment experiment, a shorter pretreatment duration of 3-day (72-hr) was tested in the SS pretreatment experiment. This decision was based on results from both the preliminary tests and previous work, which indicated that maximum FNA impacts on SS digestibility occurred within three days (Meng et al., 2020; Zahedi et al., 2017). During the 72-hr pretreatment experiment, samples were collected every 24 hrs for analyzing pH, sCOD, sPN, and sPS, while NO_2^-, TS, and VS were measured at the beginning and end of the experiment (0 and 72 hr).

Pretreatment of food waste (FW) and sewage sludge (SS) mixture: Five bench-scale reactors (named R8, R9, R10, R11, and R12) with 165 mL working volume were used in this experiment. Similar reactor setup was applied as described in the FW-only and SS-only pretreatment experiments, but using mixture of FW and SS at a ratio of 50:50 (based on VS content). As shown in Table 1, following the adjustment of pH to 5.5 ± 0.1, NO_2^- stock solution was added to R8, R9, R10, R11, and R12 to achieve five FNA concentrations of 0, 0.3, 1.0, 2.2, and 5.2 mg FNA-N/L, respectively. The oxygen condition was controlled by flushing with He for 2–3 mins to ensure anaerobic condition. During the 72-hr pretreatment, in addition to the samples analysis for pH, sCOD, sPN, sPS, NO_2^-, TS, and VS, the concentration of H_2S and SO_4^{2-} in liquid phase were measured for evaluating the potential of FNA pretreatment to control H_2S-related odor.

2.3. Biochemical methane potential (BMP) experiments

Pretreated substrates were collected at Hour 72 from all the pretreatment reactors except R9 to assess their energy production potential using BMP tests by following the established method (Meng et al., 2020). R9 was not selected in BMP test due to its NO_2^- and FNA concentrations quickly decreased to non-detectable level within 24 hrs in the pretreatment experiment. Consequently, R9 exhibited similar characteristics to the control group (R8), which did not have FNA added. The name of the reactor was updated to start with a letter "B" to indicate the reactor was part of the BMP phase of the experiments, as denoted in Table 1.

The inoculum used in the BMP test was obtained from a mesophilic inoculum reactor (113 L), with the characteristics summarized in the supplementary material. The digestion vessels (working volume = 400 mL) were filled with a mixture of inoculum and treated substrate at a ratio of 2:1 based on VS. The digestion vessels were adjusted to a pH of 7.0 ± 0.1 using 1 M HCl/NaOH, flushed with He for 2–3 mins to ensure anaerobic conditions, sealed, and incubated on an automatic mixing system (Gas Endeavour, Sweden) at 110 rpm and 35 °C. The pH, sCOD, and NO_2^- were measured at the start and end of the BMP test. Daily gas

measurements and composition analysis were taken until gas production largely ceased (approx. 30 days). A blank control group containing inoculum only was included to subtract the biogas production of inoculum from the total in the treatment groups. All experiments were performed in triplicate.

2.4. Analytical methods

The pH, TS, VS, and sCOD were measured using standard methods (APHA, 2017). The NO_2^- and NO_3^- concentrations were measured with a nutrient analyzer model AQ300 by Seal Analytical (Mequon, Wisconsin, USA). NH_4^+ was measured using Hach analyzer probe (Loveland, CO, USA). SO_4^{2-} concentrations were measured in an ion chromatograph (Dionex ICS-1100, Thermo Scientific) equipped with Dionex IonPac AS22-Fast analytical column (Li et al., 2016). H_2S concentration in the liquid phase was tested by colorimetric method using UV spectroscopy at 670 nm (Trueper et al., 1964). Different from sCOD measurement at 620 nm, the sPN and sPS measurements were performed using Bicinchoninic Acid (BCA) method with Pierce™ Rapid Gold BCA Protein Assay Kit (420 nm), and the Anthrone method (620 nm), respectively (Meng et al., 2020). The concentrations of sCOD, sPN, and sPS were determined and reported using different standards: COD, bovine serum albumin, and glucose, respectively. For all the measurements (except pH, TS, VS, and NH_4^+), collected samples were filtered (0.45 μm) prior to analysis. The composition of biogas (i.e., CH_4 and N_2) produced from BMP tests was measured by injecting 100 μL gas sample in an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-Molesieve column and thermal conductivity detector (TCD).

2.5. Data processing

The significance of differences in the results obtained from the treatment groups were determined by using MATLAB R2023a with the Statistics and Machine Learning Toolbox. The one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) test with the significance level of $p < 0.05$ was used as the statistical analysis. The reported values were given as means with standard errors. The cumulative CH_4 production data was fitted by the modified Gompertz equation (Eq. (2)) to investigate the potential inhibitory behavior due to presence of NO_2^-/FNA (Lu et al., 2019). During the calculation, the average CH_4 production in blank control (inoculum only) was subtracted from other groups to correct the results for presenting the total CH_4 production from substrate digestion.

$$B = B_0 \times \exp\{-exp\left[R_m \bullet e \bullet \frac{\lambda - t}{B_0} + 1\right]\} \quad (2)$$

where B is cumulative CH_4 production (mL/g VS); B_0 is the CH_4 production potential (mL/g VS, related to the substrate biodegradability); t is the time (day); R_m is the max. biogas production rate (mL CH_4/day/g VS); and λ is lag time (day).

3. Results and discussion

3.1. Impact of free nitrous acids (FNA) pretreatment on solubilization of organic wastes

As an indicator of the content level of bioavailable organic carbon, sCOD increase implies more cells and/or EPS are destroyed and become soluble substrates from particulate substrates (Zhang et al., 2015). Therefore, sCOD was monitored in this study to compare the solubilization of organic wastes before and after FNA pretreatment. Fig. 1 shows the results from a 28-day FNA pretreatment experiment using FW as the sole substrate. In R1 without addition of FNA, an increase of 11 ± 0 % in sCOD was observed from Day 0 to 14, followed by a significant decrease (39 %, $p < 0.01$) from Day 14 to 21. This result suggested that after adapted to the new environment, heterotrophic bacteria present in the system might grow and actively consume organic matter as a carbon source. The degradation of FW during the storage has been reported (Degueurce et al., 2020), and the results showed continued solid mass loss and gas emission over a 16-day experiment due to the biological activities. In comparison, R2 with FNA addition (5.3 mg FNA-N/L) and continuous mixing (110 rpm) showed the highest increase of sCOD. After 28 days pretreatment, sCOD in R2 increased by 44 ± 7 % (from 523.0 ± 0.7 mg sCOD/g VS on Day 0 to 752.8 ± 48.7 mg sCOD/g VS on Day 28, $p < 0.05$). Han et al. (2024) measured the dissolved organic carbon (DOC) after FNA pretreatment of FW for 24 hrs. They observed the DOC concentration increased by 15 % under the condition of 2.8 mg

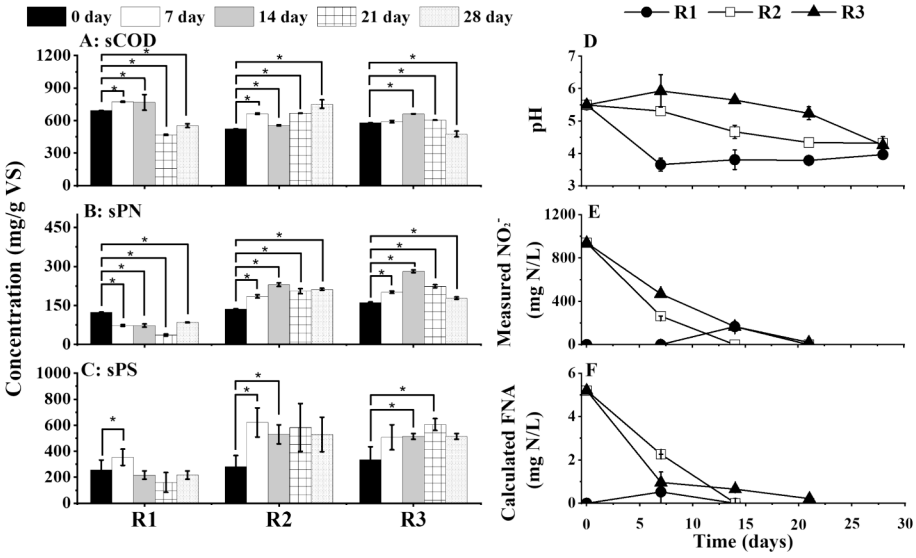

Fig. 1. The soluble chemical oxygen demand (sCOD) (**A**), soluble protein (sPN) (**B**), soluble polysaccharide (sPS) (**C**), pH (**D**), measured nitrite (mg-N/L) (**E**), and calculated effective free nitrous acid (FNA) concentration (mg-N/L) (**F**) from different pre-treatment groups with food waste (FW) as feed: R1, 0 mg FNA-N/L without mixing; R2, 5.3 mg FNA-N/L with continuous mixing at 110 rpm; and R3, 5.3 mg FNA-N/L with intermittent mixing. The intermittent mixing was conducted once every 7 days by flipping the reactor upside down for 2 mins. Error bars (shown if larger than the symbols) represent standard deviations of triplicate analysis, with significant differences from Day 0 shown. *, $p < 0.05$.

FNA-N/L and continuous shaking. A higher increase in dissolution of FW organic matters observed in R2 may be attributed to a longer pretreatment duration and the different composition of FW. However, in R3 with FNA addition (5.3 mg FNA-N/L) and intermittent mixing (once every 7 days by flipping the reactor upside down for 2 mins), the highest sCOD increase (14 ± 0 %) was observed on Day 14, followed by a decrease with the final sCOD concentration of 476.0 ± 26.9 mg sCOD/g VS on Day 28. The difference in sCOD changes between R2 (continuous mixing) and R3 (intermittent mixing) might be attributed to the distinct solid content (193.0 ± 19.2 g TS/L in R2 and 166.5 ± 18.2 g TS/L in R3), leading to different mass transfer conditions and system homogeneity. In this work, no significant VS destruction was found in the pretreatment processes (data not shown). This result is consistent with the previous study in which the increase in VS destruction after FNA pretreatment mainly occurred in AD rather than in the pretreatment step (Wei et al., 2018).

The relative changes in sPN and sPS in the reactors were analyzed by comparing them with their initial concentrations on Day 0 (Fig. 1B and 1C). The results indicated an increase in sPN by 10 – 73 % and sPS by 52 – 120 % in the reactors where 5.3 mg FNA-N/L was added (R2 and R3), while their concentrations showed a decreasing trend for sPN and no change for sPS in R1. This result is similar to Han et al. (2024) who observed the sPN and sPS increased by 27 % and 15 %, respectively, in the group with 2.9 mg FNA-N/L. As PN and PS are the main components in EPS of organic wastes, frequently responsible for binding cells and other particulate materials together (cohesion) and to the substratum (adhesion), an increase in sPN and sPS indicates a higher potential of solubilization of bound-EPS and contribute to the dissolved organic matters (Li et al., 2023; Yu et al., 2009).

In the SS-only pretreatment experiment, as shown in Fig. 2, higher FNA concentration applied in the pretreatment stage led to improved solubilization of SS. The sCOD measured in R5 (2.9 mg FNA-N/L) and R6 (5.5 mg FNA-N/L) at 72 hrs were 2.2 and 3.1 times higher than that in R4 (no FNA), respectively. The sCOD data in R7 at 72 hr was missing due to the sample damage during the preservation. These results aligned with those of Ma et al. (2015), who compared sCOD over 24 hrs of WAS pretreatment using different FNA concentrations (0.0–2.0 mg FNA-N/L). Their results demonstrated a two-fold increase in sCOD in the FNA-pretreated WAS (2.0 mg FNA-N/L). Results from sPN analysis (Fig. 2B) indicated a more substantial increase in sPN concentration in the FNA-added reactors compared to R4. Different initial sPN and sPS concentrations (at 0 hr) in the reactors were noticed. This discrepancy can be attributed to the prolonged pretreatment time (estimated to be 30 – 60 mins) spent for collecting and preparing samples to separate liquid from solids for subsequent sPN and sPS analysis.

The impact of different FNA concentrations (0–5.2 mg FNA-N/L) on the mixture of FW and SS at a ratio of 50:50 (based on VS) was also examined. The results depicted in Fig. 3 suggested that an effective FNA concentration for enhancing the solubilization of the mixed FW and SS was 5.2 mg FNA-N/L (R12), resulting in a 163 % increase in sCOD after a 72-hr pretreatment. In comparison, sCOD, sPN, and sPS in the control reactor (R8) and other FNA-added reactors (0.3–2.2 mg FNA-N/L) decreased by the end of the pretreatment. Research on the FNA pretreatment of co-substance is limited. Szypulska et al., (2022) used 8.0 mg FNA-N/L to pretreat WAS and foam for 48 hrs and observed a 5–10 folds increase in sCOD. In this study, it was observed that reactors with no or low FNA addition (R8 and R9) exhibited a more significant decrease in soluble organic contents (≥50 %) than those with higher FNA addition (R10 and R11). This might be caused by a combined effect of faster release of sCOD from cell lysis and EPS disruption, and greater inhibition of heterotrophic bacteria that consume organic matter in the reactors with FNA addition. It was reported that exposure of biomass to 1.2 mg FNA-N/L (174 mg NO_2^--N/L) for 4 hrs caused a significant biocidal effect with mortality of > 80 % (Zahedi et al., 2018). In another work, complete loss of denitrification activity was observed at 2.0 mg FNA-N/L for 1–2 days (Pijuan et al., 2012).

These results indicated FNA pretreatment with 5.2 mg FNA-N/L or higher for 24–72 hrs can effectively enhance the solubilization of both sole and co-substances, especially for SS. Although the exact mechanism of FNA on improving solubilization of the complex organic components are still not clear. Resent explorations indicated that exposure of organic wastes to FNA can trigger both membrane lysis and EPS decomposition, resulting in decreasing cell viability, increasing soluble and loosely bound EPS (S- and LB-EPS), and releasing bound water (Liu et al., 2020; Wu et al., 2018; Zhao et al., 2016). However, variations in pH and NO_2^- concentration within reactors were observed under laboratory environment. Considering the higher complexity of organic waste composition and the operational conditions in full-scale system, further studies

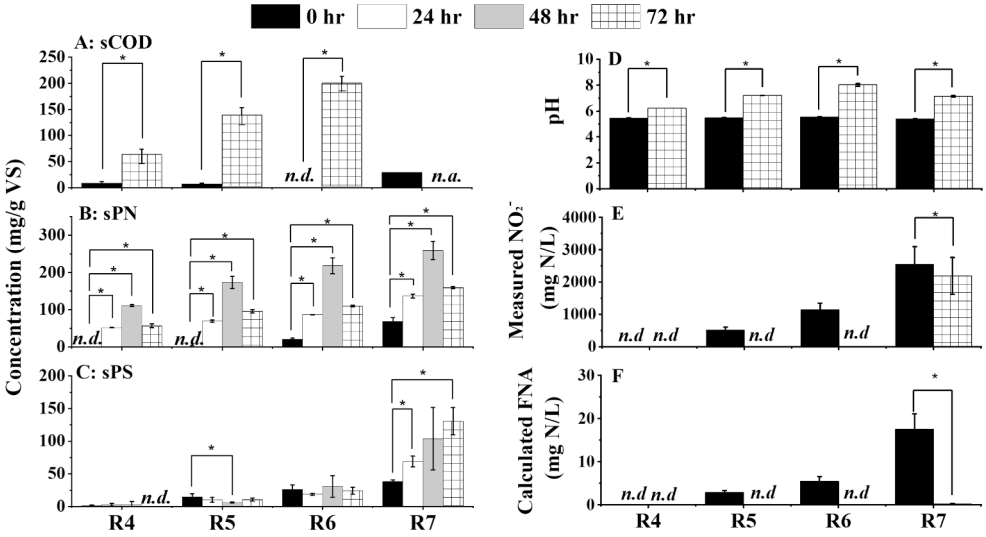

Fig. 2. The soluble chemical oxygen demand (sCOD) (**A**), soluble protein (sPN) (**B**), soluble polysaccharide (sPS) (**C**), pH (**D**), measured nitrite (mg-N/L) (**E**), and calculated effective free nitrous acid (FNA) concentration (mg-N/L) (**F**) from different pre-treatment groups with sewage sludge (SS) as feed: R4, 0 mg FNA-N/L; R5, 2.9 mg FNA-N/L; R6, 5.5 mg FNA-N/L; and R7, 17.6 mg FNA-N/L. Different initial sPN and sPS concentrations (at 0 hr) in the reactors, especially R6 and R7, can be attributed to the prolonged pretreatment time (estimated to be 30 – 60 mins) for collecting and preparing samples to separate liquid from solids for sPN and sPS analysis. *n.d.* indicates the measured value was below the detection limit for the method used. *n.a.* indicates the data was not collected due to damage of the samples. Error bars (shown if larger than the symbols) represent standard deviations of triplicate analysis, with significant differences from Day 0 shown. *, $p < 0.05$.

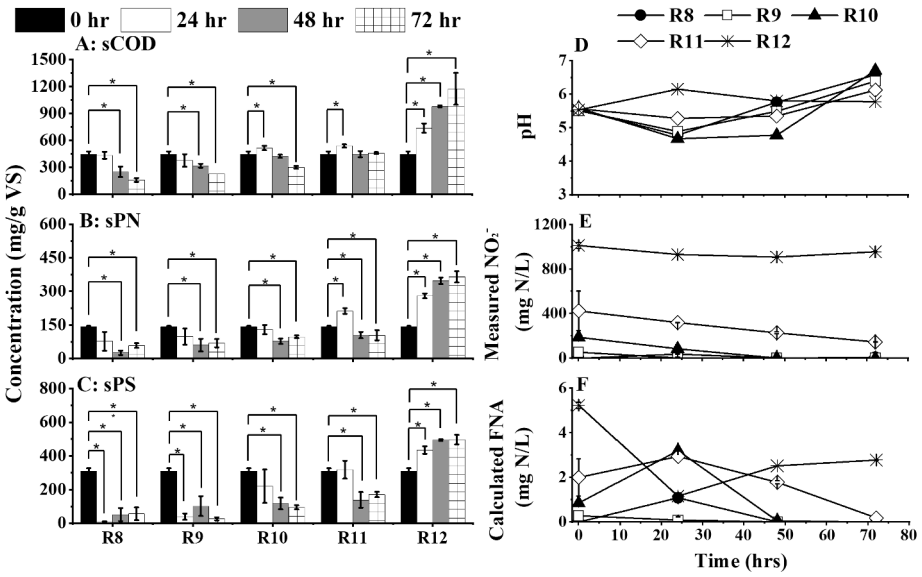

Fig. 3. The soluble chemical oxygen demand (sCOD) (**A**), soluble protein (sPN) (**B**), soluble polysaccharide (sPS) (**C**), pH (**D**), measured nitrite (mg-N/L) (**E**), and calculated effective free nitrous acid (FNA) concentration (mg-N/L) (**F**) from different pre-treatment groups with food waste and sewage sludge (FW:SS = 50:50, % volatile solids (VS)) as feed and various initial FNA concentrations during the 72-hr pretreatment. R8, 0 mg FNA-N/L; R9, 0.3 mg FNA-N/L; R10, 1.0 mg FNA-N/L; R11, 2.2 mg FNA-N/L; and R12, 5.2 mg FNA-N/L. Error bars (shown if larger than the symbols) represent standard deviations of triplicate analysis, with significant differences from Day 0 shown. *, $p < 0.05$.

are critical to comprehend the changes and maintenance of effective FNA concentration during the pretreatment process.

3.2. Effective free nitrous acids (FNA) concentration during the pretreatment process

The effective FNA concentration in each reactor was calculated by using directly measured NO_2^- concentration and pH at each sampling time. As shown in Fig. 1D, the pH in R1 (no FNA) dropped from 5.5 to 3.7 within seven days, indicating acidification due to microbial activities (Tang et al., 2023). In contrast, R2 and R3 with FNA addition showed a relatively stable pH during the initial seven days, followed by a graduate decrease, reaching a final pH of 4.3 on Day 28 in both reactors. NO_2^- concentration in R2 and R3 were also monitored (Fig. 1E), with more than 97 % of added NO_2^- disappeared on Day 21. As the result of changes in both pH and NO_2^-, calculated effective FNA concentration decreased from 5.3 mg FNA-N/L (Day 0) to 2.3 mg FNA-N/L (R2) and 1.0 mg NO_2^--N/L (R3) within seven days, respectively, while the reduction of FNA reached 100 % (R2) and 96 % (R3) on Day 21 (Fig. 1F).

A decrease in NO_2^- and FNA concentration was also observed in the reactors fed with SS (Fig. 2E and 2F) and mixture of FW and SS (Fig. 3E and 3F). A significant reduction (66–100 %) of NO_2^- and FNA was detected after 72 hr pretreatment in R5, R6, R9, R10, and R11. The disappearance of a substantial proportion of the added NO_2^- during the pretreatment was also reported by other studies (Akaniro et al., 2024; Romero-Güiza et al., 2019; Zahedi et al., 2018). For instance, Zahedi et al. (2018) detected a disappearance of 50–100 mg NO_2^--N/L after 4 hrs FNA pretreatment of WAS. The decrease in NO_2^- and FNA concentration during the pretreatment might be attributed to two reasons: 1) added NO_2^- was trapped in organic materials (e.g., EPS) in the reactor (Zahedi et al., 2018); and 2) added NO_2^- was denitrified in the presence of sCOD and low FNA concentration (Akaniro et al., 2024; Jiang et al., 2016).

For comparison, FNA concentration in R7 decreased from 17.6 mg FNA-N/L (0 hr) to 0.3 mg FNA-N/L (72 hr), while most (86 %) of the initially added NO_2^- (2559.1 ± 540.4 mg NO_2^--N/L) remained in the system. This was primarily due to the fluctuation in pH: an increase in pH can result in decrease in FNA concentration (see Eq. (1)). Similarly, after 72-hr pretreatment, the reductions in NO_2^- and FNA in R12 with initial FNA concentration of 5.2 ± 0.1 mg FNA-N/L (1013.2 ± 27.3 mg NO_2^--N/L) were 5 ± 0 % and 47 ± 0 %, respectively. The improvement of SS solubilization by NO_2^- has been reported (Lu et al., 2019; Sun et al., 2018). Sun et al. (2018) assessed the solubilization by monitoring DOC, sPN, and sPS in WAS after 24 hrs treatment using freezing alone and combined freezing + NO_2^-. Their results showed NO_2^- at 200.0 mg/L (FNA of 0.3 mg/L) was 1.5 times more effective in solubilizing particulate organics in WAS than the control without NO_2^-. In another work, Zahedi et al. (2018) concluded that the effectiveness of the FNA pretreatment on SS digestibility was linked to the nitrite levels applied rather than the FNA concentration only. Therefore, to identify the major contributor, NO_2^- or FNA, to the solubilization of SS in R7 and R12, it is important to know how long the designed FNA concentration was maintained during the pretreatment.

These results suggested that heterotrophic biological processes, such as denitrification, which remained active under low NO_2^-/FNA condition might consume both carbon sources and added NO_2^--N. Denitrifiers were reported to exhibit a high tolerance to NO_2^- (up to 2000 mg NO_2^--N/L), the reduction of NO_2^-/FNA by denitrifiers in the presence of high content of organic carbon source will impair the effectiveness of FNA pretreatment in the system (Akaniro et al., 2024; Zahedi et al., 2018; Glass et al., 1997). Previous work conducted by Ma et al. (2015) compared the carbon source from raw WAS and FNA-pretreated WAS for denitrification, and their results showed that FNA pretreatment enhanced denitrification by 76 %. This might explain why recovered viable cells (55 %) was observed after 9-hr exposure to 4.6 mg FNA-N/L in comparison to less than 10 % of viable cells after 2 and 5-hr exposure reported by Zahedi et al. (2016). Therefore, maintaining a sufficient NO_2^-/FNA concentration to inhibit the biological processes associated with nitrogen and organic carbon is critical to ensure the effectiveness of FNA pretreatment, particularly for full-scale reactors during long-term operation.

3.3. Impacts of free nitrous acids (FNA) pretreatment on H_2S-Odor control

The production of H_2S by SRB is an important source of odor and health hazards in the environment. Accumulation of H_2S in WRRFs can result in severe problems, such as corrosion of concrete and piping (Jiang et al., 2016). As shown in Fig. 4, the concentrations of inorganic

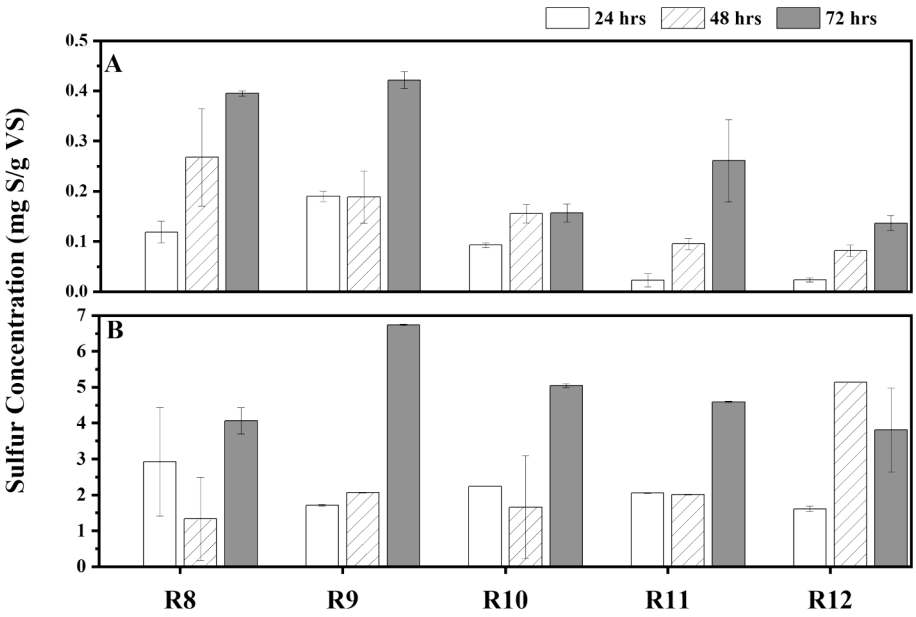

Fig. 4. The concentrations of H_2S-S (A) and SO_4^{2-}-S (B) from different pre-treatment groups with food waste and sewage sludge (FW:SS = 50:50, % volatile solids (VS)) as feed and various initial free nitrous acid (FNA) concentrations during the 72-hr pretreatment. R8, 0 mg FNA-N/L; R9, 0.3 mg FNA-N/L; R10, 1.0 mg FNA-N/L; R11, 2.2 mg FNA-N/L; and R12, 5.2 mg FNA-N/L. Error bars (shown if larger than the symbols) represent standard deviations of triplicate analysis.

sulfur species (H_2S and SO_4^{2-}) in the reactors fed with mixture of FW and SS (50:50, based on VS) at different initial FNA concentrations were analyzed during the 72-hr pretreatment. An increase in H_2S concentration, from 0.1 ± 0.0 mg S/g VS at 24 hr to 0.4 ± 0.0 mg S/g VS at 72 hr, was detected in R8 without FNA addition. This change was similar to R9 with 0.3 mg FNA-N/L (54.2 ± 0.1 mg NO_2^--N/L), indicating no biocidal effect on SRB at this added FNA concentration. When a higher FNA concentration (0.9–5.2 mg FNA-N/L) was applied, less accumulation of H_2S was observed in the reactors (R10, R11, and R12). However, even in R12 (5.0 mg FNA-N/L), which had the lowest H_2S accumulation (0.1 ± 0.0 mg S/g VS) at the end of 72-hr pretreatment, a gradual increase trend of H_2S concentration was noticed (from non-detected at 24 hr to 0.1 ± 0.0 mg S/g VS at 72 hrs, $p < 0.05$). This result was different from those of Ben-Dov et al. (2016), who reported 1.1–7.0 mg NO_2–N/L and 0.001–0.008 mg FNA-N/L effectively inhibited H_2S production through competing for the sulfite reductase enzyme and the release of reactive nitrogen species. The reduced effectiveness of NO_2^-/FNA and the accumulation of H_2S in this study might be attributed to the disappearance of NO_2^-/FNA during the pretreatment, as shown in Fig. 3E and 3F. These results suggested that pretreatment of mixed FW and SS with 0.9 – 5.2 mg FNA-N/L can provide the best control to maintain a low H_2S accumulation (<0.2 mg S/g VS) for 24–48 hrs. No significant difference was observed in different reactors for SO_4^{2-} (Fig. 4B). The increase in SO_4^{2-} within reactors at 72 hrs is suspected to be caused by the release of inorganic sulfur from cell and associated EPS.

3.4. Digestibility assessment of free nitrous acids (FNA)-Pretreated substrates

To reveal the effectiveness of FNA pretreatment in enhancing the digestibility of FW, SS, and their mixture, samples collected at 72 hrs from previous pretreatment experiments (except R9) were used for BMP tests (as shown in Table 1). The cumulative CH_4 production data was fitted using a modified Gompertz model (Eq. (2)), and the fitting curves and corresponding kinetic parameters were provided in the supplementary material. In B1 fed with FW from R1 without FNA pretreatment, the CH_4 production potential (B_0) was 359.9 ± 16.5 mL CH_4/g VS. This value is close to the reported range of FW (385–627 mL CH_4/g VS_{FW}) by Li et al. (2017). In comparison, B4 fed with SS without FNA-pretreatment showed a lower B_0 (142.8 ± 1.6 mL CH_4/g VS), close to the range of 147–206 mL CH_4/g VS_{WAS} reported by Zhang et al. (2019). The distinct portion of components in different organic wastes may attribute to different biogas production potential (Akaniro et al., 2024).

Compared with B1, no significant differences ($p > 0.05$) in total biogas and CH_4 production were observed in B2 and B3, fed with pretreated FW (Figs. 5A and 5B). Different results were observed in reactors (B5, B6 and B7) fed with pretreated SS. Compared with B4 (B_0 = 142.8 ± 1.6 mL CH_4/g VS), a higher B_0 (179.7 ± 11.2 mL CH_4/g VS, $p < 0.05$) was obtained in B5 fed with SS pretreated by 2.9 mg FNA-N/L (R5). The biodigestibility enhancement (25 ± 8 %) of SS with 72-hr FNA pretreatment in B5 was consistent with previous studies that reported an increase in CH_4 production in the range of 20 – 30 % from WAS pretreated with 1.9–3.6 mg FNA-N/L (Wang et al., 2013; Zahedi et al., 2017). Two potential reasons might explain the different effectiveness of FNA pretreatment in FW and SS: 1) FW contains a large amount composition that are already readily biodegradable and may not be further enhanced by FNA pretreatment; and 2) the lignocellulosic fractions in FW (such as cellulose, hemicelluloses, and lignin contained in fruits and vegetables) may be more resistant to FNA pretreatment (Xu et al., 2018; Zoghlami & Paës, 2019). The composition difference between FW and WAS may lead to a diminished enhancement of FW biodigestibility after FNA pretreatment than biosolids from wastewater treatment (Calderon et al., 2021; Wei et al., 2018). To elucidate the correlation between FW digestibility and FNA, further research should be conducted by characterizing the compositions of FW and assessing responses of different compositions to FNA.

The pretreated SS with different FNA concentrations also showed diverse outcomes. Compared with B5, B6 and B7 that used SS pretreated for 72-hr by higher FNA concentrations (5.5 mg FNA-N/L in R6 and 17.6 mg FNA-N/L in R7) did not further enhanced biogas production from SS (Figs. 5D and 5E). In particular, B7 showed a significant decrease in B_0 (64.0 ± 0.9 mL CH_4/g VS, reduced by 55 %) and an increase in lag time (λ = 19.6 ± 0.1 days, increased by 4.2 times) compared to B4 ($p < 0.01$). There was no gas production detected in B7 in the first 20 days of the BMP tests. The initial inhibition of CH_4 production in BMP tests was also observed by Han et al. (2024). Their results showed that addition of FNA-pretreated FW (2.8 mg FNA-N/L) to BMP tests led to a significant decrease in the daily CH_4 yield by 22–66 % in the first ten days. Another

work assessed the impact of FNA-pretreatment on the performance of lignocellulosic agro-waste in AD using BMP tests (Tamang et al., 2023). They reported a decrease of 1–11 % in the CH_4 production when wheat straw was pretreated with 3.5–5.3 mg FNA-N/L for more than 36 hrs. Romero-Güiza et al. (2019) reported an increase in lag time from 0 day to 0.9 day when increased NO_2^- concentration in BMP tests from 0 to 2.9 mg N/L. As in this experiment, a high NO_2^- concentration (2368.0 ± 569.1 mg N/L) was detected and the pH was controlled at 7.0 ± 0.1 in B7 (resulting in FNA = 0.4 mg N/L), it was suspected that NO_2^-/FNA present in B7 was responsible for this inhibition. This was supported by the observation of a higher N_2 production detected in B7 (Fig. 5F) possibly due to denitrification process that used NO_2^- as substrate. While N_2 production stopped (all NO_2^- and NO_3^- were used up within reactor) on Day 25, a positive total gas and CH_4 production were detected at the same time, suggesting such inhibition is reversible. This was consistent with the results reported by Han et al. (2024). In the later stage of BMP test (Day 25–55), methanogenesis that was previously inhibited by NO_2^-/FNA recovered drastically, even exhibited improved performance.

Similar results were found in the BMP reactors (B8, B10, and B11) fed with mixture of FW and SS (Figs. 5G and 5H). Pretreatment of the mixed substrate with FNA at the low concentrations (1.0–2.2 mg FNA-N/L) did not enhance its digestibility in AD. A higher FNA concentration (5.2 mg FNA-N/L) in the pretreatment showed a negative impact on biogas production in BMP reactor (B12), which was similar to the observation in B7. Compared with the control reactor (B8), a significant reduction in B_0 (24.9 ± 0.4 mL CH_4/g VS, reduced by 81 %) and R_m (3.4 ± 0.2 mL CH_4/day/g VS, reduced by 66 %), and an increase in lag time (λ = 15.3 ± 0.2 days, increased by 5.1 times) were observed in B12 (see supplementary material). These results suggested a limited effectiveness of FNA-pretreatment (1.0 – 5.2 mg FNA-N/L for 72 hrs) in enhancing the digestibility of mixed FW and SS. Moreover, in B12, the excessive carryover of NO_2^- (326.2 ± 52.3 mg NO_2^--N/L) from the FNA pretreatment step might negatively impact the performance of AD in two ways: 1) inhibitory impact of NO_2^-/FNA to the functional microbial processes (e.g., methanogenesis) (Banihani et al., 2009; Han et al., 2024); and 2) consumption of readily biodegradable COD (rbCOD) by NO_2^-denitrification (Akaniro et al., 2024). Assuming that 1.7 and 3.3 mg COD/mg NO_2^--N are required for denitrification and growth of denitrifiers (Bai et al., 2016), the estimated rbCOD required for a complete denitrification in B7 (2368.0 ± 569.1 mg NO_2^--N/L) and B12 (326.2 ± 52.3 mg NO_2^--N/L) are approximately 12396.9 and 1706.0 mg COD/L, respectively. Lu et al. (2019) compared the CH_4 production in the BMP reactors with and without NO_2^- addition, and found the group with 250.0 mg NO_2^--N/L produced 39 % less CH_4 than that in control group due to the consumption of rbCOD by denitrification. This was supported by their bacterial community composition analysis that showed *Vulcanibacillus*, a strictly anaerobic, moderately thermophilic nitrate-reducing bacterium, was uniquely found in the reactor with NO_2^- addition.

These results indicated that the optimal FNA concentration for pretreating FW, SS, and mixture of FW and SS for achieving enchantment of digestibility in AD may vary due to the distinct composition of organic

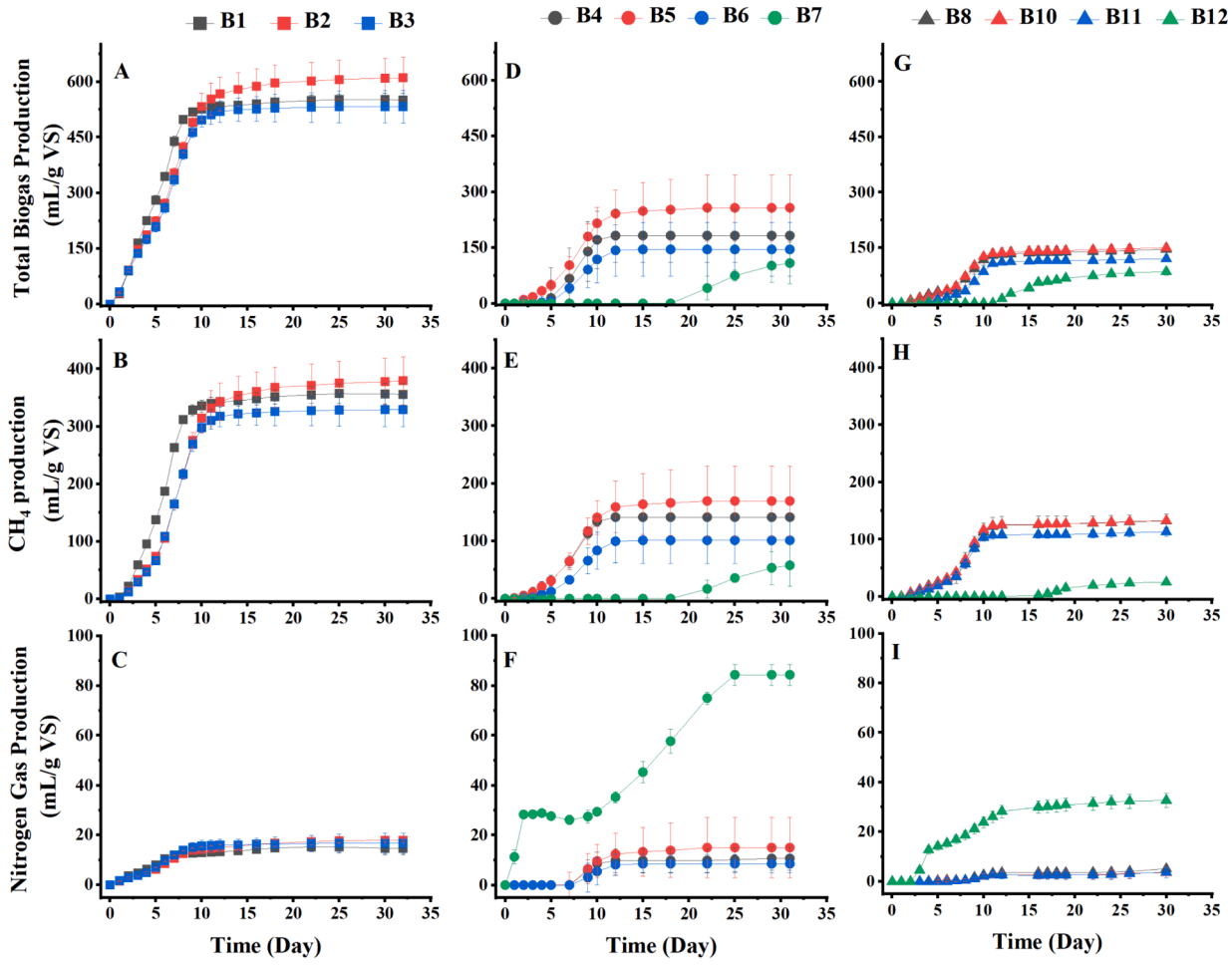

Fig. 5. The total biogas production (**A, D, and G**), total methane production (**B, E, and H**), and the nitrogen gas production (**C, F, and I**) with food waste (FW, ■), sewage sludge (SS, ●), and mixture of FW and SS (▲) The feed in the biochemical methane potential (BMP) reactors were collected from free nitrous acid (FNA) pretreatment reactors with the details provided in Table 1. If a value below 0 is obtained as the gas production in the treatment groups is less than inoculum group, gas production of 0 was used for plotting. Error bars (shown if larger than the symbols) represent standard deviations of triplicate analysis.

matters. A comprehensive investigation of the feedstock composition would be critical for developing an effective FNA pretreatment strategy to optimize the AD performance. In addition, minimizing the NO_2^- carryover from FNA pretreatment is crucial to avoid NO_2^-/FNA inhibition or competition between denitrifiers and other functional biological processes in AD for easily biodegradable organic carbon sources. Some researchers suggested that separating the pretreatment liquor before feeding it to AD can significantly reduce the amount of NO_2^-/FNA carried from the pretreatment step into the digester (Bai et al., 2016). However, additional equipment and energy input will be required for such separation, and thus reduce the technical–economic feasibility of the FNA pretreatment method.

4. Conclusion

FNA pretreatment with 5.2 mg N/L for 24–72 h demonstrated enhanced solubilization of organic wastes (i.e., FW, SS, and their mixture) and effectively controlled H_2S odor. However, the effective FNA concentration is sensitive to abiotic and/or biotic reactions that can change the pH and/or NO_2^- concentration within the system. Enhanced solubilization of organic waste by FNA pretreatment does not necessarily result in increasing biogas yield in AD. As NO_2^- carryover from FNA treatment can negatively impact the performance of AD, preliminary tests are recommended to examine the optimal conditions for pretreating target organic wastes by FNA.

5. Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used the OpenAI ChatGPT in order to improve the language and readability. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Camila A. Proano: . **Ruizhe Liu:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xueming Xu:** Methodology, Investigation, Data curation. **Seth Meisler:** Methodology, Formal analysis, Data curation. **Amro Hassanein:** Writing – review & editing, Resources, Methodology. **Stephanie Lansing:** Writing – review & editing, Supervision, Resources, Conceptualization. **Kuo Tian:** Writing – review & editing, Resources. **Guangbin Li:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Formal analysis.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.130819.

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