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An integrated strategy for sequential nitrite removal and methane recovery: Sludge fermentation driven by nitrite reduction

Xiaodi Li, Mengxue Sun, Bo Wang *, Wei Zeng, Yongzhen Peng

National Engineering Laboratory for Advanced Municipal Wastewater Treatment and Reuse Technology, Engineering Research Center of Beijing, Beijing University of Technology, Beijing 100124, PR China

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ABSTRACT

Although the treatment of sludge with free nitrous acid can effectively recover short chain fatty acids, the feasibility of sequential nitrite reduction and methane recovery without acidic pH adjustment is still scarcely studied. Therefore, this study aimed to provide insights into the effect of nitrite at different levels on nitrite reduction and methane production. The results showed that the nitrite concentrations of 100, 200, 400 and 800 mg/L were completely reduced in 1, 2, 2 and 4 days, respectively. The nitrite reduction process stimulated the fermentation of sludge to produce more organic matters, which served as electron donors for denitrification. With the nitrite concentrations increasing from 200 to 800 mg/L methane production decreased from 128.7 to 0 mg/L at the digestion time of 15 d. The toxicity of nitrite to methanogenic microorganisms and the nitrite reduction process competing with methanogens for carbon sources may lead to the inhibition of methane production by excessive nitrite. Moreover, the methane production reached 184.4 mL with 100 mg/L nitrite reduction, which was increased by 83.2 % compared with that without nitrite addition (101.1 mL). Nitrite reduction stimulated hydrolysis without negatively impacting acetogenesis, thereby providing more substrates for subsequent methanogenesis. Model-based analysis indicated that nitrite reduction enhanced the maximum methane yield and methane production rate, aligning with the aforementioned analysis. 16S rRNA analysis unraveled that the bacterial abundance associated with hydrolysis increased. This anaerobic digestion technique driven by nitrite reduction is both environmentally and economically attractive for increasing methane production.

Introduction

Anaerobic digestion (AD) has captured the attention of researchers due to its energy output (Lee et al., 2014). AD typically involves three steps: hydrolysis, acidogenesis, and methanogenesis (Li et al., 2019). Nonetheless, the limited hydrolysis capacity of waste activated sludge (WAS) often leads to unsatisfactory methane yield during AD, thereby restricting the future application of this technology (Carrère et al., 2010). Recently, many technologies have been developed to improve the efficiency of AD, such as thermal methods (Zhang et al., 2017), alkaline processes (Pang et al., 2023). Nevertheless, these pretreatment approaches are inherently complex to operate, necessitated numerous treatment facilities.

Nitrite is an intermediate product of nitrification/denitrification processes (Wang et al., 2013). Free nitrous acid (FNA) is a protonated form of nitrite and has been found to cause significant inhibition in many biological processes in wastewater treatment plants (WWTP) (Zhou et al., 2011). Recently, there has been growing interests in FNA pretreatment, for its potential of being an environmental and economical method. Studies have revealed the effectiveness of FNA pretreatment in disintegrating microbial cells and extracellular polymeric substances (EPS) (Chislett et al., 2020). Wang et al. (2013) demonstrated that pretreating WAS 24 h with FNA at concentrations ranging from 1.78 to 2.13 mg N/L not only facilitated sludge hydrolysis, but also led to an increase in subsequent methane production by approximately 30 %. Additionally, FNA pretreatment enhanced the yield of short chain fatty acids (SCFAs) during anaerobic fermentation (Li et al., 2016). Nevertheless, nitrite or FNA, which is more conducive to the accumulation of SCFAs was not clear. Therefore, Lu et al. (2019) examined the impact of nitrite and FNA on the AD of WAS and concluded that both enhanced the hydrolysis, with nitrite being more advantageous for SCFAs accumulation than FNA (Lu et al., 2019). Wu et al. (2014) demonstrated the

* Corresponding author.
E-mail address: wbo@bjut.edu.cn (B. Wang).

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efficacy of nitrite in disrupting the microbial cells in sludge at pH 7. Zhang et al. (2020a) demonstrated that adding nitrite to WAS fermentation systems accelerated hydrolysis but inhibited acidogenesis and methanogenesis, in which pH 6 was the critical condition. According to the study, FNA technology is classified as pH-dependent pretreatment, evidently, FNA can achieve optimal performance only within specific pH range (Zhang et al., 2019). Considering the influence of pH and the role of nitrite reduction, an integrated process without pH adjustment may have a synergistic effect on the nitrite removal and methane recovery, leading to cost savings. However, so far, this hypothesis has not been experimentally verified, and the corresponding mechanisms are not clear.

The purpose of this study was to elucidate the feasibility of sequential nitrite reduction and methane recovery, and exposed the relevant mechanisms through a series of batch experiments and analyses. The methane production was closely monitored with different nitrite concentrations (100, 200, 400 and 800 mg/L). Additionally, the impacts of nitrite reduction on hydrolysis, acetogenesis and microbial community were specifically analyzed. The research would effectively address the knowledge gap regarding the impact of nitrite reduction on methane production, thereby providing valuable guidance for engineers in practical applications.

Results and discussion

Sequential nitrite removal and methane recovery

The nitrite concentrations of 100, 200, 400 and 800 mg/L were completely reduced in 1, 2, 2 and 4 days, respectively (Fig. 1a). What's more, methane production was detected on the 7th, 8th, and 10th days

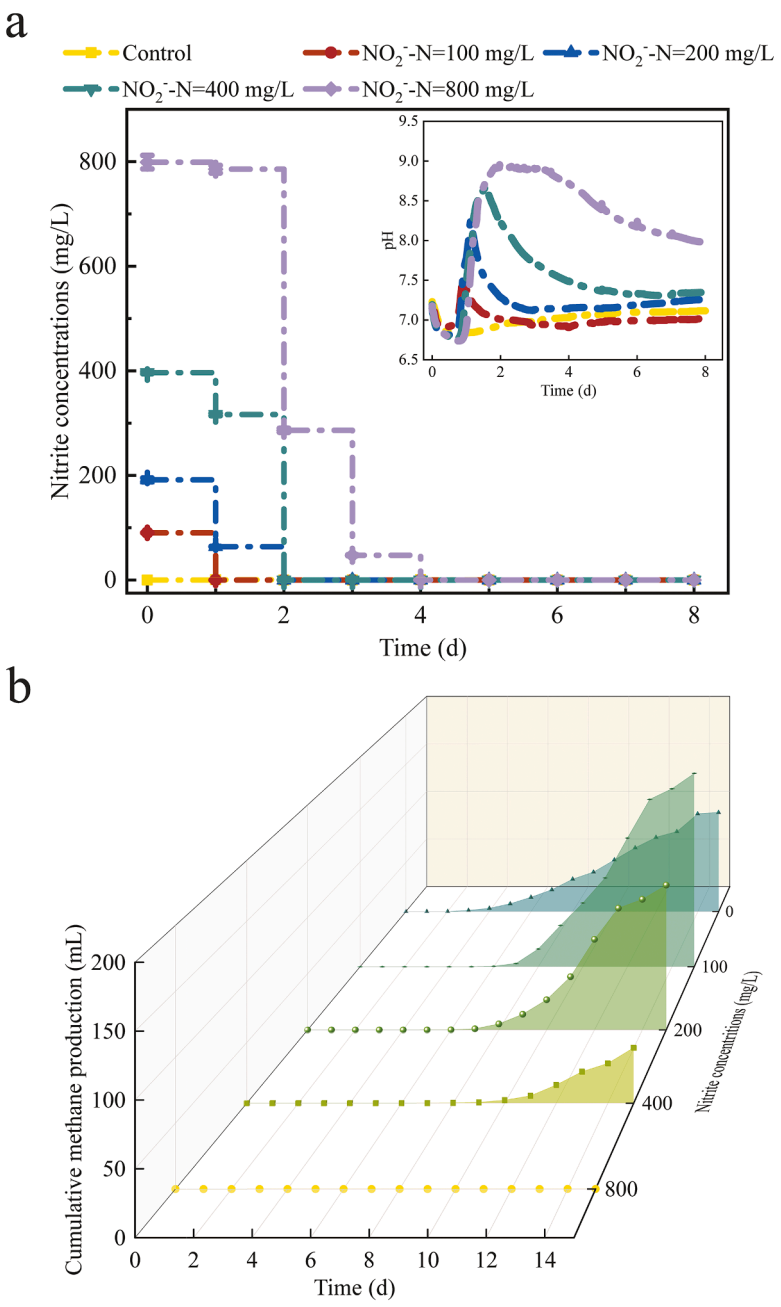

Fig. 1. (a) Performance of nitrite reduction and pH at different nitrite concentrations in Test B; (b) Cumulative methane production from anaerobic digestion of WAS in Test A.

at initial nitrite concentrations of 100, 200, and 400 mg/L, respectively, while on the 5th day in the absence of nitrite addition (Fig. 1b). The above indicated that the hypothesis of sequential nitrite reduction and methane recovery is feasible. In 800 mg nitrite-N/L reactor, it was observed that sludge fermentation and nitrite reduction did not occur simultaneously. The nitrite concentration in the reactor remained unchanged on the first day and was subsequently reduced through denitrification from the second to the fourth day. The total nitrogen removal rates in reactors B2-B5 were 0.1, 0.1, 0.2, and 0.2 kg/(m^3·d), respectively (Table 1). Compared to nitrate reduction, nitrite reduction had better nitrogen removal rates in fermentation reactors (Li et al., 2023).

The cumulative methane production with different nitrite concentrations was described in Fig. 1b. It was observed that methane production gradually increased, and the cumulative methane production was largely influenced by nitrite concentration. The highest methane production on the 15th day reached 184.4 mL with 100 mg/L nitrite, which was increased by 82.4 % compared with that without nitrite addition (101.1 mL). When nitrite concentration was increased to 200 mg/L, the cumulative methane production slightly exhibited a decline to 128.7 mL, but it was still higher than that control group. When nitrite concentration was further raised to 400 mg/L, the cumulative methane production decreased to 45.7 mL. Notably, higher nitrite concentrations (800 mg/L) significantly inhibited methane production with no methane generated within 15 days. Tugtas and Pavlostathis (2006) discovered that nitrite significantly inhibited methane production even at concentrations below 20 mg/L, persisting until nitrite was completely depleted. The likely reasons for the suppression of methane production by high nitrite levels could be the toxic effect of nitrite on methanogenic microorganisms and competition between the nitrite reduction process and methanogens for carbon substrates. Zhang et al. (2010b) described that methanogens prefer neutral environments, and they would be severely suppressed under alkaline environments, particularly when the pH was greater than 10. After 7 days of fermentation, the pH of reactors B1-B5 reached a stable level, which was 7.1, 7.0, 7.3, 7.4 and 8.0, respectively (Fig. 1a). The methane production was obviously hampered by the high pH of 9.0 in the reactor with 800 mg/L nitrite. Notely, the methane production in this study was relatively higher compared to that in other AD studies using nitrite (Romero-Guiza et al., 2019; Zhang et al., 2020a), highlighting the substantial potential of nitrite reduction technology for enhancing methane recovery.

The experimental results were substituted into the modified Gompertz equation for fitting, which suggested that the model profiles fit the real experimental data well with $R^2 > 0.99$ in all scenarios (Table 2). Nitrite reduction improved the maximum methane yield (*Gm*) and the methane production rate (*Rm*). The *Gm* was respectively attained at 28.8, 18.8 and 10.0 mL/g volatile suspended solids (mL/g VSS) in the reactors of 100, 200 and 400 mg/L, compared to 18.3 mL/g VSS in the control after 15 d of anaerobic digestion. The maximum *Rm* was 3.7 ± 0.2 mL/(g VSS·d) in the reactor with 100 mg/L nitrite, which was 2.6, 1.1, and 2.8-fold in the control group (1.4 ± 0.1 mL/(g VSS·d)), 200 mg/L group (3.3 ± 0.1 mL/(g VSS·d)), and 400 mg/L group (1.3 ± 0.1 mL/(g VSS·d)), respectively. The above results illustrated methane production was influenced by nitrite reduction, with appropriately increased nitrite concentration stimulating methane production and higher nitrite concentrations suppressing the accumulation of methane.

Table 1
Experimental conditions applied in the batch tests for assessing the effects of nitrite reduction on anaerobic methane production (Text A) and hydrolysis (Text B) and acidogenesis (Text C).

Test	Reactor	Substrate
methane production (Test A)	A1	500 mL WAS
	A2	500 mL WAS + 100 mg NO_2^--N/L
	A3	500 mL WAS + 200 mg NO_2^--N/L
	A4	500 mL WAS + 400 mg NO_2^--N/L
	A5	500 mL WAS + 800 mg NO_2^--N/L
hydrolysis (Test B)	B1	500 mL WAS
	B2	500 mL WAS + 100 mg NO_2^--N/L
	B3	500 mL WAS + 200 mg NO_2^--N/L
	B4	500 mL WAS + 400 mg NO_2^--N/L
	B5	500 mL WAS + 800 mg NO_2^--N/L
acidogenesis (Test C)	C1	450 mL synthetic wastewater containing 1000.0 mg/L glucose + 50 mL WAS
	C2	450 mL synthetic wastewater containing 1000.0 mg/L glucose + 100 mg NO_2^--N/L + 50 mL WAS
	C3	450 mL synthetic wastewater containing 1000.0 mg/L glucose + 200 mg NO_2^--N/L + 50 mL WAS
	C4	450 mL synthetic wastewater containing 1000.0 mg/L glucose + 400 mg NO_2^--N/L + 50 mL WAS
	C5	450 mL synthetic wastewater containing 1000.0 mg/L glucose + 800 mg NO_2^--N/L + 50 mL WAS

Effect of nitrite reduction on hydrolysis

Sludge hydrolysis serves as the rate-limiting step in AD. This study investigated the impacts of different nitrite concentrations on the sludge hydrolysis. The impact of nitrite reduction on the hydrolysis was evaluated by variations in COD, soluble protein, and EPS (Wang et al., 2017; Xu et al., 2017). The COD and protein concentrations in supernatant on the 6th day of digestion were observed (Fig. 2a). Nitrite reduction enhanced the release of COD and protein with dose-dependent manner. The COD in the control group on the 6th day was 111.3 mg/L, while in other reactors, it ranged from 492.1 to 1095.6 mg/L. The protein concentration was 8.5 mg/L in the control group, and ranged from 24.4 to 67.6 mg/L in other reactors. The above indicated that more intracellular and/or extracellular substances entered the liquid phase in the presence of nitrite reduction, which facilitated subsequent methanogenesis. Alkaline conditions are beneficial to sludge hydrolysis, according to research (Wu et al., 2014). Nitrite reduction created an alkaline environment, which may foster the hydrolysis of organic matters in the sludge.

The sludge hydrolysis is associated with cell rupture and EPS disintegration. EPS is the major components of sludge surface, accounting up to 80 % of the VSS (Xu et al., 2017). It protects cells from the effects of toxic and harmful environments. The main components of EPS are proteins, polysaccharides, and humic-acid (Luo et al., 2020). An increase in protein and polysaccharide concentrations in EPS is considered an indicator of enhanced sludge fermentation (Wang et al., 2019). In this study, the variations in EPS, (including soluble EPS (SB-EPS), loosely-bound EPS (LB-EPS), and tightly-bound EPS (TB-EPS)) were investigated (Fig. 2b, c). Nitrite reduction increased the concentration of SB-EPS and LB-EPS, while the TB-EPS gradually decreased. It was reported that the loose structure of SB-EPS and LB-EPS can easily adsorb dissolved organic matter that was released from cells and TB-EPS (He et al., 2018; Ye et al., 2012). Although TB-EPS was firmly attached to the cell surface, nitrite reduction caused the sludge flocs to break, which allowed TB-EPS to diffuse into SB-EPS and LB-EPS (He et al., 2016). This implied that the properties and structures of EPS were affected by nitrite reduction.

The organic compounds released from sludge include biodegradable compounds (such as proteins and carbohydrates) and recalcitrant compounds (such as lignin and humic), and recalcitrant compounds are

Table 2
The Gompertz model simulates the cumulative methane production.

Reactor	Kinetic model parameters			
	Gm (mL/g VSS)	Rm (mL/g VSS·d)	λ (d)	R^2
Control	18.28 ± 1.27	1.40 ± 0.04	5.31 ± 0.14	0.997
nitrite =100 mg/L	28.80 ± 1.64	3.86 ± 0.16	7.02 ± 0.13	0.997
nitrite =200 mg/L	18.79 ± 0.96	3.33 ± 0.18	8.02 ± 0.13	0.996
nitrite =400 mg/L	10.03 ± 1.36	1.31 ± 0.04	10.70 ± 0.12	0.997
nitrite =800 mg/L	–	–	–	–

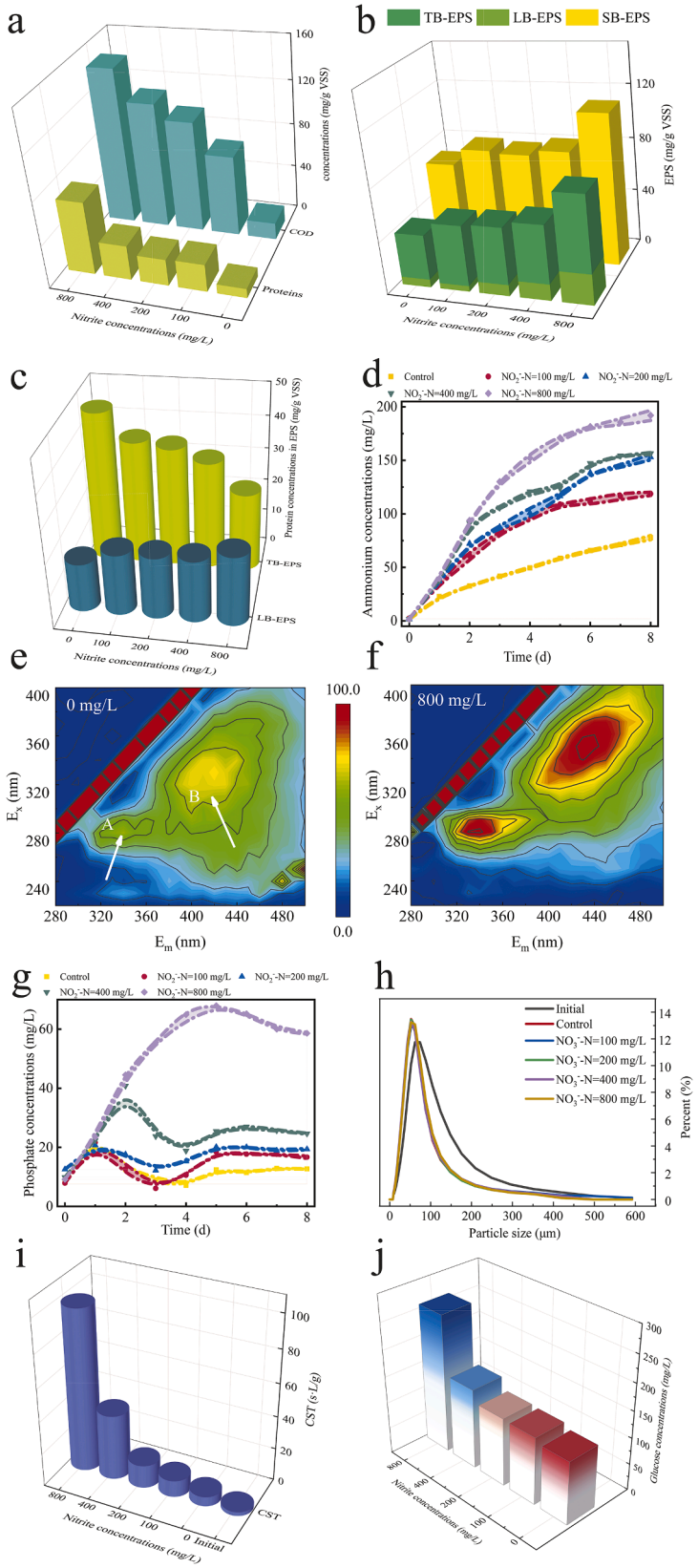

Fig. 2. (a) Concentrations of COD and proteins in the fermentation liquid on day 6; (b) Variations of EPS contents in various groups at day 6 of fermentation; (c) Release of proteins from LB-EPS and TB-EPS on day 6; Ammonium profiles (d) and phosphate profiles (g) in the mixed liquid over time; 3D-EEM spectra of organic compounds released after 6 days of digestion in 0 mg/L (e) and 800 mg/L (f); Particle size (h) and CST (i) in different groups on day 6 of fermentation; (j) variations of glucose at day 6 of fermentation in Test C.

difficult to be exploited by microorganisms during sludge fermentation (Hu et al., 2020). In this study, the impact of nitrite reduction on the release and biodegradability compounds was further evaluated using three-dimensional excitation-emission matrix (3D-EEM) technology. The 3D-EEM spectra of organic matters in the SB-EPS after 6 days of digestion were detected (Fig. 2e, f). Due to sludge hydrolysis enhanced, the peaks corresponding to polysaccharide-like substrates (Peak A) and humic acid-like substrates (Peak B) in the 800 mg/L nitrite group were higher than the control group, which indicated that nitrite reduction increased the proportion of biodegradable organic matters in the supernatant. Ji et al. (2020) demonstrated that the slowly biodegradable organic compounds can further hydrolyze and acidify into SCFAs. The findings demonstrated that nitrite reduction had a positive effect on sludge hydrolysis, leading to the release of abundant soluble substrates and promoted the conversion of refractory compounds into biodegradable compounds, which might facilitate subsequent bioprocesses.

Effect of nitrite reduction on nutrients release

During the hydrolysis, two primary nutrients (ammonium and phosphate) are released as soluble organic matter decomposes (Tong and Chen, 2009), and the release of ammonium and phosphate from the sludge phase to the supernatant would lead to an increase the discharge loading. In the experimental and control groups, ammonium concentration continued to increase over time at all nitrite concentrations (Fig. 2d). At the end of day 8, the ammonium concentrations in B1-B5 were 77.8, 118.7, 153.1, 156.4, and 192.0 mg/L, respectively. The main pathway for ammonium release is the hydrolytic acidification and deamination of proteins and nitrogen-containing compounds by various bacteria, fungi, and actinomycetes in the sludge digestion system (Zhang et al., 2013). The release of phosphate exhibited different trends compared to ammonium (Fig. 2d). When nitrite reduction was complete, the phosphate concentration reached its peak. Afterwards, the phosphate concentration rapidly decreased, then slowly increased and fluctuated. The phosphate concentrations in B1-B5 were 12.7, 16.7, 19.4, 24.6, and 58.6 mg/L, respectively, after 8 d digestion. In the AD system, the phosphate increase is mainly due to the decomposition of phosphorus containing organic matter. The variation of ammonium and phosphate levels can indicate the extent of hydrolysis and acidogenesis (Tian et al., 2023). The above findings indicated that the hydrolysis efficiency was affected by nitrite reduction, and that WAS was released sufficient organic substrates.

Effect of nitrite reduction on acidogenesis

The production of SCFAs, a significant by-product specifically generated during the acidogenesis, serves as a crucial intermediary in facilitating resource recovery (Zhang et al., 2020b). Glucose is recognized as one of the fundamental substrates for acidogenesis. Hence, Test-C aimed to assess glucose degradation to elucidate the impact of nitrite reduction on acidogenesis. Nitrite reduction decreased the glucose concentrations with dose-dependent manner. The glucose concentrations were 119.4, 127.6, 129.2, 151.3, and 260.4 mg/L, in the reactors with 0, 100, 200, 400 and 800 mg/L of nitrite after 6 d digestion (Fig. 2h). Compared to the control group, the nitrite addition had no negative effect on the acidogenesis, inconsistent with the findings of Zhang et al. (2020a). The likely reason for this discrepancy may be that the pH was not adjusted in this study.

Effect of nitrite reduction on sludge dewatering

The effect of nitrite reduction on sludge dewatering was evaluated. The capillary suction time (CST) and particle size were conducted (Fig. 2d). The CST of the sludge gradually raised, and the particle size reduced. The CST on the 8th day of digestion at nitrite 100, 200, 400 and 800 mg/L was, respectively, 10.1, 14.0, 39.8 and 98.5 s·L/g VSS, while CST in the control group was only 5.8 s·L/g VSS. Typically, WAS is metabolized by anaerobic microorganisms during AD, resulting in a reduction in the average particle size of the sludge. The deterioration of sludge dewatering performance might be attributed to the increase of CST and the reduction of sludge particle size. This was in line with the discoveries made by Sun et al. (2018), as they reported that high nitrite concentration reduced filtration properties.

Microbial community analysis at different initial nitrite concentrations

To delve into the impact of nitrite reduction on microbial communities, sludge samples were collected from the B1-B5 reactors after 8 days of AD. The samples were subjected to analysis using 16S rRNA high-throughput sequencing technology (Fig. 3). The Chao1, Shannon and Ace indexes are used to reveal the community abundance and diversity (Zhao et al., 2023). The Shannon index was 8.9 in the control group while it decreased to 8.4, 8.3, 7.0 and 6.1 in the reactors of 100, 200, 400 and 800 mg/L after 15 d (Fig. 3a), indicating that the microbial diversity decreased. The Chao1 and Ace were 1647.1 and 1654.1 in the control group while they gradually decreased to 838.7 and 841.5, respectively, indicating that diversity and richness of microbial community were slightly declined, primarily due to the toxic effects of nitrite on microorganisms. Moreover, the principal component analysis showed that B2-B4 samples were far from the control sample (Fig. 3b), indicating significant differences in the microbial composition of these experimental samples. Despite varying nitrite concentrations, the diversity of the microbial community diminished and core microbial community remained consistent under the condition of nitrite reduction. These similarities and differences suggested that nitrite reduction had a significant impact on the microbial structure.

Fig. 3c showed the evolvement of microbial community structure at the genus level under different nitrite concentrations. The dominant bacteria gradually changed from *Caldilinea* to *Proteiniclasticum* (a rumen protein-degrading bacterium) after 8 d digestion. *Caldilinea*, belonging to the *Chloroflexi* phylum, has the ability to degrade macromolecular organic substances and plays a momentous role in hydrolysis. *Proteiniclasticum* is an anaerobic bacterium capable of hydrolyzing proteins and is typically associated with SCFAs production (Wang et al., 2017). When the initial nitrite concentrations were 0,100, 200, 400 and 800 mg/L, respectively, the relative abundance of *Caldilinea* in the samples was 17.5 %, 3.2 %, 2.0 %, 0.2 % and 0.2 %, respectively, and the relative abundance of *Proteiniclasticum* in the samples was 0.1 %, 0.5 %, 2.5 %, 18.6 % and 19.8 %, respectively. *Dechloromonas* is widely present in activated sludge and is a typical genus involved in denitrification. The relative abundance of *Dechloromonas* in reactors was 1.2 %, 0.7 %, 0.6 %, 1.9 % and 0.5 %, respectively. Under anaerobic conditions, *Dechloromonas* can use NO_x^--N as an electron acceptor, converting aromatic compounds into carbon dioxide, thereby achieving denitrification (Shu et al., 2015; Wang et al., 2016). *Petrimonas* is a fermentative bacterium that mainly produces acetic acid, propionic acid, hydrogen, and carbon dioxide as metabolic byproducts (Maspolim et al., 2015). When the initial nitrite concentrations were 0,100, 200, 400 and 800 mg/L, respectively, the relative abundance of *Petrimonas* in the samples was 0.1 %, 2.3 %, 8.7 %, 10.3 % and 3.5 %, respectively.

Potential applications of the integrated strategy for sequential nitrite removal and methane recovery

Researches had shown that nitrite remarkably degraded WAS and produced SCFAs when used as an additive for WAS pretreatment (Sheng et al., 2023). In this study, it was demonstrated that nitrite reduction can sequentially promote the nitrite removal and methane recovery without pH adjustment. A series of batch experiments were conducted, which provided new insights for anaerobic digestion process based on nitrite reduction.

Nitrite reduction technologies is progressively being acknowledged

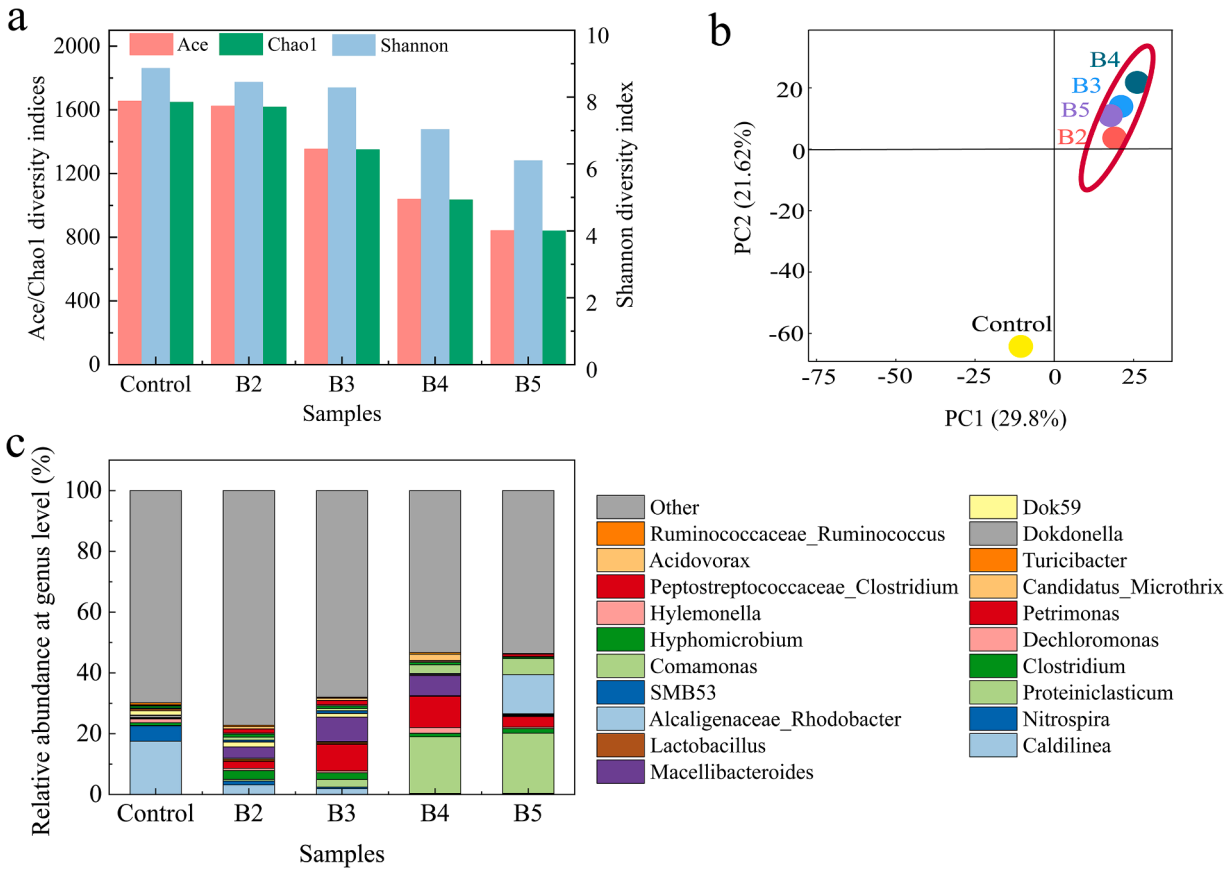

Fig. 3. Results in Test B of community diversity Ace/Chao1/Shannon at the OTU level (a), the principal component analysis (b) and microbial community structure at the genus level of different samples (c).

as cost-effective and practical approaches for recovering energy. This study proposed a feasible project for utilizing nitrite as a treatment strategy in AD of WWTP (Fig.4). Nitrite can be obtained from landfill leachate/fermentation liquor by partial nitrification. WAS is primarily obtained from the sludge thickening tank. The elevated ammonium levels in the sludge supernatant can be transformed into nitrite through side-stream treatment, thereby supplying the necessary nitrite for anaerobic digestion.

Conclusions

This study demonstrated that nitrite reduction can sequentially promote the nitrite removal and methane recovery during the AD of sludge. The results showed that methane production was significantly

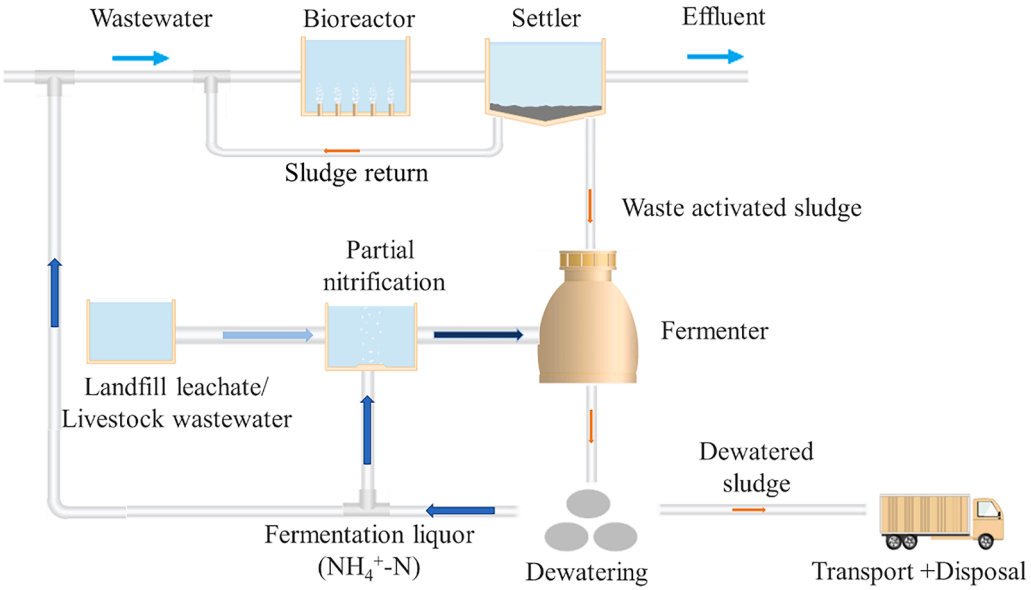

Fig. 4. Overall implication through nitrite reduction in WAS fermentation.

increased by 83.2 % when nitrite concentration was 100 mg/L. Mechanism analyses showed that nitrite reduction not only facilitated hydrolysis but also increased the proportion of biodegradable organic matter in the supernatant. Furthermore, nitrite reduction had no negative effect on acetogenesis. Microbial community analysis unraveled that nitrite reduction enriched the abundance of hydrolysis microorganisms, declined the diversity and richness of microbial community.

Materials and methods

Characteristics of WAS

The raw WAS utilized in this study was sourced from the return sludge of the secondary sedimentation tank in an urban WWTP, running with anaerobic-anoxic-aerobic (A^2/O) mode. The return sludge was immediately placed in a refrigerator at 4℃ after sieving large impurities through a 0.9 mm sieve and subsequently used within 3 days in experiments. The thickened WAS has following major properties: pH 6.8 ± 0.2, total suspended solids (TSS) 15,250.0 ± 189.0 mg/L, volatile suspended solids (VSS) 8070.0 ± 31.0 mg/L, total chemical oxygen demand 11,869.5 ± 132.2 mg/L, chemical oxygen demand (COD) 54.1 ± 8.7 mg/L, soluble proteins 15.5 ± 1.2 mg /L, soluble carbohydrates 7.1 ± 0.7 mg/L.

Batch test to assess anaerobic methane production

Five serum bottles, each has a capacity of 600 mL, were arranged for the biochemical methane potential (BMP) batch test (designated Test-A) (Table 1). Each bottle received 500 mL of WAS, serving as both the fermentation substrate and inoculum. A1 was the control group without adding sodium nitrite. 246.3 mg, 492.7 mg, 985.6 mg and 1971.3 mg sodium nitrite were added to A2-A5, representing 100, 200, 400 and 800 mg nitrite-N/L, respectively. The mixture in serum bottles was mixed thoroughly and nitrogen gas was then sparged into each bottle for a duration of 5 min to eliminate oxygen. After that, all reactors were sealed, and cultivated in a BMP instrument at a temperature of 35.0 ± 1.0℃ and a speed of 200 rpm, and gas bags were used to collect the gas at the outlet. The TSS and VSS were 15,250.0 ± 189.0 mg/L and 8070.0 ± 31.0 mg/L. The appropriate pH for methanogenic archaea is 6∼8, and the initial pH of the reactors was adjusted and maintained at 7.0 ± 0.3 (Zhang et al., 2010a). The methane production was measured daily using a gas chromatograph (GC, Agilent 7890A, USA) equipped with a flame ionization detector (FID) and a 30 m × 0.53 mm Agilent HP-PLOT/Q column.

Batch test to assess the effect of nitrite reduction on hydrolysis and acidification

Test-B was set up to appraise the effect of nitrite reduction on hydrolysis process. Identical serum bottles with an effective working volume of 600 mL were used as digesters. Each bottle being fed with 500 mL of WAS. Designed dosages of nitrite were also added at 0, 100, 200, 400 or 800 mg/L, followed by pH being adjusted to 7.0 ± 0.3. Compared with Test-A, every reactor was grown within in a water bath stirring apparatus and the fermentation process lasted for 8 days.

The degradation of glucose in Test-C was employed to evaluate the impact of nitrite reduction on the acidogenesis. Identical serum bottles with an effective working volume of 600 mL were used as digesters. Compared with Test-A, synthetic wastewater containing 1000.0 mg/L glucose (model monosaccharide compound) was introduced into C1-C5 reactors, and 10 % (volume ratio) WAS was utilized as inoculum. The initial nitrite concentrations of C1-C5 were maintained at 0, 100, 200, 400 and 800 mg/L, respectively. The TSS and VSS were 1525.0 ± 18.9 mg/L and 807.0 ± 3.1 mg/L. Temperature, pH, stirring rate and other reaction conditions were consistent with those of Test-A.

Analysis of microbial community based on 16S rRNA high-throughput sequencing

The variation of the microbial community in the reactors was studied by high-throughput sequencing analysis. Sludge samples were taken from each reactor at 8 d and freeze-dried with a lyophilizer (LABCONCO, Model 195, England). Polymerase chain reaction (PCR) amplification was performed with primer pairs 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). Based on purification and quantification, the amplification products were equimolarized and sequenced in pairs (2 × 300) on the Illumina MiSeq platform (Illumina, San Diego, USA).

Model analysis for methane production

To further assess the impact of nitrite reduction on methane production from AD of WAS, the Gompertz equation (Eq (1)) (Lay et al., 1997; Wang et al., 2018) was adopted to describe the methane production obtained from Section 2.2.

$$G = Gm \times \exp\left\{-\exp\left[\frac{Rm \times e}{Gm}(\lambda - t) + 1\right]\right\} \quad (1)$$

Where G, *Gm, Rm, λ, t* and e were the cumulative methane production (mL/g VSS), the maximum methane yield (mL/g VSS), the methane production rate (mL/g VSS·d), the lag phase time of methane production (d), the digestion time (d), and exp (l), respectively. *Gm, Rm*, and λ were kinetic parameters and can be derived through exponential equations using Origin2023 software.

Analytical methods

The sludge samples were collected from the reactors were promptly centrifuged at a speed of 10,000 rpm for 5 min and filtered through 0.45 μm millipore filter membrane to remove impurities before measuring the soluble organic matter. Concentrations of TSS, VSS, nitrite, ammonium and phosphate contents were measured according to the Standard Methods (APHA, 1998). A COD quick-analysis apparatus (Lianhua5B-3C, China) was used to analyze COD. The modified phenol-sulfuric acid method (Jimenez et al., 2013) was used to determine carbohydrates, and the modified Lowry method (Lowry et al., 1951) was used to determine proteins and humic acid. The 3D-EEM was used to comprehensively analyze the produced dissolved organic matter in the sample (Hitachi F-7100, Hitachi Nacho Office, Japan). The capillary suction time (CST) of sludge samples was determined by a CST quick-analysis device (Triton Type 304 M, UK), which equipped with an 18 mm diameter sludge sample column and Whatman No 17 standard filter paper (Sun et al., 2018). The CST were normalized to the concentration of VSS (s·L/g VSS). The particle size of sludge samples was determined by a laser particle size analyzer (Microtrac-S3500, USA).

EPS extraction

EPS was extracted by a revised treatment method (Fan et al., 2022). Firstly, took about 15 mL of mixed liquor into a 50 mL tube and added distilled water to 40 mL. The sample was filtered through 0.45μm membrane after centrifugation at 4000 rpm for 15 min. The supernatant liquid named SB-EPS. Then, residual mixture added 0.05 % NaCl (70℃) to 40 mL and was vortexed for 1 min. The sample was filtered through 0.45μm membrane after centrifugation at 4000 rpm for 10 min. The supernatant liquid named LB-EPS. Finally, 0.05 % NaCl (room temperature) to 40 mL was added again to the residual mixture. The sample was placed in water at 60℃ for 30 min and shaken every 5 min. The sample was filtered through 0.45μm membrane after centrifugation at 4000 rpm for 15 min. The supernatant liquid named TB-EPS.

CRediT authorship contribution statement

Xiaodi Li: Writing – original draft. **Mengxue Sun:** Investigation. **Bo Wang:** Writing – review & editing, Validation, Supervision. **Wei Zeng:** Supervision. **Yongzhen Peng:** 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.

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Data availability

Data will be made available on request

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