

Bioproduction of volatile fatty acid from the fermentation of waste activated sludge for *in situ* denitrification

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Waste activated sludge (WAS) fermentation integrated with denitrification (the reduction of nitrite to dinitrogen gas) at different pHs was investigated in batch-mode reactors over a 24-day period. The results showed that in comparison with controlled pHs, the volatile fatty acid (VFA) bioproduction for *in situ* denitrification was significantly improved at uncontrolled pH. VFA fermented from WAS was quickly consumed by denitrification at uncontrolled pH, which accelerated sludge degradation. On the other hand, sludge digestion was benefited from the alkalinity produced from denitrification, while methanogenesis was prohibited by alkalinity and nitrite. The integrated sludge fermentation and denitrification can be cost-effectively applied to wastewater treatment plants, so that organic substrates (e.g., VFAs) are produced for denitrification via simultaneous sludge fermentation, which enables WAS reutilization and enhances nitrogen removal efficiency without the need of external carbon sources.

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[**Key words:** Waste activated sludge; Fermentation; Denitrification; pH; Volatile fatty acid]

Acidogenic fermentation of waste activated sludge (WAS) is a promising approach to produce volatile fatty acid (VFA) for biological nitrogen removal (BNR) from low C/N wastewater. By adding alkalis, sludge hydrolysis and acidification can be significantly increased (1,2), but denitrification, a process consuming fermented liquid always occur at neutral pH, so that the pH of fermented sludge should be adjusted to enhance VFA production and avoid the inhibition of denitrification in wastewater treatment plants (WWTPs). Considering substantial amounts of sludge fermentation liquids produced in WWTPs, the carbon sources needed for denitrification can be saved. Therefore, maximizing the bioproduction of VFAs from WAS fermentation and applying VFAs for BNR yields have a profound significance for cost-effective operation of WWTPs.

Simultaneous sludge fermentation and denitrification could be a solution (3). The sludge dewatering liquor is fed to an anoxic/aerobic reactor, where most of ammonium is converted to nitrate. Afterwards, a portion of the effluent is pumped into the reactor, where the co-occurrence of sludge fermentation and nitrate reduction is achieved. However, compared with chemical oxygen demand (COD) for nitrate reduction (C/N = 2.86), the demand for nitrite reduction is lower (C/N = 1.71). Additionally, partial nitrification, the indispensable pretreatment for denitrification, is easy to achieve in a sequencing batch reactor (SBR) by controlling the duration of operational cycles, dissolved oxygen concentration, pH or free nitrous acid (4,5). Therefore, simultaneous sludge fermentation and denitrification (SFD) might be a

more easily and cost-effective process for utilization of VFA fermented from WAS.

Recently, sludge acidification with nitrite addition under acidic and alkaline conditions was assessed, and the results showed that adding nitrite improved the acidification of WAS with high VFA accumulation (6). In this study, the concept of SFD was further expanded at uncontrolled pH, which is more realistic for sludge disposal and nitrogen removal in WWTPs. Lab-scale batch-mode reactors with or without controlled pH were assessed and compared to evaluate the feasibility of enhancing VFA bioproduction for *in situ* denitrification. In addition, because BNR has been frequently found to compete with methanogenesis for organic carbon (7,8), methane emission was also monitored in the reactors.

MATERIALS AND METHODS

Sources of seeding sludge and WAS Two types of seeding sludge were used in this study, with one from an anaerobic sludge fermentor (termed as F) and the other from a reactor of sludge fermentation integrated with denitrification (termed as FD). WAS (substrate sludge) was obtained from a pilot-scale SBR (working volume: 7 m³) treating municipal wastewater (Beijing, China). The sludge was concentrated by settling at 4°C for 24 h. In the WAS, the predominant bacterial phylum was *Proteobacteria* that accounted for 50.1%, which was similar to the analytical results of bacterial communities in other sludge samples, such as 14 sewage treatment plants (in which *Proteobacteria* accounted for 36–65% of total effective bacterial sequences) (9), nitrification reactor (10), fermentation reactors (11), biofilm and suspended sludge (12) and so on. The other dominant phyla were *Bacteroidetes* (21.5%), *Chloroflexi* (9.1%) and *Chlorobi* (6.6%). Before batch-mode tests, both of seeding sludge and WAS were washed three times to minimize the concentrations of various substances in the supernatant liquid. The volatile suspended solids (VSS) of WAS, F and FD were 12.9 g/L, 4.2 g/L, and 5.1 g/L, respectively. Other details of sludge characteristics are shown in Table 1.

Batch-mode experiments A 24 L of WAS was divided equally into twelve identical batch-mode reactors (working volume: 2.5 L) operated at the temperature

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TABLE 1. Characteristics of seeding sludge and WAS.

Parameter	Sewage sludge	Seeding sludge (F)	Seeding sludge (FD)
pH	7.1 ± 0.1	8.2 ± 0.2	8.3 ± 0.1
Total suspended solids (TSS)	14247.8 ± 124.8	5340.0 ± 69.3	6401.1 ± 89.1
Volatile suspended solids (VSS)	12942.2 ± 113.5	4248.9 ± 63.2	5146.7 ± 72.9
Soluble chemical oxygen demand (SCOD)	48.1 ± 3.2	66.4 ± 5.7	49.6 ± 1.1
Total chemical oxygen demand (TCOD)	24093.1 ± 221.6	9586.9 ± 103.5	9530.6 ± 99.8
Total carbohydrate (as COD)	2084.6 ± 45.6	370.2 ± 23.4	530.9 ± 32.1
Total protein (as COD)	12337.7 ± 76.0	3751.2 ± 43.1	3531.2 ± 19.8
Nitrite (as N)	0.7 ± 0.1	0.3 ± 0.1	0.3 ± 0.1

All values are expressed in mg/L except pH.

of 21 ± 1.5°C. Six reactors (termed as set A) were fed with 0.5 L of sludge FD (fermentation and denitritation) in each reactor. The other six reactors (termed as set B) were fed with 0.5 L of sludge F (fermentation) in each reactor. The mass ratio (in terms of volatile suspended solid, VSS) between the seeding sludge and substrate sludge was 1:10. A relatively broad pH range of 6.0–10.0 was selected to represent all the possible pHs occurring in fermentation and denitritation processes (13,14). Therefore, the pH values in both sets were the uncontrolled pH and the adjusted pH (6.0, 7.0, 8.0, 9.0, and 10.0) by adding 3M sodium hydroxide (NaOH) or 3M hydrochloric acid (HCl).

The reactors were purged with nitrogen gas to remove dissolved oxygen and sealed immediately afterwards. On a daily base, 5 mL of 25 gN/L sodium nitrite (NaNO₂) was transiently added to the Set A reactors for denitritation, while there was no nitrite addition into the Set B reactors. All of these reactors were magnetically stirred at 200 rpm. Samples (sludge or gas) were taken periodically from the reactors to determine the performance of fermentation and denitritation.

Analytical methods Sludge samples were filtered through a microfiber membrane (pore size: 0.45 μm). The filtrate was analyzed for VFA, SCOD, NO₂⁻ - N, NH₄⁺ - N, PO₄³⁻ - P, carbohydrate, and protein, while the filter was assayed for VSS and TSS. The analyses of TSS and VSS were conducted according to Standard Methods (15). NH₄⁺ - N, NO₂⁻ - N, and PO₄³⁻ - P were assayed using a LACHAT flow injection system (Quikchem8500 Series 2, Lachat Instrument, Milwaukee, WI, USA). Temperature and pH were monitored with pH/Oxi 340i analyzers (WTW Company, Germany). COD concentration was measured using a 5B-1 type COD Meter (Lian-hua Tech Co., Ltd). Carbohydrate, protein and VFA concentration was measured as previously described (6).

VFA consumed for *in situ* denitritation (VFA-De), in terms of COD, was defined as VFA consumed for denitrifiers to reduce NO₂⁻ to N₂ (Eq. 1).

$$C_{\text{VFA-De}} = 1.72 \times (C_{\text{A-N}} - C_{\text{N}}) \quad (1)$$

where $C_{\text{VFA-De}}$ is the concentration of VFA-De (mg COD); $C_{\text{A-N}}$ is the initial NO₂⁻ concentration when nitrite was added in the reactors (mg N/L); C_{N} is the concentration of observed NO₂⁻ - N when sampling (mg N/L); and 1.72 is oxygen equivalent of nitrite (kg O₂/kg NO₂⁻ - N) (16).

Methane was measured using a gas chromatography (7890A, Agilent, USA) equipped with a thermal conductivity detector (TCD) and a 30 m × 320 μm × 3 μm GS-CARBONPLOT column. The temperature of the injector, column and detector was set at 40°C, 50°C, and 90°C, respectively. Helium was used as the carrier gas at a flow rate of 30 mL/min. Methane production (based on COD) was calculated as previously described (17).

RESULTS AND DISCUSSION

Bioproduction of VFA for *in situ* denitritation at uncontrolled pH Previous studies found that VFA accumulation at uncontrolled pH was lower than that at controlled pHs, and the VFA concentration under alkaline conditions was higher than that under acid or neutral conditions (13,18). The lower temperature of the anaerobic fermentation was associated with high pH values required to reach a maximum VFA production, and the optimum pH at room temperature was 10.0 (19). These studies only focused on sludge fermentation, and the criteria was established based on VFA accumulation, by which higher VFA accumulation indicated a better sludge fermentation.

However, the criteria for the integrated sludge fermentation and *in situ* denitritation (i.e., SFD) was the consumption of VFA by denitritation, instead of VFA accumulation. Thus, the lower nitrogen remaining in the system or the more VFA available for *in situ*

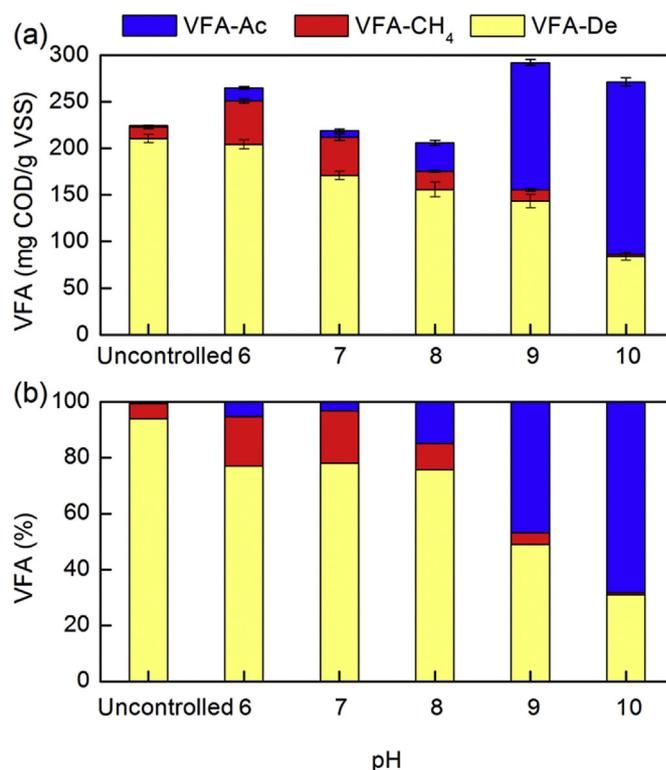


FIG. 1. VFA distributions at different pHs. (a) VFA accumulation (VFA-Ac), VFA consumption for *in situ* denitritation (VFA-De) and VFA consumption for methane production (VFA-CH₄); (b) percentages of VFA-Ac, VFA-De and VFA-CH₄ accounting for total VFA. Error bars represent standard deviation of triplicate samples.

denitritation indicates the better performance of the integrated process. The VFA consumption for *in situ* denitritation (VFA-De) at different pHs (Fig. 1a) showed that at the controlled pHs, the VFA-De decreased as pH value increasing from 6.0 to 10.0. In contrast, the maximum VFA-De occurred at uncontrolled pH, indicating that the integrated sludge fermentation and *in situ* denitritation was better than that at the controlled pHs. During the 24-day operational period, the nitrite in the reactor of the uncontrolled pH was nearly consumed, while there was still nitrite remaining in the reactors of the controlled pHs (data not shown).

The VFA balance with the COD mass was established at different pHs (Fig. 1b). At pH 6 and 7, the percentage of VFA being consumed by methane (CH₄) production (VFA-CH₄) was 17.7% and 18.7%, respectively. At pH 8, 9 and 10, although the VFA-CH₄ decreased with pH, the VFA accumulation (VFA-Ac) increased, which indicated that the low VFA-De under alkaline conditions was caused by the inhibition of denitritation rather than the deficiency of organic compounds. However, at uncontrolled pH, the VFA-CH₄ was only 5.5% and there was almost no VFA accumulation.

Mechanisms of enhancing VFA bioproduction for *in situ* denitritation at uncontrolled pH

In an SFD system, denitritation consumes substrates, and provides a positive feedback to acidification, hydrolysis, and sludge disintegration, and thus more soluble organic substrates will be generated (20). At the uncontrolled pH, the specific denitritation rate (k/X) reached the peak value on day 4 (Fig. 2a). During 24-day operational period, the k/X was higher than that at the controlled pHs except 7.0, implying that the rapid consumption of VFA triggered more WAS degradation. Although the maximum k/X at uncontrolled pH was lower than that at the pH of 7.0, shorter time was required to reach this value than that at pH 6.0 and 9.0 (Fig. 2a). Moreover,

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