Using excess sludge as carbon source for enhanced nitrogen removal and sludge reduction with hydrolysis technology
Yong-Qing Gao, Yong-Zhen Peng, Jing-Yu Zhang, Jian-Long Wang and Liu Ye

ABSTRACT
In order to improve the nitrogen removal efficiency and to achieve the sludge reduction in traditional wastewater treatment plants, a combined hydrolysis-anoxic-oxic (H-A-O) pilot-scaled reactor was used in this study to investigate the possibility and validity of using excess activated sludge (EAS) fermentation liquids to enhance the nitrogen removal. The results clearly showed that sludge acidification rate in fermentation reactor can reach to 43.2%. The percentages of acetic acid, propionic acid and butyric acid in the fermentation liquids were 68.4, 25.3 and 6.3%, respectively, while those in domestic wastewater were 73.0, 12.2 and 13.8%, respectively. Bioavailability of soluble chemical oxygen demand (SCOD) from fermentation liquids and domestic wastewater were investigated in batch reactors with nitrate as the electron accepter as well. The corresponding specific denitrification rates were 0.15 g NO₃⁻-N/g VSS d⁻¹ and 0.09 g NO₃⁻-N/g VSS d⁻¹. When the substances were enough, the denitrification reaction appeared to follow the zero-order kinetics. The results also showed that, when the H-A-O pilot-scaled reactor was operated continuously and sludge fermentation liquids were applied as additional carbon source in the A-O reactor, the removal efficiencies of SCOD, NH₄⁺-N and total nitrogen (TN) were higher than 90, 95 and 79%, respectively. EAS reduction rate in this system was able to reach 40.4%, and the sludge VSS/SS ratio decreased from 0.82 to 0.59 after hydrolysis step.

Key words | enhanced nitrogen removal, excess sludge fermentation, sludge reduction, volatile fatty acids (VFAs) composition

INTRODUCTION
An essential approach for eutrophication control in water systems is to remove the enriched nitrogen and phosphorus in wastewater before discharge. Biological nutrient removal (BNR) processes have been extensively studied and widely used in wastewater treatment plants (WWTPs) all over the world. However, challenges for wastewater treatment still remain. The percentage of readily biodegradable carbon source (C-source) in wastewater is usually considered to be a limiting factor for nitrogen and phosphorus removal (Tsuneda et al. 2005, Peng et al. 2006). Low ratio of the carbon to nitrogen (C/N) in domestic wastewater is a serious problem existing in most of WWTPs in China, especially in southern China, where there usually is a longer rain season and larger quantity of water utilization. Commercial external C-source has been added into wastewater treatment process, but it increases the operational cost and sludge yields. Thus, the alternative C-source which will be both economic and beneficial to sludge reduction is urgently needed.

So far, it has been reported widely that there is abundant of waste C-source in EAS and primary sludge.
The hydrolysis and acidification of either EAS or PS will produce large amount of volatile fatty acids (VFAs), which can be used as additional C-source for BNR systems (Bouzas et al. 2007; Jiang et al. 2007; Cokgor et al. 2009). Using sludge fermentation liquids instead of chemical organic materials (i.e. acetate, ethanol, and glucose) as C-source has been believed to increase the wastewater treatment efficiency and decrease the yields of EAS (Tong & Chen 2009).

In recent research, many parameters have been studied to enhance VFAs yields in sludge fermentation, including pH (Chen et al. 2007; Cokgor et al. 2009; Doğan & Sanin 2009), temperature (Cokgor et al. 2009; Feng et al. 2009), solids retention time (SRT) (Bouzas et al. 2007; Feng et al. 2009), microwave irradiation (Doğan & Sanin 2009) and so on. Ucisik & Henze (2008) studied the effect of sludge type and origin on VFAs yields and composition. They found that PS fermentation could produce more VFAs than EAS under the same studying conditions. It meant that VFAs production had a relationship with sludge type. It has been reported that PS fermentation could provide about 14 mg SCOD to a liter of wastewater, and the biodegradable SCOD level could increase by 5%, moreover, nutrient release could be negligible (Cokgor et al. 2009). PS C-source obtained by mesophilic fermentation could enhance the nitrate removal by 4~10% (Canziani et al. 1995).

However, EAS produced in WWTPs is much more than PS. Thus, reducing the amount of EAS and utilizing the EAS as C-source are much more crucial and favorable to WWTPs. It has been reported that 90% of the phosphorus in wastewater could be removed by utilizing EAS fermentation liquids as C-source (Ucisik & Henze 2008). Tong & Chen (2009) also discovered that when using EAS alkaline fermentation liquids after NH$_4^+$-N and PO$_4^{3-}$-P recovery, both the PO$_4^{3-}$-P and TN removal efficiencies in wastewater were increased to 92 and 83.2%, and the volume ratio of EAS fermentation liquids to municipal wastewater was 1:35. It's apparent that the unavoidable problem existing in EAS fermentation liquids utilization is the nitrogen and phosphorus released from sludge cells.

Mass balance and utilization of sludge C-source for nitrogen and phosphorus removal have been either evaluated by calculation or tested by batch experiments. However, there are few reports about pilot-scaled application of EAS fermentation liquids. EAS reduction by hydrolysis and acidification function was rarely reported, either. Therefore, this research tries to use a combined process which contains a sludge fermentation column with a working volume of 21 L and an anoxic/oxic (A/O) reactor with a working volume of 50 L to study the EAS reduction. After 4 months' operation of this combined system, the applicability and stability of using EAS fermentation liquids as additional external C-source in pilot-scaled nutrient removal process were investigated, which can provide useful and practical information for its application in WWTPs.

**MATERIALS AND METHODS**

**Continuous experiments**

Continuous experiments were conducted in a pilot-scaled H-A-O system. It consisted of a hydrolysis column which was an upflow anaerobic sludge blanket (UASB) reactor for EAS hydrolysis and acidification, and an A/O reactor for enhanced wastewater nitrogen removal. This combined system had a total working volume of 95 L, and the schematic of this system was shown in Figure 1. The UASB column had a working volume of 21 L. The hydraulic retention time (HRT) was 3.5 h, and the anabranch was set...
at the mid-column for the purpose of complete sludge mixture in the suspended part. The A/O reactor had a valid volume of 50 L, and the ratio between anoxic section and aerobic section was 4:6. Mechanical stirrer was used in anoxic section and air compressor was used to control DO (dissolved oxygen) concentration in aerobic section around 1.2 mg/L–4.0 mg/L. The mixed liquid suspended solids (MLSS) concentration was 3.0 g/L, HRT was 8 h, SRT was 12 d, internal recirculation rate \(R_i\) was 300% and sludge recirculation rate \(R_e\) was 50%. This continuous experiment was conducted at a room temperature range between 15°C–30°C due to the seasons changed. When the system was conducted continuously, EAS concentrated by settling at 4°C was pumped into the column within 1 hour. Then fermentation products were elutriated out continuously by domestic wastewater at the flow rate of 6.2 L h\(^{-1}\) and then the effluent flowed into the anoxic part of the A/O reactor for nitrogen removal.

EAS used in UASB column was obtained from the secondary sedimentation tank after the A/O reactor. The domestic wastewater used was from a septic tank in the utility area of Beijing University of Technology, China. The characteristics of EAS and domestic wastewater were listed in Table 1.

**Batch experiments**

Batch experiments were carried out at 25 ± 1°C to compare the effect of different SCOD origin (SCOD\(_{FL}\) from fermentation liquids and SCOD\(_{Dw}\) from domestic wastewater) on nitrate denitrification. Two identical reactors with the valid volume of 1.5 L were used. Sludge obtained from the anoxic part of A/O reactor was washed for three times by the effluent of the setting tank. Then the washed sludge was centrifuged at 4,000 rpm 4°C for 5 min. Sludge fermentation liquids obtained were utilized by three times dilution. When the experiments started, 0.5 L of prepared sludge and 1 L of domestic wastewater or diluted fermentation liquids were added to two reactors for denitrification. The mixed liquid volatile suspended solids (MLVSS) concentration in each reactor was about 4.0 ± 0.2 g/L. Magnetic mixture machine with temperature control was used for complete mixing. The whole batch experiments were repeated three times until consistent results were received. The initial parameters were shown in Table 2.

**Analytical methods**

Samples were centrifuged at 3,000 rpm for 2 min and then filtrated by 0.45 μm acetate cellulose membranes.

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**Table 1** | Characteristics of origin EAS, domestic wastewater and fermentation liquids

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Excess activated sludge</th>
<th>Domestic wastewater</th>
<th>Fermentation liquids</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (g/L)</td>
<td>7.8 (0.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>VSS (g/L)</td>
<td>6.3 (0.3)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 (0.1)</td>
<td>7.6 (0.1)</td>
<td>7.0 (0.1)</td>
</tr>
<tr>
<td>TCOD (g/L)</td>
<td>9.4 (0.6)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SCOD (mg/L)</td>
<td>56.0 (4.0)</td>
<td>223.0 (3.0)</td>
<td>695.2 (10)</td>
</tr>
<tr>
<td>TN(^+) (mg/L)</td>
<td>41.9 (2.2)</td>
<td>66.0 (2.5)</td>
<td>142.2 (5.1)</td>
</tr>
<tr>
<td>NH(_4^+)-N (mg/L)</td>
<td>0.6</td>
<td>54.0</td>
<td>110.1</td>
</tr>
<tr>
<td>PO(_4^3-) -P (mg/L)</td>
<td>3.4</td>
<td>6.0</td>
<td>27.1</td>
</tr>
<tr>
<td>HAc (mg COD/L)</td>
<td>8.3</td>
<td>67.6</td>
<td>205.3</td>
</tr>
<tr>
<td>HPPr (mg COD/L)</td>
<td>4.3</td>
<td>12.9</td>
<td>75.8</td>
</tr>
<tr>
<td>HBu (mg COD/L)</td>
<td>0</td>
<td>22.0</td>
<td>18.9</td>
</tr>
<tr>
<td>VFA(_t) (mg COD/L)</td>
<td>12.6</td>
<td>90.9</td>
<td>300.1</td>
</tr>
<tr>
<td>Carbo.(_t) (mg COD/L)</td>
<td>13.0 (1.1)</td>
<td>27.0 (1.0)</td>
<td>48.1 (1.2)</td>
</tr>
<tr>
<td>Prote.(_t) (mg COD/L)</td>
<td>24.2 (0.9)</td>
<td>92.2 (0.5)</td>
<td>181.7 (1.1)</td>
</tr>
</tbody>
</table>

\(^{1}\)Soluble substances.
HAc: Acetic acid, HPPr: Propionic acid, HBu: Butyric acid, Carbo.: Carbohydrates, Prote.: Proteins (They are the same meanings when appeared in other tables and figures). Standard deviation is shown in brackets.
The analyses for SCOD, NH₄⁺-N, PO₄³⁻-P, NO₂⁻-N, NO₃⁻-N, TSS and VSS were conducted in accordance with Standard Methods (APHA 1998). Proteins were measured by Lowry-Folin method (Lowry et al. 1951) and carbohydrates were analyzed by phenol-sulfuric method (Herbert et al. 1971). VFAs were analyzed by gas chromatography (Agilent 6890N) with flame ionization detector (FID) and a 50 m × 30 μm × 1 μm Agilent DB-WAXetr column. Nitrogen was used as the carrier gas. The temperature of injection port and detector were 220 °C and 250 °C, respectively. VFAs concentration was last converted to COD concentration by using conversion factors as 1.07 for acetic acid, 1.51 for propionic acid and 1.82 for butyric acids.

### RESULTS AND DISCUSSION

**SCOD and VFAs composition in fermentation liquids and domestic wastewater**

In order to confirm the suitability of EAS fermentation liquids as C-source for BNR systems, the comparison of SCOD and VFAs composition between domestic wastewater and UASB effluent was conducted (Figure 2). The proportion of acetic acid, propionic acid and butyric acid in VFAs in fermentation liquids was 11.0:4.0:1.0, which were different from the ratio of 5.4:0.9:1.0 in VFAs in domestic wastewater. In both UASB effluent and domestic wastewater, acetic acid was always the dominant organic acid, and the concentration in UASB effluent was three times of that in domestic wastewater. In domestic wastewater butyric acid was the second dominant acid. In UASB effluent, propionic acid became the second one which accounted for 25.3% of the total VFAs, but butyric acid only accounted for 6.3%. Moser-Engeler et al. (1998) and Li et al. (2008) had reported that VFAs composition was important to nitrogen and phosphorus removal especially the proportion of acetic acid and propionic acid.

Proteins, carbohydrates and VFAs were the three main types of SCOD existing in domestic wastewater and in EAS fermentation liquids (shown in Figure 2). In fermentation liquids, there were 43.2% of VFAs, 26.2% of proteins and 6.9% of carbohydrates. However, in domestic wastewater the data were 39.8% of VFAs, 41.4% of proteins and 12.1% of carbohydrates. During the sludge hydrolysis and acidification process, carbohydrates were firstly hydrolyzed into cellobiose and glucose, and then degraded into VFAs (Colberg 1988). Proteins were reported to be hydrolyzed to amino acids first and then amino acids were degraded into ammonium, CO₂, H₂ and VFAs (Pavlostathis & Giraldo-Gomez 1991). Due to the complexity of EAS, there was about 23.8% of unknown SCOD form in fermentation liquids which was 17.0% higher than that in domestic wastewater. From Table 1 it can be seen that the total SCOD concentration in UASB effluent was three times higher than that in domestic wastewater. Thus, it can be concluded that sludge fermentation liquids have the potential and advantages to be used as the supplement C-source for BNR systems.

<table>
<thead>
<tr>
<th>Initial parameters in denitrification batch reactors</th>
<th>SCOD origin</th>
<th>NO₃⁻-N (mg/L)</th>
<th>Prote. Carbo. HAc (mg COD/L)</th>
<th>HPr (mg COD/L)</th>
<th>HBu (mg COD/L)</th>
<th>SCOD (mg/L)</th>
<th>SCOD/NO₃⁻-N (Initial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCODDw*</td>
<td>26.0</td>
<td>61.1</td>
<td>12.4</td>
<td>64.2</td>
<td>10.4</td>
<td>20.4</td>
<td>168.5</td>
</tr>
<tr>
<td>SCODFl†</td>
<td>24.9</td>
<td>46.2</td>
<td>11.5</td>
<td>65.1</td>
<td>22.0</td>
<td>5.3</td>
<td>152.7</td>
</tr>
</tbody>
</table>

*Dw. Fl. Dw. Fl. from domestic wastewater.
†SCOD from sludge fermentation liquids.

![Figure 2](https://iwaponline.com/wst/article-pdf/62/7/1536/446719/1536.pdf)
Biological availability comparison of fermentation liquids and domestic wastewater

In order to ascertain the effect of EAS fermentation C-source on denitrification more clearly, a series of batch experiments were conducted. The initial SCOD to NO₃⁻-N ratio was approximately close to 6.5:1, and the original concentrations of VFAs and carbohydrates in diluted fermentation liquids and domestic wastewater were nearly the same except proteins (Table 2). However, the denitrification rate and organic substances consumption rate varied distinctly (Figures 3 and 4). The nitrate denitrification with SCODFl and SCODDw finished respectively at 60 min and 90 min, and the responding specific nitrate denitrification rate (Rdn) were 0.15 g NO₃⁻-N/g VSS d⁻¹ and 0.09 g NO₃⁻-N/g VSS d⁻¹. In reaction with SCODFl, a more obvious nitrite accumulating phenomenon appeared, because the nitrate denitrification rate was faster than the rate with SCODDw. When there was abundant of biodegradable C-source, denitrification followed the zero order kinetics.

One interesting phenomenon in both reactors was that a fast VFA consumption period existed. VFA Dw consumption rate was extremely high during the first 15 minutes, while VFAFl was consumed fast during the first 45 minutes. After the turning point, VFAs consumption rate slowed down as shown in Figure 4. When the reaction finished, the average consumption rate were 14.55 mg COD/g VSS h⁻¹ for VFAFl and 9.44 mg COD/g VSS h⁻¹ for VFA Dw, respectively. This difference might be resulted from the different composition and proportion between VFAFl and VFA Dw (shown in Figure 2(a)).

During the reactions, the average carbohydrates consumption rate were 1.00 mg COD/g VSS h⁻¹ for denitrification with SCODFl and 0.85 mg COD/g VSS h⁻¹ for denitrification with SCODDw. Carbohydrates consumption in these two reactors was similar, which indicated that the compositions of carbohydrates from fermentation liquids and domestic wastewater were approximately the same, including monosaccharides and oligosaccharides available to denitrification bacteria. Proteins concentration in both reactors represented a slow decreasing trend, which indicated that neither proteins in fermentation liquids nor proteins in domestic wastewater would be chose as C-source by denitrification bacteria when VFAs or carbohydrates existed. These results showed that EAS C-source could help to enhance the nitrogen removal by increasing the denitrification rate dramatically.

Enhanced nitrogen removal in a combined H-A-O system

As a concern to the concentration of SCOD and NH₄⁺-N in the influent, this domestic wastewater used during the whole pilot-scaled experiment is a kind of typical domestic wastewater with low C/N ratio (as shown in Table 1) and wide fluctuation range. Thus, the results obtained in batch study were necessary to be tested in a pilot-scaled system. When domestic wastewater and EAS fermentation products
flowed from the UASB column to the A/O reactor as shown in Figure 1, the SCOD concentration in the setting tank effluent was less than 50 mg/L, and the average removal efficiency was higher than 90% (Figure 5). It demonstrated that A/O reactor represented a stable removal effect on organic compounds. By calculation, more than 50% of SCOD was consumed in the anoxic step, and less than 20% was removed during the aerobic step.

NH$_4^+$-N and TN variations in the A/O reactor can be seen in Figure 6. NH$_4^+$-N concentration in the effluent was less than 2 mg/L, and the average removal efficiency was higher than 95% which indicated that NH$_4^+$-N removal was not affected by the addition of EAS fermentation liquids. The same results had been obtained by Lee et al. (1997). Table 1 showed that NH$_4^+$-N concentration in the UASB effluent was higher than that in domestic wastewater, which was the result of EAS hydrolysis and acidification (Boehler & Siegrist 2006). In the hydrolysis step, microorganism cells were disintegrated and internal organic compounds were discharged. Some organic nitrogen compounds were deamminated and transformed into inorganic nitrogen. Thus, the sludge fermentation step increased the nitrogen load in BNR systems. However, additional VFAs products were supplied to A/O reactor and they were able to counteract those additional nitrogen. In domestic wastewater, the ratio of SCOD to NH$_4^+$-N was 4.1, but in the UASB effluent, it increased to 6.3 (Table 1).

Finally, TN removal efficiency was higher than 79% under the condition of HRT 8 h and $R_i$ 300%. When $R_i$ increased above 300%, the nitrogen removal efficiency decreased, because the hydrolysis column couldn’t supply more easily bio-degradable C-source under the current study condition. It’s proved that enhanced nitrogen removal could be realized in pilot-scaled systems by adding EAS fermentation C-source, although it would increased the nitrogen load. Moreover, it can be assumed that EAS fermentation C-source will increase the TN removal efficiency in WWTPs if sludge hydrolysis and acidification technology is introduced.

**Excess sludge reduction during fermentation step**

Excess sludge reduction rate ($R_e$) was investigated as shown in Figure 7. $R_e$ was calculated according to the Equation (1) below. In this research, no sludge was discharged from the hydrolysis column. Figure 7 showed that, in the first 5 weeks, as sludge was pumped into the column, the total sludge production rate increased from 3.8 g/d to 4.5 g/d, and the VSS/SS ratio increased gradually from 0.66 to 0.82. It seemed that no sludge reduction occurred. Until 6 weeks later, sludge production rate slowed down, and the VSS/SS ratio began to decrease, too. It indicated that sludge reduction by hydrolysis and acidification technology became remarkable. During the last 5 weeks, EAS reduction rate in the UASB column fluctuated around 40.4%, and the VSS/SS ratio finally decreased to 0.59. Easily bio-utilizable substances in sludge had been transformed into soluble substances, but hardly bio-degradable compounds accumulated, which would need a longer time to be fermented. During this step, anaerobes and facultative aerobes in EAS survived and became supplement to fermentation bacteria,
but the aerobes died and were degraded into micro-
molecule substances. If the bacteria survived were con-
sidered, excess sludge reduction would be more significant.

$$R_e = \frac{\Delta MLSS_{\text{return}} - \Delta MLSS_{\text{sample}} - \Delta MLSS_{\text{hydrolysis}}}{\Delta MLSS_{\text{return}}} \times 100\%$$  

(1)

Mass balance calculations

The advantages of EAS fermentation liquids on nutrient removal had been validated. However, the addition of fermentation liquids had inevitably increased the nitrogen load in A/O reactor. Moreover, in this research, domestic wastewater was used to elutriate the EAS C-source out. Thus, it’s necessary to figure out the contribution of EAS C-source to the nitrogen removal, which means to exclude the SCOD, VFs and NH$_4^+$-N existing in domestic wastewater and only consider the C-source released by sludge. As the inflow quantity of the UASB was 6.2 L/h, thus, there were about 8.4 g NH$_4^+$-N and 70.3 g SCOD from EAS were added into the A/O reactor every day. Within these SCOD, there were 31.2 g VFs. Considering both nitrogen from domestic wastewater and sludge fermentation, the A/O reactor had an average nitrogen treating capacity of 16.4 g N/d. According to the theoretical C/N ratio of 8.6 g COD/g NO$_3^-$-N (Chen et al. 1991; Chung & Bae 2002), it seems that 140.9 g COD/d would be needed to complete the denitrification. It was evident that EAS fermentation could not supply enough C-source for complete denitrification. However, it could help to obtain a theoretical TN removal efficiency of 73.5%, which was close to the data achieved in the pilot-scaled experiments. At the end of these pilot-scaled experiments, in the UASB column, TSS was 10.11 g/L, the total quantity of sludge was 212.31 g, and the dissolved substances yields was 82.5 g/d. Thus, the sludge reduction rate by calculations could be 38.9% which was a little lower than the real reduction rate.

CONCLUSIONS

EAS reduction by fermentation technology and enhanced nitrogen removal by using EAS fermentation liquids as C-source were investigated in a set of pilot-scaled H-A-O system. The findings of this work can be summarized as follows:

1. In the hydrolysis and acidification column, VFs accounted for 43.2% of the total SCOD, and the proportion of acetic acid, propionic acid and butyric acid was 11.0:4.0:1.0, while the ratio in domestic wastewater was 5.4:0.9:1.0;
2. The specific denitrification rate ($R_{dn}$) with SCOD$_{Fl}$ could reach to 0.15 g/g VSS d$^{-1}$, and the rate with SCOD$_{Dw}$ was only 0.09 g/g VSS d$^{-1}$;
3. By utilizing the EAS fermentation liquids as additional C-source, the removal efficiencies of SCOD, NH$_4^+$-N and TN in the combined pilot-scaled system were higher than 90, 95 and 79%, respectively;
4. The excess sludge reduction rate obtained by fermentation technology reached to 40.4%. Data achieved by mass balance calculation were close to that obtained in experiments. It’s proved that EAS fermentation technology can not only supply C-source to nutrient removal, but also achieve waste sludge reduction.

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