Study of the treatment of domestic sewage using PVA gel beads as a biomass carrier
Yibo Wang, Yonghong Liu, Minquan Feng and Lina Wang

ABSTRACT
Technologies for domestic sewage treatment with low excess sludge yield rate and highly efficient biological treatment methods are needed, and the moving-bed biofilm reactor has great promise for meeting this need. To provide technical support for the treatment of typical domestic sewage, this paper provides an estimate of the bacterial diversity in polyvinyl alcohol (PVA) gel beads determined by a 16S-rRNA gene-based polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) approach, proposes a method for reducing the excess sludge yield rate, and describes the startup and continuous operation of the PVA technology. Domestic sewage was treated by a moving-bed biofilm reactor system using PVA gel beads as a biomass carrier. A significant amount of sphalerite, filamentous bacteria and bacillus were observed on the surface and internal structure of the PVA-gel beads by scanning electron microscope. Clostridiaceae bacterium, Alpha proteobacterium, Phenyllobacterium haematophilum and Rhodobacter were identified as dominant bacteria strains using 16S-rRNA PCR-DGGE. The active sludge tanks were found to play a significant role in the reduction of excess sludge.

Key words | domestic sewage, organic loading rates, PCR-DGGE, PVA-gel beads, sludge yield rate

INTRODUCTION
Domestic sewage commonly contains a low-concentration of organic pollutants, a high suspended solid content and many pathogenic bacteria and worms. If it is directly discharged into bodies of water or groundwater, it will pollute the water and cause eutrophication of lakes (Hench et al. 2005). The conventional activated sludge (CAS) process is widely used for treatment of both municipal wastewater and industrial effluents (Mohammadi et al. 2011), but there are problems with it. It yields too much sludge with extremely complex composition, so the sludge needs to be handled properly to avoid secondary pollution of water, soil and groundwater (Zhu et al. 2016). The excess sludge yield rate associated with the CAS process is approximately 0.5 g of suspended solids (SS) per g of chemical oxygen demand (COD) removed (Metcalf 1991). The cost for disposal of excess sludge has increased, accounting for about 50–60% of the total operation costs in water resource recovery facilities (Low & Chase 1999; Wei et al. 2005). Another problem for the CAS process is that because the total number of microorganisms in per unit volume is low, its treatment efficiency is low, and a larger area is required to construct activated sludge tanks. Therefore, technologies with low excess sludge yield rate and highly efficient biological treatment methods are needed.

Because the biomass carrier process has some advantages such as high biomass concentration, good performance of solid–liquid separation and high treatment efficiency, the
technology has become a research hotspot (Rahimi et al. 2014). The moving-bed biofilm reactor is one of the promising biomass technologies (Gani et al. 2016). This process can be added to existing plants to enhance the treatment efficiency of CAS without major new construction.

Recently, several researchers have explored adding polyvinyl alcohol (PVA) gel beads to moving-bed biofilm reactors as a biomass carrier. The PVA gel is composed of 4 mm spherical beads with a network of 10–20 micron pores in each bead that allow cultivation of bacteria in a sheltered environment and thus reduces sloughing of the biomass (Hoa et al. 2006; Gani et al. 2016). Hoa et al. (2006) treated synthetic wastewater with a fluidized-bed reactor, and their total nitrogen removal efficiency was up to 83%. Rouse et al. (2007) treated municipal wastewater with a moving-bed biofilm reactor, COD removal efficiency was more than 90%, and biological oxygen demand (BOD₅) removal efficiency was also more than 90%. Zhang et al. (2009) treated high-concentration corn steep liquor with an anaerobic fluidized-bed reactor, and the COD removal efficiency was in excess of 90%. Khang & Phong (2012) treated aquaculture wastewater with an expanded granular sludge bed reactor, yielding up to 92% COD and over 90% total suspended solids removal efficiencies. They all used PVA-gel beads as a biomass carrier in the reactors.

However, little information is known regarding microbial communities that inhabit this treatment system, so to provide technical support for the treatment of typical domestic sewage, this paper provides an estimate of the bacterial diversity in PVA-gel beads determined by a 16S-rRNA polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) approach, proposes a method for reducing the excess sludge yield rate, and describes the startup and continuous operation of the PVA technology.

MATERIALS AND METHODS

Materials

Domestic sewage was collected from a drainpipe at Xi’an University of Technology (Xi’an, China). The sewage had pH of 7.5–8.5, COD of 140–270 mg/L, NH₄⁺-N of 20–50 mg/L, BOD₅ of 92–254 mg/L, turbidity of 33–68 NTU, and SS concentration of 180–380 mg/L. The PVA-gel beads were provided by Kuraray Co., Ltd, Japan, each bead is spherical with a network of 10–20 micron pores, diameter of 4 mm and density of 1.03 g/cm³ (Hoa et al. 2006; Zhang et al. 2009; Gani et al. 2016). Activated sludge was collected from the Third Water Resource Recovery Facility in Xi’an, China.

Experimental device and method

The experimental system mainly consisted of a PVA reactor, two active sludge reactors, a sedimentation tank and an ultraviolet disinfection device. The aerating and heating devices were arranged at the bottom of the PVA and active sludge reactors. The ultraviolet disinfection device (GPH845T5L/49) was provided by Light Tech Lightsources Co., Ltd, USA. A flow diagram of the PVA biological process is shown in Figure 1.

During the startup phases, 2.5 L of PVA-gel beads with a diameter of 4 mm and 20 L of activated sludge were added to the
the PVA reactor. At the beginning of the experiment there was no activated sludge in the two sludge tanks, however, with time, almost all of the activated sludge in the PVA reactor flowed to the first and second sludge tanks. A grill prevented the PVA-gel beads from leaving the PVA reactor, the mesh size of the grill being 2 mm. The dissolved oxygen (DO) concentrations in the PVA reactor and in the two sludge tanks were kept in the range of 4–5 and 2–3 mg/L, respectively.

**Analytical methods**

COD, BOD₅, NH₄⁺-N and SS were determined by applying the standard methods of the Chinese National Environmental Policy Act (NEPA) (NEPA 2002). The DO was determined using a JPB-607A DO meter (INESA Scientific Instrument Co., Ltd, Shanghai, China). Turbidity was determined using a WZS-185 Turbidity meter (INESA Scientific Instrument Co., Ltd, Shanghai, China). Biomass attachment to the PVA-gel was measured by the weight method (Zhang et al. 2014). Microbial morphology was observed with a VEGA II XMU scanning electron microscopic (TESCAN Europe). Clump count was determined with an SN/T 1897-2007 Compact Dry (3 M Co., Ltd, USA). Total DNA was extracted using a D5625 DNA extraction kit (OMEGA, America), and it was amplified through a nest-PCR to obtain the target fragment for DGGE analysis (Pereira et al. 2002).

**The extraction and amplification of DNA of bacteria**

In the study of microbial diversity, the first step is DNA extraction, using the D5625 DNA extraction kit.

The 16S rRNA genes were amplified using two types of PCR primers. One type of general primer included 357F (5’-CCGCGCGCGCGCGCGCCGCCGCCGCCCCC-3’) and 518R (5’-ATTACCGCGGCTGCTGG-3’), and the other included 16S-1 (5’-CGGCCGCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCC-3’) and 16S-2 (5’-TGTCCTCAGTCCGATGTGCC-3’).

**Cloning and sequencing of DGGE bands**

In order to further analyze the dominant bacterial strains and microbial diversity, the target bands were collected, the bacteria in the bands were cloned, and the microbial species were identified. The target bands were cloned by the Takara pMD™18-T Vector (TaKaRa Biotechnology Co., Ltd, Dalian, China). Cloned inserts were amplified from lysed colonies by PCR with the plasmid-vector specific primers M13F (5’-GTAAAACGACGGCCAG-3’) and M13R (5’-CAGGAAACAGCTATGAC-3’). Clones were sequenced with an ABI 3730 automated sequencer (Invitrogen, Shanghai, China). The sequencing results of DGGE bands were compared to sequences available in public databases using the Basic Local Alignment Search Tool from the National Center of Biotechnology Information (Altschul et al. 1997).

**RESULTS AND DISCUSSION**

**PVA reactor startup**

Influent pH ranged from 7.5 to 8.5, the reactor was started with an organic loading (OLR) of 0.5 kg COD/m³ d, on the 7th day, influent COD, BOD₅ and NH₄⁺-N were 221.81, 141.83 and 36.86 mg/L, respectively. On the 14th day, the removal rates of COD, BOD₅ and NH₄⁺-N were more than 73, 90 and 80%, respectively. The removal efficiency of the PVA reactor was stable, which implied that the PVA reactor start-up was finished.

Using active sludge as an inoculum, the start-up of the CAS process has to take four months or more (Third et al. 2008; Nutchanat & Suwanchai 2011). However, the start-up of the PVA reactor can be achieved in 14 days.

**Increase of OLR**

When the BOD₅ removal efficiency was above 90% for more than 2 days, the authors increased the OLR in stages by 0.5 kg COD/m³ d for each step. The treatment efficiency of the PVA process for the 30-day experiment is shown in Figure 2.

Figure 2 shows that as the OLR was increased to 1.0 kg COD/m³ d from the 11th to the 14th day, the COD removal efficiency constantly increased to a peak of about 90%. As the OLR was increased to 1.5 kg COD/m³ d from the 15th to the 21st day, the COD removal efficiency fluctuated but remained above 75%. When the OLR was increased to
2.0 kg COD/m³ d on the 23rd day, the hydraulic retention time (HRT) in the PVA reactor decreased to around 0.5 h. Due to inadequate HRT, the COD was not completely degraded by microorganisms in the PVA reactor. The COD removal efficiency dropped to 55% from the 27th to the 30th day, and the removal efficiency was steady at around 68%. During the whole process, the BOD₅ removal efficiency was always above 90%. In addition, when the OLR was increased to 1.0 kg COD/m³ d, NH₄⁺-N removal efficiency dropped to 30%, and as the OLR increased to 1.5 kg COD/m³ d, the NH₄⁺-N removal efficiency increased gradually to 78%. However, when the OLR increased to 2.0 kg COD/m³ d, the NH₄⁺-N removal efficiency was steady at around 50%.

The results show that as the OLR was increased to 1.5 kg COD/m³ d, the PVA process improves the treatment of this sewage. The removal efficiency of COD and BOD₅ reached more than 73 and 90%, respectively, and the NH₄⁺-N removal efficiency reached 78%.

### Optimization of operating temperature

Previous studies have shown that temperature has a great effect on the efficiencies of biological treatment processes (Krishna & Van Loosdrecht 1999; Gomec et al. 2008; Khanh et al. 2011), so operating temperature of 20, 25 and 30 °C were tested. The results are shown in Table 1.

As shown in Table 1, the removal efficiency decreased with the decreasing of temperature. The temperature had a great influence on the removal efficiency of COD and NH₄⁺-N, but it had less of an effect on BOD₅. When the authors set the operating temperature at 30 °C (OLR = 1.5 kg COD/m³ d), the COD, BOD₅ and NH₄⁺-N removal efficiency reached more than 68, 90 and 61%, respectively. Based on this, in the following study, the operating temperature was set at 30 °C.

### Performance of the PVA process

In order to test the performance of the PVA treatment process, the authors continued the experiment with an HRT of 2–3 hours (OLR, 1.5 kg COD/m³ d). The results are shown in Figure 3.

Figure 3(a) shows that the influent COD, NH₄⁺-N and BOD₅ were 141–259, 22–49 and 97–155 mg/L, respectively. Effluent COD, NH₄⁺-N and BOD₅ were 14–57, 0.1–15 and

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**Table 1 | Optimization of operating temperature**

<table>
<thead>
<tr>
<th>Days</th>
<th>Temperature (°C)</th>
<th>COD removal efficiency</th>
<th>NH₄⁺-N removal efficiency</th>
<th>BOD₅ removal efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>Standard deviation %</td>
<td>Standard deviation %</td>
</tr>
<tr>
<td>33–45</td>
<td>30</td>
<td>68–79</td>
<td>3.10</td>
<td>61–92</td>
</tr>
<tr>
<td>46–58</td>
<td>25</td>
<td>52–69</td>
<td>5.73</td>
<td>58–76</td>
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<tr>
<td>59–71</td>
<td>20</td>
<td>47–56</td>
<td>3.04</td>
<td>40–51</td>
</tr>
</tbody>
</table>
6–10 mg/L, respectively. Removal efficiency of the COD, \( \text{NH}_4^+ \)-N and \( \text{BOD}_5 \) were 68–93, 66–99 and 93–96%, respectively.

**Figure 3(b)** shows that the influent SS and turbidity were 180–380 mg/L and 57–68 NTU, respectively. Effluent SS and turbidity were reduced to 20–38 mg/L and 2–4 NTU, respectively. Removal efficiencies of SS and turbidity were 88–94 and 92–97%, respectively.

In addition, the colony count of the influent, the effluent from sedimentation tank and the effluent from ultraviolet disinfection were around \( 4.5 \times 10^8 \), \( 1.5 \times 10^8 \) and \( 1.0 \times 10^4 \) CFU/L, respectively, indicating that the colony count of the sewage was reduced.

**Removal efficiency of every processing unit**

In order to determine which processing unit played the most important role in the PVA process, we made a comparison of the effluent quality of every processing unit. The results are shown in **Figure 4**.

**Figure 4** shows that 65–85% of the COD and 59–98% of the \( \text{NH}_4^+ \)-N were removed by the PVA reactor. Only 3–8% of
COD and 1–7% of NH$_4^+$-N were removed by the two active sludge tanks.

Next, under the same operating condition, the two sludge tanks were placed first, followed by the PVA reactor. The performance of this sequence is shown in Table 2.

Table 2 shows that 20–44% of the COD and 29–44% of the NH$_4^+$-N were removed by the two active sludge tanks, and 21–46% of the COD and 12–38% of the NH$_4^+$-N were removed by the PVA reactor. The final effluent COD and NH$_4^+$-N were higher than the previous experiment (the PVA reactor was placed first, followed by the two sludge tanks), therefore, it is more suitable that the PVA reactor was used as the lead unit.

Observations of the PVA-gel beads surface

In order to analyze the characteristics of the microorganisms in the PVA process, the authors observed the change in the PVA-gel beads and the enrichment of microorganisms.

The color of PVA-gel beads surface changed from the initial white to yellow after 30 days of operation, and

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Performance of every processing unit when the two sludge tanks were placed first, followed by the PVA reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>Influent COD (mg/L)</td>
</tr>
<tr>
<td>1</td>
<td>205.7</td>
</tr>
<tr>
<td>2</td>
<td>155.7</td>
</tr>
<tr>
<td>3</td>
<td>177.4</td>
</tr>
<tr>
<td>4</td>
<td>168</td>
</tr>
<tr>
<td>5</td>
<td>259.8</td>
</tr>
<tr>
<td>6</td>
<td>175.1</td>
</tr>
<tr>
<td>7</td>
<td>190.6</td>
</tr>
<tr>
<td>8</td>
<td>150.8</td>
</tr>
<tr>
<td>9</td>
<td>169.1</td>
</tr>
<tr>
<td>10</td>
<td>178.5</td>
</tr>
</tbody>
</table>

Figure 4 | Performance of every processing unit.
mature PVA-gel beads became red brown after 85 days, as shown in Figure 5.

The red brown strains seen in Figure 5 on the surface of the beads after 85 days shows that the quantity of microorganisms attached to the PVA-gel beads increased with time. At this time, the NH$_4^+$-N removal efficiency reached 96%. Related studies have found that when ammonia nitrogen sewage is treated with PVA-gel beads, the color of the beads changes to red brown (Ali et al. 2015).

It can be speculated that some strains inside the PVA-gel beads were nitrifying bacteria, and this is why the process had high NH$_4^+$-N removal efficiency.

**Microorganism enrichment in PVA-gel beads**

In order to investigate the characteristics of microorganisms attached to the surface and the internal structure of PVA-gel beads, the authors observed the PVA-gel beads by scanning electron microscope (SEM). Figure 6 shows the results.

As shown in Figure 6(a), an unused PVA-gel bead has a large number of micro-holes. Figure 6(b) shows a layer of dense bio-film formed around the PVA-gel bead after 127 days, indicating a large number of microorganisms attached to the surface. Figure 6(c) and 6(d) show a large number of *Sphaerita, Filamentous fungi* and *Bacillus* attached to the PVA bead surface and internal structure, leading to an efficient degradation of pollutants. Biomass attachment to the PVA-gel beads was up to 0.41 g VSS/g PVA-gel bead after 127 days, suggesting that the PVA-gel beads perform well for adsorption of microorganisms.

**Denaturing gel gradient electrophoresis of PCR product**

The denaturing gel gradient electrophoresis (DGGE) technology can separate the PCR products and analyze the

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**Figure 5** Observation of PVA-gel beads: (a) surface on day 1, (b) after 30 days, and (c) after 85 days. Please refer to the online version of this paper to see this figure in color: 10.2166/wrd.2017.181.

**Figure 6** SEM micrographs of PVA-gel beads: (a) surface of an unused PVA gel bead (×5,000), (b) surface of PVA gel bead after 127 days (×100), (c) bacteria on surface of the bead after 127 days (×5,000), and (d) bacteria inside the PVA gel bead after 127 days (×5,000).
properties of bacteria strains. Figure 7 shows the DGGE profile of bacterial amplicons from PVA-gel beads samples.

There are two lanes in the DGGE profile. The first lane was created by the primers 357 F and 518 R. There are fewer bands on the first lane, and the separate bands are fuzzy. The second lane was generated by the primers 16S-1 and 16S-2. There are more bands than the first lane, and the separate bands are clearer. Bands 7 and 9 are particularly bright, indicating that the dominant bacterial strains may exist in those bands. The rest of bands are dark compared to bands 7 and 9, but they can still be seen clearly. The bacterial diversity is striking. Bacterial 16S rRNA gene sequences are shown in Table 3.

Based on the similarity and consistency of the test sequences, Table 3 shows that operational taxonomic unit (OTU) 1 includes six cloned bands. These bands belonged to Clostridiaceae bacterium with a similarity percentage of 99%. OTUs 2, 3 and 4 included two cloned bands with similarity percentages of 97%. OTU 2 belonged to Alpha proteobacterium, OTU 3 belonged to Phenylobacterium haematophilum, and OTU 4 belonged to Rhodobacter sp. For OTU 5–16, each OTU included one clone band. Again, it

<table>
<thead>
<tr>
<th>Operational taxonomic unit (OTU)</th>
<th>Number of cloning</th>
<th>Best match database (GenBank accession number)</th>
<th>Representative sequence (GenBank accession number)</th>
<th>Similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Clostridiaceae bacterium WN011</td>
<td>AB298726</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Alpha proteobacterium A0839</td>
<td>AF236002</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Phenylobacterium haematophilum (T); type strain: LMG 11050</td>
<td>AJ244650</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Rhodobacter sp. HME8657</td>
<td>JX219390</td>
<td>97</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>Uncultured bacterium; 136ds10</td>
<td>AY212586</td>
<td>96</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>Schlegelella thermodepolymerans</td>
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<td>97</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>Uncultured bacterium copi29</td>
<td>AY563456</td>
<td>96</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>Sphingopyxis sp. Geo24</td>
<td>DQ137852</td>
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<tr>
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<td>1</td>
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<td>FJ660540</td>
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<td>Uncultured bacterium</td>
<td>JX133355</td>
<td>95</td>
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<tr>
<td>16</td>
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<td>Lysobacter brunescens ncd2191d04c1</td>
<td>KC252873</td>
<td>95</td>
</tr>
</tbody>
</table>
can be inferred that there are a wide variety of bacterial strains in the biological treatment system.

These bacteria use their metabolites to degrade the nitrogen in sewage. For example, butyric acid can convert nitrates to N₂ and N₂O. These bacteria metabolize carbohydrates, peptones, and metabolic intermediates, converting this organic matter into simpler substances.

.alpha proteobacteria are divided into five series (\(\alpha, \beta, \gamma, \delta\) and \(\epsilon\)). Some play an important role in the degradation of organic compounds, some are ammonia oxidation bacteria, some are denitrifying bacteria, and some are nitrite-oxidizing bacteria. These bacteria play an important role in the degradation of nitrogen in sewage.

Clostridiaceae bacterium, Alpha proteobacterium, Phenylobacterium haematophilum and Rhodobacter were found to be the dominant bacteria strains in the PVA-gel beads. These results suggest that the PVA performs well in the treatment of sewage.

### Study of excess sludge reduction

The excess sludge yield (Y) was calculated using Equation (1) (Ghyoot & Verstraete 2000):

\[
Y = \frac{g_{SS_{\text{end}}} - g_{SS_{\text{start}}}}{g_{COD_{\text{removed}}}}
\]

The variable \(g_{SS_{\text{start}}}\) represents the total mass of SS present at the start of the experiment, and \(g_{SS_{\text{end}}}\) represents the sum of the masses of the SS present at the end of the experiment plus the SS that have left the reactor with the effluent and waste sludge. On the first day, 20 L of activated sludge (concentration, 9.42 g/L) was added to the PVA reactor, so \(g_{SS_{\text{start}}}\) was 188.40 g. Because no sludge was discharged during the whole operation period, and because there was almost no sludge in effluent, \(g_{SS_{\text{end}}}\) was the total mass of the sludge in the PVA reactor, the two sludge tank and the sedimentation tank at the end of the experiment. After 138 days of operation, the total sludge was collected in a plastic bucket, and the weight of the total sludge was 1,698.50 g, which is \(g_{SS_{\text{end}}}\). According to the record of COD and the amounts of water ingress each day, the total mass of \(COD_{\text{removed}}\) was calculated as 14,512.50 g. Therefore, the excess sludge yield rate was about 0.10 g SS/g COD_{removed}.

Compared to the CAS process, which has an excess sludge yield rate of 0.5 g SS/g COD_{removed}, the excess sludge yield rate of the PVA process is greatly reduced. The PVA process has two active sludge tanks, and only 3–8% of the COD and 1–7% of the NH₄\(^+\)-N were removed by the tanks, so the PVA reactor plays a main role in treatment efficiency and the two tanks play a role in reduction of excess sludge. Some new excess sludge is degraded in these tanks. According to the previous study by Singh et al. (2016), using an applied moving bed biofilm reactor and one active sludge tank to treat municipal wastewater leads to an excess sludge yield rate of 0.18 g SS/g COD_{removed}. This supports the claim that the sludge tanks play a role in the reduction of excess sludge.

### CONCLUSIONS

Rapid start-up of the PVA process was successfully achieved. A large number of microorganisms attached to both the surface and internal structure of the PVA-gel beads, so the process can effectively treat domestic sewage with OLR of 1.5 kg COD/m³·d, the process performed well in treatment of this domestic sewage. Clostridiaceae bacterium, Alpha proteobacterium, Phenylobacterium haematophilum and Rhodobacter were the dominant bacteria strains in the treatment system. The process displayed a good performance in sludge reduction, and the active sludge tanks were found to play a role in the reduction of excess sludge. The excess sludge yield rate was only about 0.10 g SS/g COD_{removed}. This technology provides a new way for upgrading the CAS process without major new construction.

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