A novel integrated step-feed biofilm process for the treatment of decentralized domestic wastewater in rural areas of China

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Abstract

For wastewater treatment in rural areas, a novel three-stage step-feed wastewater treatment system, combined with a drop-aeration biofilm process, was tested in the laboratory to investigate its performance in removing suspended solids (SS), chemical oxygen demand (COD), NH$_4^+$-N, total nitrogen (TN), and total phosphorus (TP). The removal rates of SS, COD and NH$_4^+$-N were 90%, 80%, and 90% in effluent concentrations less than 10 mg/L, 50 mg/L and 8 mg/L, respectively. The TP removal rate was less satisfactory. The C/N ratio in the raw wastewater was often less than 3.5, and the removal efficiency of TN was therefore limited. A carbon-release batch experiment was carried out to measure the feasibility of enhancing denitrification at low influent C/N ratios. The result showed that the C/N could be over 9.0 in the supernatant. Polymerase chain reaction denaturing gradient gel electrophoresis technology was used to reveal the changes in the bacterial community during different stages of the integrated step-feed biofilm process. The results showed that banding patterns and the distribution of dominant bands for the same experimental period in different aerobic zones were similar. Phylogenetic analysis indicated that lanes 10, 11 and 12, which presented three aerobic zones at the same operation period, had the closest phylogenetic relationship among the lanes.

Key words: step-feed; biofilm; removal efficiency; PCR-DGGE; C/N ratio

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Introduction

Currently in rural areas of China, more than 96% of domestic wastewater is discharged directly into aquatic environments without any treatment (MHURDPRC, 2005). To protect lakes, rivers, and other natural water bodies from heavy pollution, the Chinese government has developed and established corresponding laws and criterion. One of the most significant pollution problems in China is the treatment of decentralized domestic wastewater in rural areas, which urgently requires solutions that not only consider actual conditions but also satisfy environmental standards for effluent.

Biological processes are widely used for wastewater treatment. In order to enhance nutrient removal efficiency, many researchers have focused on the development and use of step-feed biological nitrogen removal (SFBNR) (Qiu and Ding, 2003; Tchobanoglous et al., 2003). This SFBNR process generally consists of several stages of denitrification-nitrification reactors in series. Moreover, the energy for internal recycling is saved and the solid retention time (SRT) can be increased by a suspended solids gradient along the reactors (Zhu et al., 2006). Over the last decade or so, many researchers have considered the parameters of design and operation for the step-feed process through theoretical analysis and simulation (Shigeo, 1996; Ju et al., 2006; Zhu and Peng, 2006). The dimensionless model and optimization algorithm for the design and operation of the step-feed process was proposed (Ayesa et al., 1995, 1998), with the four-stage step-feed process able to decrease the whole reactor volume by approximately 25% compared to the conventional denitrification-nitrification process (Larrea et al., 2001). The nitrogen removal efficiency in the step-feed process might be 66% when the influent flow rate of the second stage was 35% among the total influent flow (Kitaya et al., 1994). The influent flow rate distribution ratio along the step-feed process under different influent C/N ratios has showed that total nitrogen removal efficiency was high, over 95%, and could be achieved without an internal nitrate cycle or external carbon source under some influent flow rate distributions (Zhu et al., 2005, 2007). In 1996, the step-feed process was adopted in the Newtown Creek Wastewater Treatment Plant reconstructing project to achieve higher nitrogen removal efficiency. The results observed from January 1997 to June 1998 proved that the removal efficiency of bio-chemical oxygen demand (BOD), suspended solids (SS) and total nitrogen (TN) was 82%–86%, 84%–89% and 76%–85%, respectively (Fillos et al., 2002; Sakai and Koike, 1998).

The aim of this study was to develop a decentralized
domestic wastewater treatment system suitable for application in rural areas. The treatment system should be one of low-cost, easy-maintenance, and high efficiency. The SFBNR process mentioned above is a type of activated sludge process. As such, the sludge volume index (SVI) value should increase under high flow rate distribution ratios, leading to sludge bulking (Zhu et al., 2009), high energy consumption for aeration, and sludge returning.

In this study, drop-aeration biofilm technology was combined with the step-feed process. Consequently, a suspended spherical carrier was set in the three-stage anoxic/oxic reactors, thus the energy for sludge recycling was unnecessary. As the effluent from the anoxic unit drops into the next oxic unit, energy consumption is reduced. The overall performance of the step-feed biofilm process was investigated, the microbial community at different stages was studied by molecular biological detection, and the carbon source release under anoxic conditions was tested to optimize the reactor structure for enhancing nitrogen removal.

1 Materials and methods

1.1 Integrated step-feed biofilm process

The experimental system is shown in Fig. 1. A three-stage integrated step-feed biofilm reactor made of plexiglass with a working volume of 42 L was used. The working volume of the three-stages was 15.5, 15.5 and 11 L, respectively. Each stage consisted of an anoxic and oxic zones (separated by clapboards), and the volume ratio of anoxic to oxic was 1:3.5, 1:3.5 and 1:3.25, respectively. The effluent from the anoxic zone flowed down into the next oxic zone with the height difference of 300 mm. Two kinds of suspended spherical carrier with diameters of 40 mm and 20 mm were set in all the stages. The process was an anoxic/oxic alternant submerge biofilm. One air compressor was used for aeration, and three air flow meters were utilized to control the airflow rate. A vertical secondary clarifier was set in the inner cylinder with the working volume of 9.5 L.

1.2 Operating conditions

The start-up of the integrated step-feed biofilm process was initiated by seeding domestic wastewater of a residential area in the Research Center for Eco-Environmental Sciences (RCEES), Chinese Academy of Sciences. The inoculated sludge was obtained from an integrated oxidation ditch with vertical circle wastewater treatment system in RCEES. The process was operated in batch mode to offer the initial colonization and accumulation of microorganisms in the beginning 10 days, and then was operated in continuous inflow mode with a gradually increasing flow to enhance bacterial growth. After 30 days of operation, a steady state was obtained and a compact biofilm was formed on the suspended spherical carrier.

### Table 1 Operation parameters of the process

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First stage</th>
<th>Second stage</th>
<th>Third stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>12–26</td>
<td>12–26</td>
<td>12–26</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>&lt; 0.2</td>
<td>&lt; 3.5</td>
<td>&gt; 0.2</td>
</tr>
<tr>
<td>Inflow ratio</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>HRT (hr)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

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Fig. 1  Integrated step-feed biofilm process for wastewater treatment: unwrapping along with generatrix. (I) first stage; (II) second stage; (III) third stage; (IV) secondary clarifier; (1) influent tank; (2) check valve; (3) feed pump; (4) diffuser; (5) air flow meter; (6) air compressor; (7) perforated pipe; (8) suspended spherical filler in anoxic reactor; (9) suspended spherical filler in oxic reactor; (10) effluent; (11) waste sludge.
The operation parameters of the testing process are listed in Table 1. During the experimental period, the temperature of the wastewater in the integrated step-feed biofilm process was 12–26°C. The dissolved oxygen (DO) levels for the three aerobic zones were maintained at 2.0–3.5 mg/L, while the DO levels for all the anoxic zones were below 0.5 mg/L. The values of the oxidation reduction potential (ORP) in all the anoxic zones were maintained between –200 to –100 mV. The influent flow ratio among the three process stages was controlled at 5:4:3, and the total hydraulic retention time (HRT) was 12.3 hr (including 2.3 hr in the secondary clarifier). Moreover, it was hard to provide the HRT of each stage in the integrated step-feed biofilm process (Zhu, 2006). The raw wastewater was pumped into the influent tank regularly after passing through a grit chamber. Three peristaltic pumps (Mode Z1515, Lange Bump Co., China) were used to control the influent flow to each stage. There was no sludge return in the process; therefore, the energy required for return pump consumption was reduced. A small amount of waste sludge was released to the municipal sewage system approximately once every 80 days. Moreover, the intensive fluctuation of the wastewater quantity and quality could be buffered due to the three stages influent.

1.3 Carbon released in anoxic condition

A batch experiment was carried out to evaluate the ability of activated sludge to release carbon under long-term anoxic (anaerobic) situations. The sludge used in this study was obtained from the aerobic zone tank of a municipal wastewater treatment plant in Beijing (A²/O process, 400,000 m³/day), and was conditioned to 7.33 g/L. It was then transferred into 250 mL triangle bottles with a volume of 200 mL. Nitrogen gas was used to excrete the air, and the bottles were sealed with a rubber plug immediately to create the appropriate anoxic (anaerobic) conditions. The samples were placed in a continuous shaker at 30°C and 160 r/min. The samples were taken out at different times, and polyethylene syringes were used to extract the supernatant from the solution, which was then filtered through a 0.45-µm filter paper. The concentrations of chemical oxygen demand (COD), NH₄⁺-N and TN in the filtrate were then analyzed.

1.4 Analytic methods

The temperature, DO and ORP were regularly measured with a WTW-Multi 340i analysis instrument (Germany), and the probes were CellOx325 and SenTix ORP Electrode, respectively. The SS, COD, NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, TN, and total phosphate (TP) in both influent and effluent were analyzed weekly according to standard methods (APHA, 1998; CEPB, 2004).

A number of suspended spherical carriers at different stages were removed at four different periods and transferred into glass beakers. After adding the sterile water, the mixed solution, which included an abundant microbial community, was obtained after ultrasound treatment. The microbial communities in the mixed solution were determined by PCR-DGGE technology.

DNA was extracted according to the instructions on the test kit (Omega), agarose gel electrophoresis of 8 g/L was used to observe possible DNA strap. The PCR primers for 357f (5’-CGC CCG CCG CGC CGG GGC GCG GGG GCA CGG GGG GGC TAC GGG AGG CAG CAG-3’) and 518r (5’-TTA CCG CCG CTG CTG G-3’) (Sánchez et al., 2007) were used to amplify the segment of subbacterial 16S rDNA. The GC-clamp was added to the PCR primers to facilitate the DGGE. The PCR amplification reaction was performed using a bio-rad PTC-200 Peltier thermal cycler at a final volume of 50 µL PCR. The reaction mixture contained 20 pmol of each primers, 20 µmol of each dNTPs, 5 µL of 10 × buffer, and 1.25 units of Taq DNA polymerase. The temperature of cycling conditions were 94°C for 3 min, followed by 31 cycles at 94°C for 30 sec, 55°C for 30 sec and 72°C for 1 min. The final extension was at 72°C for 10 min. A 5-µL aliquot of the PCR product was separated with a 1.5% (W/V) agarose gel at 100 V for 30 min to verify amplification prior to DGGE.

For the DGGE analysis, the PCR product generated from each sample was separated by an 8% (W/V) acrylamide gel with a linear denaturant gradient increasing from 30% to 60% (Bio-Rad, USA). DGGE was performed using 30 µL of the PCR product in 1 × TAE buffer at 60°C and 90 V for 720 min. The gel was then visualized using Gel Red (Biotium, USA). The images were analyzed by Quantity One Software (Bio-Rad, USA). The bacterial diversity was estimated by the following Shannon index (H) equation:

\[ H = - \sum (n_i/N) \ln(n_i/N) \]

where, \( i \) is the number of bands in each DGGE gel profile, \( n_i \) is the peak height of each band \( i \), and \( N \) is the sum of the peak heights in a given DGGE gel profile.

2 Results and discussion

2.1 Performance of integrated step-feed biofilm system

The performance of the integrated step-feed biofilm system for wastewater treatment was examined during 290 days. The removal efficiencies for SS, COD, NH₄⁺-N, and TP are shown in Fig. 2. For SS removal during the steady operation period, removal efficiency was above 90% for concentrations in the effluent of less than 10 mg/L. In addition, SS in the influent ranged from 30 to 220 mg/L, and the concentrations during the middle operational period (summer) were lower than in the initial (spring) and ending phase (winter), respectively. The concentration of COD in the influent showed a similar tendency, with a range of 51–508 mg/L and a removal efficiency around 80%. The perfect state for NH₄⁺-N removal was obtained after 40 days, as the nitrobacteria needed a relatively long time to reach stable growth. The results of the high removal efficiency of NH₄⁺-N could be attributed to the characteristics of the biofilm adhered on the carrier, which offered long sludge retention time for nitrobacteria accumulation. The concentration of NO₃⁻-N
Fig. 2 Removal efficiency of SS, COD, NH\textsubscript{4}\textsuperscript{+}-N and TP.

Fig. 3 Concentration of NO\textsubscript{2}\textsuperscript{−}-N in effluent.

Fig. 4 DGGE patterns of amplified 16S rDNA from the media samples in the step-feed biofilm process during operation. Lanes of 1, 2, and 3; 4, 5, and 6; 7, 8, and 9; and 10, 11, and 12 represented the strains in the three aerobic zones on day 130, day 180, day 220 and day 260. Arrows indicate functional bacteria in the microbial community.

The number of bands revealed the relative diversity of the bacterial community, while the intensity of each band was correlated to the degree of abundance constituting every bacterial group (Chung, 2007). As shown in Fig. 4, the lanes of 1, 2, and 3; 4, 5, and 6; 7, 8, and 9; and 10, 11, and 12 depicted the bacterial community of the three aerobic reactor zones in the three-stage step-feed biofilm process for the four operational periods, respectively. Lanes 13, 14, and 15 represented the population diversities in the three anoxic zones for the different stages during winter. According to the DNA-fingerprints (Fig. 4), generally, banding patterns and the distribution of dominant bands in different aerobic zones (lanes 1–12) did not change significantly, which indicated that the contamination in

in the effluent was also monitored (Fig. 3), and the results revealed that the nitrite was not accumulated in the step-feed reactor, as NH\textsubscript{4}\textsuperscript{+}-N was efficiently transformed to NO\textsubscript{3}\textsuperscript{−}-N by the nitrobacteria. Thus, the SS, COD and NH\textsubscript{4}\textsuperscript{+}-N in the effluent were all less than the values (10, 50 and 8 mg/L) required in the standard of class A (GB 3838-2002, Environmental Quality Standards for Surface Water). However, the removal capacity of TP in the integrated step-feed biofilm system was not as effective as that for SS, COD and NH\textsubscript{4}\textsuperscript{+}-N. Results showed that TP efficiency was typically below 15%, with the highest value only at 40%. Therefore, an ecological treatment unit could be followed for further TP removal.

2.2 Detection of differences and changes in microbial community composition by PCR-DGGE

PCR-DGGE was used to compare the 16S rRNA gene fragment profiles of the eubacteria in the samples collected during different operational periods and at each stage of the integrated step-feed biofilm wastewater treatment process.
the raw wastewater was relatively stable. The variation in the composition of the bacterial community in the three aerobic reactor zones, however, demonstrated that the number of bands increased over 290 days of operation.

One remarkable band A (lane 1) was observed on day 130, which became weaker over the following days. A reasonable explanation is that strain A was exposed to higher contaminant levels during the early stages of the experiment; however, as time passed the concentration of the substrate that induced band A decreased. Therefore, the density of the band became lighter. The dominant and intense band B (lane 7), which appeared on day 220, was darker than during former operational periods (lanes 1–6). A similar shift trend was observed in lanes 8 and 9 during the same period. This might be the result of a rapid increase in B-degrading bacteria that acclimated to the corresponding contaminant. However, according to the DNA-fingerprints of lanes 10, 11, and 12, the density of band B became obviously lighter in the following months. Compared with other bands, the intensity of band E showed no obvious change during the 290 days of the experimental period, which indicated that both the number of strain E and its corresponding substrate concentration were relatively stable. Based on the observations of the bacterial community in Fig. 4, the bands A, D, E, and I were found in almost all lanes, irrespective of aerobic or anoxic reactor zones. This indicates there were certain facultative bacteria that were adaptive to both aerobic and anoxic conditions.

The DGGE gel profile was also analyzed statistically using the phylogenetic chart and the Shannon index. The phylogenetic relationship of different lanes is shown in Fig. 5. The results revealed that the phylogenetic relationship between lanes 10, 11, and 12, which belonged to the same period in different aerobic zones, was the closest among all of the lanes. The second closest relationship was for lanes 13, 14, and 15, which were in three anoxic zones, on day 260. The Shannon index revealed the relative intensity of the bands and the diversity of the population (Ding et al., 2008). The distinction of the Shannon index among the lanes is shown in Fig. 6, and reflects the altered diversity of different bacterial communities. In aerobic reactor zones (lanes 1–12), the total diversity of the population in three lanes of the same operational period, showed an increasing trend with time, that is: lanes 10, 11, 12 > lanes 7, 8, 9 > lanes 4, 5, 6 > lanes 1, 2, 3. This is likely the result of increasing substrate concentration (i.e., COD and NH$_4^+$-N in the influent).

2.3 Effect of C/N ratio for nitrogen removal efficiency

The capacity of the integrated step-feed biofilm process in TN removal is shown in Fig. 7. The TN in the influent fluctuated intensively during the operational period, with values that ranged from 40 to 138 mg/L. Generally, the TN concentration in the effluent in the middle phase (summer) was lower than in the initial (spring) and ending phases (winter), as was the change of its removal efficiency. One reasonable explanation may be that the temperature in winter was often below 15°C, and the denitrification bacteria was temperature sensitive, thereby decreasing the denitrification rate. In addition, the ratio of C/N in the influent was lower than 3.5 for most of the operational period (Fig. 7). Due to previous research, however, when the influent C/N ratio was more than 4.91, good denitrification could be obtained (Zhu, 2006). In a conventional four-stage step-feed biological nitrogen removal process, when the influent C/N was at 3.59, 2.52, 2.14 and 1.92, the TN removal efficiency could be enhanced to 68.2%, 62%, 55.2% and 46%, respectively, through adjusting the influent flow distribution in different stages and optimizing the volume of the anoxic and oxic zones (Zhu et al., 2009). In this study, considering the practical application in rural areas, the volumes of all the anoxic/oxic zones were fixed. In addition, raw wastewater quality continuously fluctuates unlike the synthetic wastewater in previous research (Zhu et al., 2007; Zhu et al., 2009). The performance of TN.
therefore, was not so perfect, with removal rates ranging from 38% to 75% when the influent C/N was at 2.0–3.5.

2.4 Carbon release in anoxic condition

As shown in Fig. 7, carbon was often scarce in the raw wastewater, which resulted in poor TN removal capacity. Previous research on supernatant in sludge thickener revealed that many organic compounds would be released from condensed sludge by anaerobic digestion (Hu et al., 1999). In order to enhance the TN removal efficiency under low C/N ratios, the carbon source release under long time anoxic situations was investigated by a batch experiment (Fig. 8). In the initial 12 hr, the COD was increased slowly, after which the concentration of the COD in the supernatant increased sharply to a stable level. Until hour 96, the COD concentration tended to be in equilibrium. The small molecular weight organic compounds held major parts after 24 hr (Hu et al., 1999) which might be utilized by denitrification bacteria as a carbon source. In addition, the concentration of NH$_4^+$-N and TN did not change in the initial 12 hr but did begin to accumulate after this time. The C/N ratio in the supernatant, however, was always kept over 7.0, and 9.0 was obtained from the 48th hr to the 120th hr. When the influent C/N ratio reached 6.75, 90% TN removal efficiency could be achieved in a four-stage step-feed system (Zhu et al., 2007). The result of the batch experiment indicated that aerobic sludge under extended anoxic conditions could supply a carbon source as compensation when the influent C/N ratio was low. Thus, in order to obtain high sludge concentration and age, the volume of the anoxic zone in different stages of the step-feed process should be designed large enough to deposit and accumulate the sludge from the prior aerobic zone. In addition, the inflow loads should be decreased to maintain longer HRT as the denitrification rate is influenced by temperature variation, that is, denitrification rate decreases when the temperature is low.

3 Conclusions

The integrated step-feed biofilm wastewater treatment process performed very well for SS, COD and NH$_4^+$-N removal during the steady operational periods, and their removal efficiencies were measured at 90%, 80%, and 90%, respectively. The ability of this process to remove TP was poor however.

The TN removal efficiency was affected by the C/N ratio in influent. The results of the batch experiment revealed that the aerobic sludge released carbon and thus the C/N ratio could be increased to 9.0 under specific anoxic conditions. This indicated that large volume anoxic zones would be of benefit for denitrification.

The results of the PCR-DGGE analysis revealed that the diversity of bacterial populations increased as the substrate concentration varied, and certain facultative bacteria, (bands A, D, E and I) were distributed in almost all lanes and in both aerobic and anoxic reactor zones. The lanes 10, 11, and 12 were the closest among the twelve lanes according to the phylogenetic chart, which proved that the dominant strains were similar in the three aerobic zones at the same study period.

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References


