Enhancing nitrogen removal efficiency in a dyestuff wastewater treatment plant with the IFFAS process: the pilot-scale and full-scale studies
Yanjun Mao, Xie Quan, Huimin Zhao, Yaobin Zhang, Shuo Chen and Tao Liu

ABSTRACT
The activated sludge (AS) process is widely applied in dyestuff wastewater treatment plants (WWTPs); however, the nitrogen removal efficiency is relatively low and the effluent does not meet the indirect discharge standards before being discharged into the industrial park’s WWTP. Hence it is necessary to upgrade the WWTP with more advanced technologies. Moving bed biofilm processes with suspended carriers in an aerobic tank are promising methods due to enhanced nitrification and denitrification. Herein, a pilot-scale integrated free-floating biofilm and activated sludge (IFFAS) process was employed to investigate the feasibility of enhancing nitrogen removal efficiency at different hydraulic retention times (HRTs). The results showed that the effluent chemical oxygen demand (COD), ammonium nitrate (NH₄⁺-N) and total nitrogen (TN) concentrations of the IFFAS process were significantly lower than those of the AS process, and could meet the indirect discharge standards. PCR-DGGE and FISH results indicated that more nitrifiers and denitrifiers co-existed in the IFFAS system, promoting simultaneous nitrification and denitrification. Based on the pilot results, the IFFAS process was used to upgrade the full-scale AS process, and the effluent COD, NH₄⁺-N and TN of the IFFAS process were 91–291 mg/L, 10.6–28.7 mg/L and 18.9–48.6 mg/L, stably meeting the indirect discharge standards and demonstrating the advantages of IFFAS in dyestuff wastewater treatment.

Key words | dyestuff wastewater, integrated free-floating biofilm and activated sludge (IFFAS), nitrogen removal, simultaneous nitrification and denitrification (SND), upgrading

INTRODUCTION
Large quantities of dyestuff wastewater in China are generated from dyestuff-producing and dyestuff-consuming industries each year. Nitrogen-containing dyestuffs (such as azo dyes) account for approximately 70% by mass of all dyestuffs used worldwide, making them the largest group of synthetic dyestuffs which are released into the environment. Since these dyestuffs are nitrogen-containing polycyclic and heterocyclic compounds, the dyestuff wastewater is not only toxic, but also contains significant amounts of total nitrogen (usually higher than 50 mg/L) (Li et al. 2015; Le et al. 2016). Thus, nitrogen removal is necessary in the dyestuff wastewater treatment. Biological nitrogen removal (BNR) processes as conventional processes are often the most economical technologies when compared to other treatment methods. Among these BNR processes, the anaerobic/aerobic (A/O) process with activated sludge (AS) system has been widely used in most dyestuff wastewater treatment plants (WWTPs), because of the rich practical experiences obtained from the long-term application (Bai et al. 2016).

Nitrogen removal in the AS process is typically achieved by autotrophic nitrification under aerobic conditions followed by heterotrophic denitrification under anoxic conditions. Autotrophic nitrifiers have low growth rates, long generation cycles (5–8 days), and their growth can be inhibited due to the competitive weakness (e.g. dissolved oxygen; DO) with heterotrophic bacteria during the degradation process of the substrate; therefore nitrification is generally the rate-limiting step in nitrification–denitrification process (Hibiya et al. 2000). Hence, a longer hydraulic retention time (HRT), directly affecting the infrastructure and
operating costs in the A/O process, it is necessary for a longer sludge retention time (SRT) for the growth of nitrifiers and denitrifiers to accomplish nitrification and denitrification (Xia et al. 2016). In addition, the long HRT of the AS process could result in reduced pollutant removal efficiency and increased operating costs. Hence, an effective method for modifying the A/O process with the AS system to enhance nitrogen removal efficiency is needed to ensure that effluent nitrogen concentration meets the increasingly stringent discharge standard.

The integrated free-floating biofilm and activated sludge (IFFAS) process, a hybrid process combining microorganisms between flocs and biofilm by incorporating suspended carriers into aeration reactors, has been successfully used as the favored biological treatment system (Ge et al. 2014). Compared with the AS process, the IFFAS process has higher volumetric loading rates and thus shorter HRTs for economical operation. Also, it can provide a longer SRT for the growth of nitrifiers and denitrifiers, owing to the attachment of the biomass on the carriers (Li et al. 2012).

As a result, with prolonged SRT, the IFFAS process is capable of improving nitrogen removal efficiency at a short HRT (Bassin et al. 2012). Furthermore, simultaneous nitrification and denitrification (SND) can occur in the IFFAS process, because nitrifiers and denitrifiers can coexist on the suspended carriers in aerobic reactors (Xia et al. 2008). Therefore, the IFFAS process could be an attractive option for enhancing nitrogen removal efficiency in dyestuff wastewater treatment.

A pilot-scale IFFAS process was operated at different HRTs to test the full-scale process design, and the performance of the IFFAS process was evaluated in comparison with that of the full-scale AS process. Furthermore, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and fluorescence in-situ hybridization (FISH) were used to evaluate the difference in microbial community composition and distribution between the IFFAS and AS processes. Subsequently, the IFFAS process was applied to the upgrading of a full-scale AS process, and the pollutants removal efficiency and process stability were further investigated.

MATERIALS AND METHODS

Pilot-scale experimental set-up and operating strategy

The AS process-based dyestuff WWTP was located in an industrial park of Northeast China. Its effluent was required to meet the indirect discharge standards (DB 21/1627-2008: chemical oxygen demand (COD) <300 mg/L, NH4+-N <30 mg/L and total nitrogen (TN) <50 mg/L) before being discharged into the industrial park’s WWTP. The biological treatment phase of the dyestuff WWTP consisted of an anoxic tank (2,000 m³) and an aerobic tank (3,400 m³). The treatment capacity was nearly 2,000 m³/d, resulting in a total HRT of 64 hours. The process was operated at both constant sludge recirculation ratio and nitrate liquor recirculation ratio of 100%.

To match the operating conditions of the original AS process (G2), the pilot-scale IFFAS process (G1) consisted of an anoxic reactor (6 m³), an aerobic IFFAS reactor (10 m³) and a secondary sedimentation reactor (6 m³), respectively (Figure 1). The sludge recirculation ratio and nitrate liquor recirculation ratio were 100%. The SRTs were controlled at 10–13 days by discharging the surplus sludge. The DO concentration in the aerobic reactors of these two processes was controlled in the range of 2.0–3.0 mg/L, and the wastewater temperature was in the range of 24–29 °C during the operating period. The suspended carriers with the size of Φ25×10 mm and the density of 0.97 g/cm³ were added into the aerobic reactor of G1 with 30% of the packing ratio. To accelerate the start-up of the moving bed biofilm process and promote the growth of both nitrifiers and denitrifiers, the electrophilic-modified polyethylene (PE) carrier, which was developed by our laboratory and has been commercialized (Mao et al. 2017), was used in the IFFAS process. In order to evaluate the influence of HRT on nitrogen removal efficiency, the inflow rate of G1 was controlled from 16 m³/d to 8 m³/d, resulting in the HRT ranging from 24 to 48 hours. The

![Figure 1](https://iwaponline.com/wst/article-pdf/77/1/70/211739/wst077010070.pdf)
Physicochemical analysis of water quality

The concentrations of COD, NH$_4^+$-N, TN, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were analyzed according to Standard Methods (SM 5220 for COD, SM 4500-NH$_4^+$ for NH$_4^+$-N, SM 4500-N for TN, SM 2540 for suspended solids) (APHA 2005). Biofilm solids were determined by the difference in weight of dried carriers (105 °C for ≥1 hour) before and after removal of biofilm. Removal of biofilm solids was done in H$_2$SO$_4$ (4 M) by mechanical shaking and ultrasonication, followed by thorough brushing. Temperature, DO and pH were measured by WTW Multi 340i meter with DO and pH probes (WTW Company, Germany).

DNA extraction and PCR-DGGE analysis

The carriers with mature biofilm were obtained from the reactor and kept in 1.0 mM phosphate buffer solution (PBS). The biofilm was scraped from the surface of carriers and homogenized using ultrasonic vibration for 10 min. The carriers were subsequently washed three times in PBS and then centrifuged at 12,000 g for 10 min at 4 °C to collect cells. Suspended sludge samples were collected from the aerobic reactor. The sludge samples were used for PCR-DGGE and FISH analysis as follows.

Genomic DNA extraction was performed according to the methods described previously (Lakay et al. 2007). The universal primers for bacteria were 541F (forward primer, containing a 40-bp GC clamp) and 907R (reverse primer), targeting the V3 region of 16S rDNA sequences. Appropriately sized PCR products were confirmed by electrophoresis through 1% agarose gel electrophoresis in 0.5×TAE buffer, followed by staining with Genfinder (Dalian TaKaRa, China). The PCR products were applied to a 6% polyacrylamide DGGE gel with a linear denaturing gradient ranging from 30 to 60% (e.g., 100% denaturing gradient contains 7 M urea and 40% formamide). Electrophoresis was performed at a constant voltage of 180 V for 6 hours in the 1×TAE buffer. Subsequently, the gels were stained with SYBR Gold (Dalian TaKaRa, China) in 1×TAE buffer for 40 min, and gel digital images were obtained using the Gel Doc 2,000 System (Bio-Rad Laboratories, USA). The 16S rRNA fragments were sequenced with Sequencing System ABI PRISM 3730 by a commercial service (Applied Biosystems, USA). Sequence data from 16S rRNA gene fragments were submitted to NCBI for homology searching.

FISH analysis

The sludge samples of the suspended sludge and the biofilm biomass were fixed with 4% paraformaldehyde solution according to the procedure described by Amann et al. (1995). The used probes were labeled with the fluorochromes Cy3, Cy5 and FITC. Hybridization was performed at 46 °C for 180 min adjusting formamide concentrations at the different percentages shown in Table 1. The mounted biofilm samples were visualized with a fluorescence microscopy.
microscope (OLYMPUS IX71, Japan). The number of probe-hybridized cells was counted using Image-Pro Plus 6.0 image analysis software (Media Cybernetics, USA) (Li et al. 2012).

**Calculation of nitrogen removal by SND**

The amount of nitrogen removal through SND and SND efficiency ($R_{SND}$) can be calculated according to Equations (1)–(4).

$$R_{TN} = \frac{N_{inf} - N_{eff}}{N_{inf}} \times 100\%$$  

$$R_{den} = \frac{(A_{inf} - A_{eff}) \times (R + 1)}{N_{inf}} \times 100\%$$  

$$R_{assi} = \frac{MLSS_{surplus} \times V_{surplus} \times f_{VSS/SS} \times f_{N/biomass}}{N_{inf} \times Q} \times 100\%$$  

$$R_{SND} = R_{TN} - R_{den} - R_{assi}$$

where $R_{TN}$ is TN removal efficiency; $R_{den}$, $R_{assi}$ and $R_{SND}$ are the nitrogen removal efficiencies through denitrification, biomass assimilation and SND, respectively; $N_{inf}$ and $N_{eff}$ are the amounts of nitrogen in the influent and effluent, respectively (g/L); $A_{inf}$ and $A_{eff}$ are the TN in the influent and effluent of anoxic reactor, respectively; $R$ is the nitrate liquor recirculation ratio (100%); $Q$ is the influent flow (L/d); $MLSS_{surplus}$ is the surplus sludge concentration (g/L); $V_{surplus}$ is the daily discharge amount of surplus sludge (L); $f_{VSS/SS}$ is the ratio of MLVSS to MLSS in the surplus sludge; $f_{N/biomass}$ represents the nitrogen ratio of the total biomass (12.39%) (Bai et al. 2015).

**RESULTS AND DISCUSSION**

**Effects of HRT on COD and nitrogen removal efficiencies in G1 and G2 reactors**

The COD and nitrogen removal efficiencies of G1 and G2 under different HRTs are summarized in Figure 2(a). With the influent COD fluctuating between 1,795 and 3,119 mg/L during the operating period, the COD removal efficiencies in G1 were 89%, 94% and 95% at the HRT of 24, 32 and

**Figure 2** COD (a), NH$_4^+$-N (b) and TN (c) removal efficiencies of G1 and G2 at different HRTs.
48 hours, respectively (with effluent COD concentrations of 205–291 mg/L, 152–264 mg/L and 106–186 mg/L, respectively). By contrast, the effluent COD concentrations in G2 were 406–499 mg/L, 321–426 mg/L and 310–403 mg/L, with COD removal efficiencies of 80%, 84% and 86%, respectively. The noticeably higher COD removal efficiencies in G1 compared to G2 were probably attributable to the higher biomass concentration in the carriers in G1 (the biofilm concentration was more than 3,600 mg/L, corresponding to 14 g TSS/m² of carrier).

Figure 2(b) illustrates the NH4+-N removal efficiencies of G1 and G2 operating at different HRTs. NH4+-N removal efficiencies clearly increased with the increase in HRT in both G1 and G2, indicating better nitrification performance with longer HRT (Liu et al. 2010). After nine days, the effluent NH4+-N concentrations of G1 were 25.2–35.7 mg/L, 13.2–18.7 mg/L and 4.3–8.6 mg/L, respectively (removal efficiencies of 52%, 76% and 91%), whereas the values for G2 were 29%, 45% and 55% at the HRTs of 24, 32 and 48 hours, respectively. It is worth mentioning that at the HRT of 32 hours, the effluent NH4+-N concentrations of G1 (<30 mg/L) could meet the indirect discharge standard. This result demonstrates that the IFFAS process can improve nitrification performance (Regmi et al. 2011).

Figure 2(c) indicates that longer HRTs favored better TN removal performance. When the influent TN concentrations were 115.8–222.9 mg/L, the effluent TN concentrations of G1 were 55.4–76.5 mg/L, 32.7–44.3 mg/L and 29.0–38.1 mg/L with removal efficiencies of 55%, 78% and 85%, at the HRTs of 24, 32 and 48 hours, respectively. Similar to NH4+-N removal performance, the effluent TN concentration of G1 was less than 50 mg/L at the HRT of 32 hours, which was below the TN discharge standard. By contrast, even when the HRT increased to 48 hours, the effluent TN of G2 was 52–70.4 mg/L, and could not meet the discharge standard. These results demonstrated that, although the IFFAS process was operating at the lower HRT, it could still achieve higher COD and nitrogen removal efficiencies.

As shown in Table 2, TN removal efficiencies via the denitrification (Rden) and biomass assimilation (Rass) were almost the same for G1 and G2 at the same HRT. However, there was a significant difference in the effluent TN from the aerobic reactor, which might be explained by the occurrence of SND in the aerobic reactor (G1). In an aerobic reactor with biofilm carriers, the biofilm on the surface of carriers was divided into different sub-microenvironments on the basis of DO gradient: DO was hard to transfer into the interior of the biofilm, providing an aerobic zone at the surface for nitrifiers and an anoxic/anaerobic zone inside for denitrifiers, and thus facilitated the occurrence of SND. As a result, the TN removal efficiency via SND (R(SND)) of G1 was 21.1–24.9% (Table 2), much more than that of G2 (3.2–3.8%).

### Bacterial community structure analysis

The PCR-DGGE technology was adopted for comparison and analysis of the microbial community in the IFFAS process (G1) and the AS process (G2). The fingerprints and the unweighted pair group mean average (UPGMA) cluster analysis results are presented in Figure 3. Samples S1 and S2 from the AS of G1 and G2 showed a high similarity (95.6%) in the microbial community structure. However, an obvious difference in the community structures was observed between AS and biofilm samples, indicating that the biofilm contained a more diverse microbial community than the suspended sludge, which is in agreement with the report on the microbial diversity of a full-scale printing and dyeing wastewater treatment system by Yang et al. (2012).

The results of species identification are summarized in Table 3. Bands 8, 12, 13 and 14 that existed in the biofilm
sample (S3) showed 99%, 95%, 97% and 98% similarity to uncultured bacterium clone TN4, Azonexus caeni strain Slu-05, Thauera sp. and Denitratisoma oestradiolicum, respectively. Uncultured bacterium clone TN4 (band 8) was the nitrite-oxidizer, which can oxidize nitrite to nitrate (Keluskar et al. 2013). Azonexus caeni strain Slu-05 (band 12), Thauera sp. (band 13) and Denitratisoma oestradiolicum (band 14) were identified as denitrifiers, which exist mainly in anoxic reactors and can use diverse organics for denitrification (Etchebehere et al. 2001; Heylen et al. 2008; Leal et al. 2016). The coexistence of nitrifiers and denitrifiers on the biofilm created favorable conditions for SND in the aerobic reactor of the IFFAS process.

**FISH analysis**

To investigate the bacterial distribution ratios at different HRTs in G1 and G2, the distribution of nitrifier, denitrifier and total bacteria was examined by FISH (Figure S1, available with the online version of this paper). The relative distribution ratios of nitrifiers and denitrifiers to total bacteria in the biofilm of the surface of suspended carriers and AS were examined by FISH technology (Figure 4).

As shown in Figure 4, the distribution ratios of nitrifiers (average 37.4%) and denitrifiers (average 15.4%) in the samples from biofilm of G1 (S3) were higher than those from AS of G2 and G1 (S2 and S1). Hence, more nitrifiers and denitrifiers in the biofilm might be one of the main reasons for the higher nitrification and SND efficiency in G1 than in G2.

During the whole operating period, the distribution ratios of nitrifiers in S3 were relatively stable (in the range of 36.4–39.2%). There were sufficient nitrifiers in G1 (IFFAS process) to maintain high nitrification efficiency. However, the distribution ratios of nitrifiers from S1 and S2 were 22.2% and 24.7% at the short HRT of 24 hours,

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**Table 3** | Sequence analysis of dominant bands obtained from DGGE

<table>
<thead>
<tr>
<th>Band</th>
<th>Closest sequences</th>
<th>Accession number</th>
<th>Identity (%)</th>
<th>Phylogenetic division</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Uncultured sludge bacterium A10</td>
<td>AP234758</td>
<td>97</td>
<td>Alphaproteobacteria</td>
</tr>
<tr>
<td>2</td>
<td>Nitrosomonas sp. JL21</td>
<td>AB000700</td>
<td>98</td>
<td>Betaproteobacteria</td>
</tr>
<tr>
<td>3</td>
<td>Rhodobacter gluconicum</td>
<td>AB077986</td>
<td>97</td>
<td>Alphaproteobacteria</td>
</tr>
<tr>
<td>4</td>
<td>Nitrospira mosconiensis</td>
<td>X82558</td>
<td>97</td>
<td>Nitrospirae</td>
</tr>
<tr>
<td>5</td>
<td>Sphingopyxis sp. SS10.25</td>
<td>KC160718</td>
<td>99</td>
<td>Betaproteobacteria</td>
</tr>
<tr>
<td>6</td>
<td>Betaproteobacterium HTCC304</td>
<td>AY429720.1</td>
<td>98</td>
<td>Betaproteobacteria</td>
</tr>
<tr>
<td>7</td>
<td>Alphaproteobacterium ‘Mena 25/4-1’</td>
<td>Y11584.1</td>
<td>96</td>
<td>Alphaproteobacteria</td>
</tr>
<tr>
<td>8</td>
<td>Uncultured bacterium clone TN4</td>
<td>JX143773.1</td>
<td>99</td>
<td>Betaproteobacteria</td>
</tr>
<tr>
<td>9</td>
<td>Bacterium enrichment culture clone EBa13</td>
<td>KU399797.1</td>
<td>100</td>
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</tr>
<tr>
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<tr>
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<td>Bacterium J10</td>
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<tr>
<td>12</td>
<td>Azonexus caeni strain Slu-05</td>
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<td>95</td>
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</tr>
<tr>
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<td>Thauera sp.</td>
<td>AJ277704.1</td>
<td>97</td>
<td>Betaproteobacteria</td>
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<tr>
<td>14</td>
<td>Denitratisoma oestradiolicum</td>
<td>AY879297</td>
<td>98</td>
<td>Betaproteobacteria</td>
</tr>
</tbody>
</table>
which were lower than those at HRTs of 32 and 48 hours. The probable reason was that shortening the HRT could enhance the wastewater treatment load, but nitrifiers and nitrifying populations, which had low growth rate and poor growth yields, suspended their growth in the AS process, and could easily run out with the effluent under faster flow velocity from the AS system. Therefore the shorter HRT could not provide enough SRT for the growth of nitrifying populations, which had low growth rate and poor tolerances to the fluctuations of influent COD, NH₄-N and TN removal efficiencies in full-scale IFFAS WWTP were almost the same as the pilot-scale G1 reactor (12 hours for the anoxic tank and 20 hours for the aerobic tank), and the COD and nitrogen removal efficiencies in full-scale IFFAS WWTP are shown in Figure 5.

During the operating period of the full-scale IFFAS process, the effluent COD, NH₄-N and TN concentrations were 91–291 mg/L, 10.6–28.7 mg/L and 18.9–48.6 mg/L, respectively, which could stably meet the indirect discharge standard, and the COD, NH₄-N and TN removal efficiencies were 93%, 67% and 77%, respectively. These results indicated that the IFFAS process significantly enhanced not only the tolerance to the fluctuations of influent pollutant concentrations (influent COD, NH₄-N and TN concentrations of 1,744–3,456 mg/L, 48.7–95.0 mg/L and 100.2–208.8 mg/L, respectively), but also the nitrogen removal efficiency.

**CONCLUSIONS**

The IFFAS process was successfully applied to upgrade the AS process for dyestuff wastewater treatment to enhance the nitrogen removal efficiency and wastewater treatment capacity at a shorter HRT. Compared with the original AS process, the COD, NH₄-N and TN removal efficiencies of the pilot-scale IFFAS process improved noticeably, and the
The effluent could meet the indirect discharge standards before being discharged into the industrial park’s WWTP. More nitrifiers and denitrifiers co-existed in the IFFAS process, which benefited from the occurrence of SND in the aerobic reactor of the IFFAS process. Over six months of operation after upgrading, the effluent COD, NH$_4^+$-N and TN of IFFAS process could stably meet the discharge standards. This study provides a simple and economical upgrading strategy for high nitrogen-contained WWTPs for enhancing nitrogen removal efficiency by applying the IFFAS process.

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