Nitrogen removal from wastewater in an anoxic–aerobic biofilm reactor

M. F. Hamoda and R. A. Bin-Fahad

ABSTRACT

A pilot plant, using a four-compartment reactor packed with Biolace media, was operated in the anoxic/aerobic submerged fixed-film (A/ASFF) and the aerobic (ASFF) modes at loadings 0.03 to 0.3 g BOD g⁻¹ BVS d⁻¹, 0.01 to 0.11 g NH₃ g⁻¹ BVS d⁻¹, HRTs 0.7 to 8 h, C/N of 6, and 28 ± 2 °C. The system proved to be very effective in treating municipal wastewater, achieving removals up to 98% for biological oxygen demand (BOD), 75% for chemical oxygen demand (COD) and 97% for ammonia. Performance was not adversely affected by a 10-fold increase in loading rate. Both modes of operation showed high specific nitrification rates up to 96 mg N g⁻¹ BVS d⁻¹, but the A/ASFF was more stable and efficient at higher loadings. Its anoxic stage removed more than 90 and 60% for BOD and COD, respectively. The A/ASFF reactor also achieved denitrification, which eliminated 3.35 mg BOD (or 6.6 mg COD) versus 1 mg denitrified NO₃-N, that resulted in higher organic removals. Denitrification rate increased linearly with the TON (total oxidised nitrogen) loading applied, and specific substrate removal reached up to 114 mg TON g⁻¹ BVS d⁻¹.

Key words | fixed-film processes, nitrification–denitrification, substrate removal rates, wastewater treatment

INTRODUCTION

In line with developments in the water reuse sector, the concerned authorities in arid and semi-arid countries have implemented policies calling for construction of new wastewater treatment plants and upgrading existing plants to cope with increasing flows and produce treated effluents suitable for reuse in irrigation. Currently, treated wastewater effluents in the Arabian Gulf countries are reused extensively in landscape and greenery irrigation and, to a lesser extent, in agricultural lands (Hamoda 2004).

Biological suspended growth systems, such as the activated sludge process, are commonly used for the secondary treatment of municipal wastewaters (Metcalf & Eddy 2003). However, such systems generally suffer from poor nitrification and excessive sludge production. On the other hand, attached growth (fixed-film) systems are gaining much attention for their merits (Ong et al. 2004; Von Sperling 2007; Love et al. 2010).

Attached growth processes are biological systems in which the microorganisms responsible for biodegradation and stabilization of organic matter are attached or fixed to a solid inert media forming a biofilm. Such processes have the advantages of long solids retention, better nitrification, good sludge settleability, low sludge production and stable operation (Lessel 1994; Metcalf & Eddy 2003; Farabegoli et al. 2004; Ryu et al. 2008; Love et al. 2010). A number of innovative processes have been developed, such as the aerated submerged fixed-film (ASFF) process (Hamoda 1989). This process employs a four compartment-in-series reactor equipped with an array of submerged media (fixed ceramic plates) for biomass attachment that is maintained under continuous diffused aeration. Modification of the ASFF process to operate in the anoxic–aerobic (A/ASFF) mode could have some advantages based on studies on other biological systems (Hao & Huang 1996). Moreover, combining both attached growth and suspended growth of microorganisms...
has become a viable option to upgrade the activated sludge process (Odegaard & Rusten 1990; Su & Ouyang 1996; Hamoda & Al-Sharekh 2000). Performance of such hybrid systems requires further investigation.

This study was conducted in order to investigate the effect of hydraulic loading on nitrogen removal in the ASSF hybrid system. A new approach was to alternate the anoxic–aerobic mode in an A/ASFF bioreactor in order to promote nitrogen recovery from wastewater for potential water reuse.

MATERIALS AND METHODS

Description of the pilot plant and experimental set-up

The aerated submerged fixed-film (ASFF) bioreactor was used for conducting the pilot-scale experiments. This reactor is made of 6-mm thick plexiglass sheets and is divided into four equal-sized compartments connected in series. Each reactor has a total liquid volume of 115 l. A pilot plant was installed at Al-Awir WWTP in Dubai (Figure 1).

The experimental programme involved in-parallel testing of ASFF and A/ASFF reactors, each packed with the ‘Biolace’ support medium. Biolace is a structured medium of cross-linked textile fibres which is fixed vertically and stretched in a high-grade stainless steel cage. Five sheets of the Biolace (each 390 mm long and 230 mm wide), spaced at 25 mm, were fixed in each cage, occupying approximately 44% of the compartment’s volume. The Biolace is manufactured by UTS, Germany. It has a specific surface area of 17.5 m²/m³. Each reactor was operated continuously at a preset feed flow rate. Different flow rates were tested in each reactor over a total period of 9 months to obtain HRTs (hydraulic retention times) in the range of 0.7 to 8 hours. Aeration was provided in the second to fourth compartments in the A/ASFF and in all compartments in the ASFF reactors through medium-to-fine, tubular-membrane, air diffusers placed underneath the media and operated at a constant pressure of approximately 200–250 kPa.

Operating parameters

The operating parameters applied in the ASFF and the A/ASFF bioreactors are summarized in Table 1. The reactors were operated at ambient temperature of 28 ± 2 °C. The mass loadings were based on filtered (soluble) biological oxygen demand (BOD) and chemical oxygen demand (COD) concentrations. There are some differences in the operation of the two bioreactors used in this study in terms of estimating the hydraulic and mass loadings. The ASFF bioreactor is classified as an ‘attached growth process’ with minimal suspended growth. In contrast, the A/ASFF
bioreactor involves a hybrid-growth biological process where both ‘attached and suspended growth patterns’ exist because of sludge recirculation. Therefore, estimation of loading rates based on available surface area for fixed-film growth could not be applied to the A/ASFF bioreactor. It will generally undermine the clear contribution of the suspended growth biomass which constituted about 30% of the total biomass.

Loading rates were calculated per total biomass present in the system; that is, per total attached and suspended volatile biomass solids. Mixed liquor volatile suspended solids (MLVSS) and attached volatile solids (AVS) were added up to express the biomass volatile solids (BVS). Hence, hydraulic loading was estimated as m³ per kg BVS per day and mass loading as g BOD (or g COD) per g BVS per day. Internal recirculation was achieved in the A/ASFF bioreactor by: (1) recycling the thickened nitrified sludge (NRS) into the first compartment (anoxic zone) to support anoxic conditions; and (2) circulating the effluent nitrified mixed liquor (NML) into the first compartment to further support the anoxic process, especially at the shorter hydraulic retention times (HRTs) of 1 h or less. Therefore, such internal loading was accounted for in the overall hydraulic loading applied to the system.

### Analytical methods

Samples were collected daily from each reactor and analysed on the same day of collection. The samples were filtered using Whatman Qualitative Filters size 4. The following parameters were determined on the filtrate: BOD₅, COD, ammonia (NH₃-N), nitrites (NO₂-N), nitrates (NO₃-N), and total oxidized nitrogen (TON). Unfiltered samples

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**Table 1 | Operating parameters applied to the pilot plant**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>(a) The ASFF bioreactor</td>
<td></td>
</tr>
<tr>
<td>Flow rate (m³ d⁻¹)</td>
<td>0.346</td>
</tr>
<tr>
<td>BVS (g)</td>
<td>291.55</td>
</tr>
<tr>
<td>Hyd. load (m³ kg⁻¹ BVS d⁻¹)</td>
<td>1.185</td>
</tr>
<tr>
<td>BOD load (g BOD g⁻¹ BVS d⁻¹)</td>
<td>0.154</td>
</tr>
<tr>
<td>COD load (g COD g⁻¹ BVS d⁻¹)</td>
<td>0.330</td>
</tr>
<tr>
<td>NH₃N load (g NH₃-N g⁻¹ BVS d⁻¹)</td>
<td>0.049</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>(b) The A/ASFF bioreactor</td>
<td></td>
</tr>
<tr>
<td>Flow rate (m³ d⁻¹)</td>
<td>0.173</td>
</tr>
<tr>
<td>NRS (m³ d⁻¹)</td>
<td>0.173</td>
</tr>
<tr>
<td>NML (m³ d⁻¹)</td>
<td>N/A</td>
</tr>
<tr>
<td>Total inflow (m³ d⁻¹)</td>
<td>0.346</td>
</tr>
<tr>
<td>Recycle ratio</td>
<td>1.0</td>
</tr>
<tr>
<td>BVS (g)</td>
<td>694.69</td>
</tr>
<tr>
<td>Hyd. load (m³ kg⁻¹ BVS d⁻¹)</td>
<td>0.497</td>
</tr>
<tr>
<td>BOD load (g BOD g⁻¹ BVS d⁻¹)</td>
<td>0.035</td>
</tr>
<tr>
<td>COD load (g COD g⁻¹ BVS d⁻¹)</td>
<td>0.076</td>
</tr>
<tr>
<td>NH₃N load (g NH₃-N g⁻¹ BVS d⁻¹)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

HRT: hydraulic retention time; NRS: nitrified return sludge; NML: nitrified mixed liquor; BVS: biomass volatile solids.
were used for other measurements such as suspended solids (SS) and volatile suspended solids (VSS). The pH, dissolved oxygen (DO) concentration and temperature were measured on all samples collected. Compartmental attached biofilm mass was determined at the end of each experimental run. Representative compartmental samples of each medium were collected and oven-dried at 105°C. The volatile (organic) fraction of the attached and suspended solids was determined by further burning the samples at 550°C. All laboratory analyses were performed according to *Standard Methods* (1998).

**RESULTS AND DISCUSSION**

Major performance parameters such as organics removal, nitrogen transformation and nitrification–denitrification rates were used to compare the performance of the ASFF and A/ASFF bioreactors operating at constant, but different hydraulic loading rates.

**Removal of organics**

Tables 2 and 3 present percentage BOD and COD removal, respectively, along the compartments in both the A/ASFF and the ASFF bioreactors at different hydraulic retention times. The BOD and COD removal profiles, generated at all HRTs in each of the bioreactors, indicated that the majority of organic removal occurred in the first compartment, in the range of 85–92% and 60–67% for BOD and COD, respectively. No appreciable removal occurred in the remaining compartments as the substrate removal followed a first-order kinetic model (IWA 2006). The overall BOD and COD removal efficiencies obtained in the ASFF and A/ASFF bioreactors at different HRTs are shown in Figure 2. It is evident that the A/ASFF bioreactor performance was slightly better than that of the ASFF bioreactor.

The first compartment of the A/ASFF bioreactor had DO concentrations between 0.1 and 0.2 mg l⁻¹. This is due to the long retention time in the settling tank, thus...
return sludge was recycled with low DO. The NML, on the other hand, was recycled with a high DO concentration of approximately 4–5 mg l⁻¹, but no drawback on the anoxic process was observed at the short HRTs applied of 1 and 0.7 h. Meanwhile, low DO concentration in the first compartment was a good sign of attaining anoxic conditions. This was the great advantage of implementing the anoxic process where the carbonaceous substrate (BOD and COD) can be utilized as a carbon source for the denitrifying bacteria. This is achieved without any outside energy source (oxygen) and without encountering any operational difficulties such as attached growth bridging (excessive biomass growth). In the second, third and fourth aerated compartments, the DO concentrations increased rapidly to 6 mg l⁻¹. In general, high DO concentrations prevailed at all HRTs regardless of loading. Usually, as was the case with the ASFF bioreactor, longer HRTs resulted in longer contact periods between substrate and microorganisms compared with shorter HRTs, thus allowing better removal efficiencies and exhibition of higher DO concentrations.

It is evident that, apart from the advantage of implementing the anoxic stage in improving the carbonaceous substrate removal, having high mixed liquor solids concentration resulting from recirculation of return sludge and additional recycling of mixed liquor solids, had greatly helped to support high removal rates where the process could be classified as hybrid growth (i.e. attached and suspended growth). Analysis of the specific oxygen consumption rate for the aerobic compartments has shown that suspended growth was superior in the removal efficiency especially at high loadings. This in turn had a positive impact in maintaining thinner biofilms that allow for better substrate and oxygen penetration.

**Nitrogen transformation**

The biological process of nitrogen transformation is currently the most economical and widely employed means of removing nitrogen from wastewater (Metcalf & Eddy 2003). Nitrogen is primarily removed from wastewater by two biological processes: nitrification (oxidation of ammonium to nitrate nitrogen by aerobic autotrophic bacteria) followed by denitrification (reduction of nitrate to nitrogen gas by facultative heterotrophic bacteria). At normal pH values, ammonia in wastewater is actually in the form of the ammonium cation (NH₄⁺). Bacteria from the genera *Nitrosomonas* convert ammonia to nitrite nitrogen (NO₂⁻), then bacteria from the genus *Nitrobacter* further convert nitrite to nitrate (NO₃⁻), i.e nitrification is a two-step process. On the other hand, denitrification is classified into heterotrophic and autotrophic denitrification according to electron donor. Heterotrophic denitrification requires an organic carbon source as food for the bacteria to live. Facultative bacteria can get their oxygen by taking dissolved oxygen out of the water and by taking it off the nitrate molecules. Denitrification occurs under anoxic conditions when oxygen levels are depleted (<0.5 mg/l) and nitrates become the primary oxygen source for microorganisms.
Recent research has demonstrated discrepancies in the ability of suspended growth treatment systems compared with attached growth systems (Metcalf & Eddy 2003). Attached growth treatment systems have shown improved rates and maintenance of ammonia removal over traditional, suspended growth treatment systems (Delatolla et al. 2010). In general nitrogen removal rates are most influenced by temperature, hydraulic retention time (i.e. hydraulic loading rates) and carbon:nitrogen (C:N) ratio (i.e. substrate loading rates) among a number of other factors (Metcalf & Eddy 2003). An understanding of the impact of C:N ratio on nitrogen removal from wastewater is imperative for optimizing the biofilm reactor (Biplop et al. 2011).

In this study, fixed organic (BOD or COD) loading was applied to simulate the growth of autotrophs since the autotrophs decrease with the incremental increase of influent COD (Ni et al. 2008). The compartmental percentage ammonia removal and the total oxidized nitrogen (TON) production of the A/ASFF bioreactor were determined as illustrated in Table 4. Generally, the observed compartmental efficiency suggests the presence of active nitrifying microorganisms mainly in the second and third compartments at all loading rates. This allowed the fourth compartment of the A/ASFF bioreactor to contribute to process stabilization and to act as a buffering zone to make up for any fluctuations in performance. Therefore, the A/ASFF bioreactor would offer stable nitrification performance and quickly adapt to fluctuations in the organic and hydraulic loading rates applied to the system. In contrast, nitrification in the ASFF bioreactor was mainly accomplished in the third and fourth compartments.

The A/ASFF bioreactor effluent was characterized by <1.0 mg l⁻¹ NH₃-N, <12.2 mg l⁻¹ NO₂-N, <16 mg l⁻¹ TON-N, <5 mg l⁻¹ BOD and <10 mg l⁻¹ SS which can easily meet the requirements for nonpotable agricultural or industrial reuse applications (Hamoda 2004). An effluent NO₃-N concentration of <3 mg l⁻¹ exists in wastewaters that are fully nitrified and denitrified. An effluent that is fully nitrified but has not been denitrified will generally contain an NO₃-N concentration of approximately 20 mg l⁻¹ (Metcalf & Eddy 2003). Meanwhile, the pH of the influent during experimentation varied in the range of: 6.9–7.2 in the influent, 7.2–7.5 in the anoxic compartment and 7.3–7.7 in the aerobic compartments. The alkalinity (as CaCO₃) averaged 290 ± 35 mg l⁻¹ for the influent, 185 ± 40 mg l⁻¹ for the anoxic compartment and 130 ± 45 mg l⁻¹ for the aerobic compartments since the nitrification reaction consumes alkalinity. Achievement of such good effluent characteristics emphasizes the favourable response of the A/ASFF bioreactor to a wide range of loading rates and the sustainability of performance without loss of nitrogen removal capacity compared with other biofilm systems (Liu et al. 1996; Chowdhury et al. 2010; Biplop et al. 2011).

**Removal rates**

Ammonia removal efficiencies were higher in the A/ASFF bioreactor compared with those observed in the ASFF bioreactor especially at shorter HRTs. This is illustrated in Figure 3 which shows percentage ammonia oxidation of up to 97% at HRTs of 6–8 h. Meanwhile, Figure 4 illustrates the mean concentrations of nitrates obtained in all

![Table 4](https://iwaponline.com/jwrd/article-pdf/2/3/165/378380/165.pdf)

**Table 4 |** Compartmental mean (steady state) percentage NH₃-N oxidized and TON produced in the A/ASFF bioreactor

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>2nd compartment</th>
<th>3rd compartment</th>
<th>4th compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% NH₃-N removal</td>
<td>% TON produced</td>
<td>% NH₃-N removal</td>
</tr>
<tr>
<td>8</td>
<td>31.40</td>
<td>52.52</td>
<td>17.68</td>
</tr>
<tr>
<td>6</td>
<td>33.02</td>
<td>54.93</td>
<td>13.23</td>
</tr>
<tr>
<td>4</td>
<td>19.81</td>
<td>55.60</td>
<td>17.98</td>
</tr>
<tr>
<td>2</td>
<td>25.14</td>
<td>34.33</td>
<td>26.12</td>
</tr>
<tr>
<td>1.5</td>
<td>19.00</td>
<td>26.20</td>
<td>17.00</td>
</tr>
<tr>
<td>1.0</td>
<td>20.00</td>
<td>11.60</td>
<td>19.00</td>
</tr>
<tr>
<td>0.7</td>
<td>12.00</td>
<td>18.60</td>
<td>13.00</td>
</tr>
</tbody>
</table>
compartments of the A/ASFF bioreactor at all HRTs. Presence of nitrates in the aerobic compartments (second, third and fourth) confirms the activity of the nitrifying bacteria in these compartments. In contrast, only traces of nitrates were observed in the anoxic first compartment where denitrification takes place. Moreover, this study was conducted at a constant C:N (COD:ammonia) ratio of about 6 in both the ASFF and the A/ASFF systems and showed a maximum ammonia removal efficiency of 97%.

Other studies reported in the literature (Ahmed et al. 2007; Biplop et al. 2011) showed that the percentage ammonia removal ranged from about 69 to 91% at C:N ratios of about 20 to 2, respectively. It is clear that the ammonia removal efficiency increases at lower C:N ratios at similar pH values and operating temperatures. Meanwhile, the nitrification rates obtained in this study are comparable to those reported in the literature as displayed in Table 5.

One of the benefits gained in adopting the anoxic step in the A/ASFF bioreactor is evident in securing steady ammonia removal at higher hydraulic loading rates than applied in the ASFF bioreactor which gives an edge to the A/ASFF bioreactor as a biological nitrogen removal (BNR) process (Tay et al. 2003; Chowdhury et al. 2010; Biplop et al. 2011). Also, high removals of both the carbonaceous and nitrogenous matter were achieved by the A/ASFF bioreactor under the anoxic conditions as compared with the aerobic systems. Moreover, aeration requirements in the A/ASFF bioreactor are much lower. It can be noticed from Table 6 that a 10-fold increase in the organic loading rate from 0.133 to 1.360 g BOD.g⁻¹BVS d⁻¹ resulted in a 10-fold increase in the denitrification rate from 0.44 to 4.74 g TON kg⁻¹BVS·h⁻¹. Such rates are higher than those reported by Galvez et al. (2003) for a submerged fixed-film reactor but are comparable to those reported by other researchers for either autotrophic or heterotrophic denitrification as presented in Table 7.

The specific substrate (TON) utilization rate increased linearly with the increase in the TON loading rate applied in the first compartment of the A/ASFF bioreactor, in the range of loadings studied. The correlation coefficient obtained was high ($r^2 = 0.9923$) as shown in Figure 5. This indicates that the system was not under TON limitation. Similarly, the carbonaceous (BOD) substrate utilization rate was not adversely affected despite the increase in mass or hydraulic loading rate applied (i.e. decrease in HRT) and followed a first-order kinetic model (IWA 2006).

The BOD consumed per TON removed (g BOD/g TON) was determined. Figure 6 shows that this ratio was increased at shorter HRTs (i.e. higher loading rates) and was associated with higher denitrification rates. The denitrification process was able to eliminate about 3.35 mg BOD (or 6.6 g...
The ASFF and A/ASFF bioreactors achieved high carbonaceous and nitrogenous substrate removal efficiency of up to 98% and 75% for BOD and COD, respectively, and up to 97% for ammonia. Process performance was not adversely affected by a 10-fold increase in loading rates in the range of 0.5 to 6.6 m$^3$ kg$^{-1}$ BVS d$^{-1}$ for hydraulic loadings and in the range of 0.03 to 0.3 g BOD g$^{-1}$ BVS d$^{-1}$ for organic loadings.

2. For nitrification at the shorter HRTs (0.7 h), the A/ASFF bioreactor was superior to the ASFF bioreactor in terms of ammonia removal efficiencies. The denitrification rate was also high in the first compartment of the A/ASFF bioreactor at the shorter HRTs. A 10-fold increase in specific denitrification rate from 11 to 114 mg TON g$^{-1}$ BVS d$^{-1}$ was associated with a 10-fold increase in specific organic loading rate from 0.133 to 1.360 g BOD g$^{-1}$ BVS d$^{-1}$. Under anoxic conditions and low aeration rates in the A/ASFF process compared with aerobic conditions in the ASFF process results in reduced operational cost.

**CONCLUSIONS**

Based on the experimental results obtained, the principal findings of this study are the following:

1. The ASFF and A/ASFF bioreactors achieved high carbonaceous and nitrogenous substrate removal efficiency of up to 98% and 75% for BOD and COD, respectively, and up to 97% for ammonia. Process performance was not adversely affected by a 10-fold increase in loading rates in the range of 0.5 to 6.6 m$^3$ kg$^{-1}$ BVS d$^{-1}$ for hydraulic loadings and in the range of 0.03 to 0.3 g BOD g$^{-1}$ BVS d$^{-1}$ for organic loadings.

2. For nitrification at the shorter HRTs (0.7 h), the A/ASFF bioreactor was superior to the ASFF bioreactor in terms of ammonia removal efficiencies. The denitrification rate was also high in the first compartment of the A/ASFF bioreactor at the shorter HRTs. A 10-fold increase in specific denitrification rate from 11 to 114 mg TON g$^{-1}$ BVS d$^{-1}$ was associated with a 10-fold increase in specific organic loading rate from 0.133 to 1.360 g BOD g$^{-1}$ BVS d$^{-1}$.
3. Success of the A/ASFF bioreactor was mainly due to the efficient performance of the anoxic stage incorporated in the first compartment. Removal efficiencies of more than 90% and 60% for BOD and COD, respectively, were achieved in this stage. For treatment of wastewater in hot climates, utilization of an anoxic process provides efficient carbonaceous/nitrogenous substrate removal without additional aeration requirements.

### REFERENCES


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