Nitrification/denitrification in swine wastewater using porous ceramic sticks with plastic rings as supporting media in two-stage fixed-biofilm reactors

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ABSTRACT

This study evaluated the performance of oxic-anoxic fixed-biofilm reactors (FBRs) using porous ceramic sticks with plastic rings as supporting media for nitrogen and organic carbon (as COD) removal from swine wastewater. Experimental results indicate that the removal efficiency of NH$_4$+N increased to 86–92% from 52–98% as the volumetric ammonium-nitrogen loading rate increased to 0.25 kg NH$_4$+N/m$^3$·d from 0.082 kg NH$_4$+N/m$^3$·d. Furthermore, during the denitrifying column test, the average removal efficiencies for COD and NO$_x$-N were 83 and 76%, respectively. Only small amounts of NO$_2$-N and NO$_3$-N accumulated in the denitrifying FBR. The average values for NO$_2$-N and NO$_3$-N in effluent from denitrifying reactor were roughly 2 mg/L and 6 mg/L, respectively. Approximately 82% of NO$_3$-N was converted into N$_2$ by denitrifying bacteria in the denitrifying FBR. Approximately 98–100% high removal efficiencies of NO$_x$-N could be reached in denitrifying FBR, when the ratio of COD$_r$/NO$_x$-N$_r$ was controlled at 9–12 throughout the test. Microscopic observations show that cell number on the ceramic sticks in denitrifying FBR was greater than that in nitrifying FBR in the final phase of colonization.

Key words | denitrification, fixed-biofilm reactors (FBRs), nitrification, swine wastewater

INTRODUCTION

Swine wastewater has garnered considerable attention due to its high organic matter, solids and nitrogen contents (Sánchez et al. 2005; Shin et al. 2005). Removing nitrogen and organic matter from swine wastewater is particularly important because ammonium and nitrate provoke health risks and can result in eutrophication (Obaja et al. 2003; Lin 2008). During the last two decades, many aerobic processes, such as activated sludge, rotating biological contactors, oxidation ditches and the anoxic/aerobic process have been used to treat swine wastewater, however, the nitrogen removal rate by these processes is unsatisfactory as total nitrogen removal efficiency is only 20–60% (Ghaly & Kok 1986; Osada et al. 1991; Pan & Drapcho 2001).

The sequencing batch reactor (SBR), intermittent aeration process and membrane bioreactor (MBR) are currently the most common and efficient methods for nitrogen removal from swine wastewater (Bortone et al. 1992; Bicudo & Svobada 1995). However, due to the sensitivity of nitrifying bacteria to environmental factors and their slow growth rate, maintaining a sufficient population of nitrifying bacteria in mixed microbial consortia is difficult. Moreover, although SBR can achieve good removal efficiencies for COD, TN and TP, a hydraulic retention time (HRT) as long as 9–16 days is needed (Tilche et al. 1999), resulting in considerable construction and running cost and power consumption (Deng et al. 2008). Therefore, treatment of swine wastewater using anaerobic fixed-bed reactors became attractive and experiments were carried out in laboratories and at full scale (Nikolaeva et al. 2002; Sánchez et al. 2002).

The anaerobic fixed-bed reactors were capable of markedly reducing the organic compound concentrations...
and amounts of pathogenic bacteria at hydraulic retention times of just a few days, which made post-treatment and final disposal easier than before (Borja et al. 2005). The use of supporting media contributed to the increase in microbial retention time, increasing the capacity of the reactor to resist shock loadings of organic compounds, changes in substrate characteristics, and the presence of inhibitory compounds. The benefits of using ceramic rasching rings as supporting media to which microorganisms attach to achieve high organic matter removal efficiencies for piggery wastewater have been demonstrated (Sánchez et al. 2005). Nacheva et al. (2008) conducted FBR packed with ceramic spheres as supporting media for domestic wastewater treatment. They found that the COD removal was 68–85% under different specific COD loading rate of 1–6 g COD/m²-d. Moreover, the plastic rings with greater void spaces reduced clogging risks in the fixed bed (Wuertz et al. 2003). However, relatively few studies have focused on simultaneous removal of nitrogen and organic carbon (as COD) using mixed supporting media, such as porous ceramic sticks and plastic rings, in a nitrifying-denitrifying FBR process.

The advantages of porous ceramic spheres with a rough surface to which microorganisms could attach easily and plastic rings used to prevent flow clogging have been reported (Wuertz et al. 2003; Sánchez et al. 2005). Therefore, two-stage laboratory-scale fixed-biofilm reactors (FBRs) with porous ceramic sticks and plastic rings as supporting media were utilized to remove nitrogen and organic carbon (as COD) from swine wastewater in this study. The main objectives of this study were to (1) observe the effects of volumetric NH₄⁺-N loading rates and COD loading rates on NH₄⁺-N and COD removal in the nitrifying FBR; (2) investigate the effect of volumetric COD loading rate on denitrification in the denitrifying FBR for treating the effluent of swine wastewater from nitrifying FBR; and (3) compare the bacterial colonization on the porous ceramic sticks in two-separate nitrifying and denitrifying FBRs during initial, intermediate and final phase of colonization. Experimental results in this study can be used to assess the feasibility of pilot-scale or full-scale design for simultaneous removal of nitrogen and organic carbon in swine wastewater.

MATERIALS AND METHODS

Microorganisms

Nitrifying bacteria obtained from a municipal wastewater treatment plant were enriched under laboratory conditions by propagating a sample in a synthetic, ammonium-nitrogen substrate. The nitrifying bacteria were enriched by adding the following mineral mediums (mg/L): (NH₄)₂SO₄, 500; MgSO₄·7H₂O, 200; CaCl₂·2H₂O, 20; chelated iron, 1; Na₂MoO₄·2H₂O, 0.1; MnCl₂·4H₂O, 0.2; CoCl₂·6H₂O, 0.002; CuSO₄·5H₂O, 0.02; and ZnSO₄·7H₂O, 0.1; in 1 L of 0.1 M phosphate buffer (Mahne et al. 1996). A soil suspension (10 g soil in 90 mL water) was utilized as the source of denitrifying bacteria that were isolated from rice paddy field sediment and identified using the BIOLOG system; the bacteria included Psychrobacter immobilis, Ochrobactrum anthropi and Alcaligenes denitrificans (Pai et al. 1999). These denitrifying bacteria were enriched by the complex medium containing the following ingredients (g/L): KNO₃, 10.0; glycerol, 10.0; peptone, 5.0; and yeast extract, 3.0; Na₂HPO₄·7H₂O, 7.9; KH₂PO₄, 1.5; NH₄Cl, 0.3; MgSO₄·7H₂O, 0.1; and 2 ml of trace element solution. The trace element solution contained the following ingredients (g/L): EDTA, 50; ZnSO₄, 2.2; CaCl₂, 5.5; MnCl₂-4H₂O, 5.06; FeSO₄·7H₂O, 5; (NH₄)₆Mo₇O₂₄·4H₂O, 1.1; CuSO₄·5H₂O, 1.57; and CaCl₂, 1.61. Medium pH was adjusted at 7.0.

Bacterial inoculum

The acclimation of the nitrifying and denitrifying bacteria into the FBR was achieved by adding about 100 mL of inoculum and mineral media from the upper part of the reactor to a working volume of 1.5 L. The nitrifying and denitrifying FBR were mixed by using air and nitrogen, respectively, to maintain oxic and anoxic conditions over 24 h for bacterial attachment.

Substrates

Swine wastewater was obtained from a primary sedimentation tank of wastewater treatment plant at a piggery with a capacity of 300 head located in Yuan-lin County, Taiwan.
The treatment process of swine wastewater primarily consists of influent of swine wastewater, solid-liquid separation, anaerobic digester, primary sedimentation, aerobic digester, and final sedimentation units. Once the swine wastewater was collected, it was immediately screened through a 2-mm sieve to remove coarse particles to prevent pumps clogging. The wastewater was then allowed to settle for 1 h to remove settleable solid particles. The supernatant was used as the substrate for experiments. The supernatant obtained was analyzed to determine the NH$_4^+$-N concentration. The average volumetric loading rate of NH$_4^+$-N was 0.082 kg NH$_4^+$-N/m$^3$-d for the first run and 0.25 kg NH$_4^+$-N/m$^3$-d for the second run. The organic concentration value in swine wastewater was approximately 18–191 mg COD/L. The alkalinity in the influent of the nitrifying FBR was adjusted to 550 mg CaCO$_3$/L by adding NaHCO$_3$. The pH value of supernatant was about 7 ± 0.2.

Table 1 lists operating parameters for influent of nitrifying FBR. Furthermore, the prepared influent wastewater containing NO$_2^-$-N, NO$_3^-$-N and organic carbon (as COD) was then pumped into the anoxic reactor for denitrification. The DO was maintained at a value below 0.5 mg/L to maintain an anoxic condition in the denitrifying FBR. Table 2 lists operating parameters for influent of denitrifying FBR.

**Supporting media**

The porous ceramic sticks were chosen as the support media for nitrifying and denitrifying biofilm attachment because they are inert and have a known surface area that supports biofilm growth. All porous ceramic sticks had a density of 1.33 g/cm$^3$ and specific bed area of 1,032 m$^2$/m$^3$. The bed media voidage was 45% and the bed pore volume was 1.66 mL/g. Additionally, the plastic Ballast$^\circledR$ rings, 1.58 cm in both diameter and height were used as packing media to prevent reactor clogging because these rings have a considerable free space. The density of the plastic Ballast$^\circledR$ rings was 0.116 g/cm$^3$ and their specific surface area was 354 m$^2$/m$^3$. The free space was 86% and the packing factor was 97.

**Experimental device**

Figure 1 shows the FBRs system for nitrification and denitrification. The reactors comprised a glass cylinder with a diameter of 8 cm and height of 40 cm. The aeration plate was placed in the oxic reactor to distribute air for nitrification. Dissolved oxygen (DO) was controlled at a value above 4.5 mg/L for nitrification by an adjustable air pump. The air supply was sterilized by filtration through polytetrafluoroethylene (PTFE) membranes with a pore size of 0.2 μm. Approximately 100 porous ceramic sticks with a diameter of 1.5 cm and height of 2 cm as supporting media were placed in oxic and anoxic reactors for biofilm attachments, respectively. Additionally, roughly 100 plastic rings with a diameter and height of 1.58 cm were placed in the oxic and anoxic reactors, respectively, for uniform distribution of influent to prevent reactor clogging.

**Table 1 | Operational parameters for the nitrifying FBR**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Value Run #1</th>
<th>Value Run #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent flow rate</td>
<td>L/d</td>
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<td>3</td>
</tr>
<tr>
<td>Volumetric COD loading rate</td>
<td>kg COD/m$^3$-d</td>
<td>0.074</td>
<td>0.271</td>
</tr>
<tr>
<td>Volumetric NH$_4^+$-N loading rate</td>
<td>kg NH$_4^+$-N/m$^3$-d</td>
<td>0.082</td>
<td>0.25</td>
</tr>
<tr>
<td>Specific surface loading rate of COD</td>
<td>g COD/m$^2$-d</td>
<td>0.0719</td>
<td>0.263</td>
</tr>
<tr>
<td>Specific surface loading rate of NH$_4^+$-N</td>
<td>g NH$_4^+$-N/m$^2$-d</td>
<td>0.079</td>
<td>0.242</td>
</tr>
<tr>
<td>NH$_4^+$-N sludge loading rate</td>
<td>kg NH$_4^+$-N/kg VSS-d</td>
<td>0.054</td>
<td>0.167</td>
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<tr>
<td>F/M rate</td>
<td>kg NH$_4^+$-N/kg VSS-d</td>
<td>4.9 × 10$^{-3}$</td>
<td>0.18</td>
</tr>
<tr>
<td>MLVSS</td>
<td>mg VSS/L</td>
<td>1,500</td>
<td>1,500</td>
</tr>
<tr>
<td>DO</td>
<td>mg/L</td>
<td>&gt;4.5</td>
<td>&gt;4.5</td>
</tr>
<tr>
<td>HRT</td>
<td>hr</td>
<td>12</td>
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</tbody>
</table>
The influent of swine wastewater was pumped into reactor using a peristaltic pump.

According to the stoichiometric relationship of NO$_3^-$N and glucose, the COD effluent concentration from the nitrifying FBR was inadequate for denitrification: the nitrate and COD effluent concentrations were 21–60 mg NO$_3^-$N/L and 6.1–42.7 mg/L, respectively. Therefore, the effluent from the nitrifying FBR flowed into a mixing tank and was mixed with 0.25–0.5 g/L glucose as an extra carbon source to provide the growth of denitrifying bacteria in the denitrifying FBR. The interval of addition of glucose was 3–9 days. The prepared influent wastewater containing NO$_2^-$N, NO$_3^-$N and organic carbon (as COD) was then pumped into the anoxic reactor for denitrification test. The DO was controlled at a value below 0.5 mg/L to maintain an anoxic condition in the denitrifying FBR. The experiment was carried out during an operating period of nearly 60 days, in which the pH was maintained at an optimal level of 7.2 ± 0.1 and the temperature was controlled at 24°C.

**Chemical analyses**

The concentrations of NH$_4^+$-N, NO$_2^-$N, NO$_3^-$N, mixed liquor volatile suspended solids (MLVSS) and COD in the influent and effluent were determined by employing procedures in *Standard Methods (2005)*. Estimation of the NH$_4^+$-N concentration was made using the phenate method which determined absorbency at 635 nm. The NO$_2^-$N concentration was estimated colorimetrically using sulfanilamide and *N*-(1-naphthyl)-ethylenediamine (NED) dihydrochloride reagents at 540 nm, while brucine sulfanilic acid method at 410 nm was used to determine the NO$_3^-$N concentration (*Pai et al. 1999*). The COD concentration was determined using the closed reflux colorimetric method at 600 nm with a spectrophotometer (Shimadzu, model UV-1700). The suspended biomass was filtered through a glass filter membrane with a pore size of 0.22 μm, washed, dried at 103°C for 1 h and then heated to 550°C for 15 min in a muffle furnace for determining the concentrations of MLVSS.

**Scanning electron microscopy (SEM)**

The microorganisms attached to the porous ceramic sticks were collected by shaking and immersion in a phosphate buffer solution three times at 10-min intervals. Biological samples were then fixed with 2.5% glutaraldehyde. Samples were stored at 4°C for 12–16 h. The fixed samples were dehydrated by equilibration for 10 min each in a sequence of aqueous acetone solutions (30, 50, 70, 90, and 100% acetone twice) (*Sich & Van Rijn 1997*). Samples were critical-point dried, fixed on aluminum stubs, sputtercoated with gold-palladium, and examined with a scanning electron microscope at 10 KV (model S-3000 N, Hitachi, Japan).
RESULTS AND DISCUSSION

Nitrification

Nitrification was conducted using two different consecutive microbial processes: nitritation and nitratation. During nitritation, ammonia is converted into nitrite due to its oxidation by the ammonia oxidizing bacteria (AOB). During nitratation, a nitrite oxidizing bacteria (NOB) converted nitrite into nitrate (Vayenas et al. 1997). During the nitrifying process, a significant difference in removal efficiency existed for different volumetric NH₄-N loading rates. Figure 2a shows the NH₄⁺-N influent and effluent concentrations as well as removal efficiency varied over time in the nitrifying FBR. In the first 10 days, low removal efficiency of NH₄⁺-N was observed due to system startup and acclimation. During the first run, the effluent of NH₄⁺-N was observed due to system startup and acclimation. During the first run, the effluent of NH₄⁺-N reached a steady-state condition approximately after 15 days and the average effluent concentration of NH₄⁺-N was approximately 8.7 mg/L during operation period of 27 days. The average removal efficiency for NH₄⁺-N during operation period of 27 days was 8.7 mg/L during operation period of 27 days. The average removal efficiency for NH₄⁺-N was 88% in the second run. Figure 2b presents the variation of removal efficiency for NH₄⁺-N with volumetric NH₄⁺-N loading rate. The removal efficiency of NH₄⁺-N increased to 86–92% from 52–98% as the volumetric loading rate increased to 0.25 kg NH₄⁺-N/m³·d from 0.082 kg NH₄⁺-N/m³·d, respectively.

A combined process of submerged membrane bioreactor (MBR) and anaerobic upflow bed filter (AUBF) for the treatment of swine wastewater was used to evaluate organics removal and nitrification (Shin et al. 2005). Their experimental results show that the removal efficiency of NH₄⁺-N was 85-100% when the volumetric nitrogen loading rate was maintained at 0.2–0.65 kg NH₄⁺-N/m³·d. The removal efficiency of NH₄⁺-N from nitrifying FBR in this study was comparable to that from AUBF-MBR process. Moreover, approximately 50% of NH₄⁺-N removal was achieved in a sequencing batch reactor (SBR) treating digested effluent of swine wastewater (Deng et al. 2008). In this study, the NH₄⁺-N removal efficiency in FBR was higher than that in the SBR, both treating digested effluent of swine wastewater.

Figure 2c presents influent and effluent concentrations of NO₂⁻-N, which varied over time at two different volumetric NH₄⁺-N loading rates. Notably, NO₂⁻-N was an intermediate compound produced by NH₄⁺-N oxidation by the AOB and converted into NO₃⁻-N by the NOB. The concentration of influent NO₂⁻-N was very low, indicating that no AOB existed in influent. In the first run, the first part of the NO₂⁻-N curve was for 5–10 days. During this period, the NO₂⁻-N production rate by the AOB was lower than its utilization rate by the NOB. The peak height of the NO₂⁻-N effluent concentration was 1.40 mg NO₂⁻-N/L. During the second period in the first run, 10–20 days, the NO₂⁻-N concentration decreased gradually because the NO₂⁻-N production rate by the AOB was lower than its utilization rate by the NOB. In the second run from day 32 to 62, the variation of NO₂⁻-N effluent concentration curve was the same as that in the first run. The peak height of the NO₂⁻-N effluent concentration exceeded 1 mg/L in the first and second run probably because overall production rate by AOB was higher than overall utilization rate by NOB. Additionally, no NO₂⁻-N accumulation in nitrifying FBR existed at the end of the first and second run.

Figure 2d plots the variation of NO₃⁻-N effluent with time. The influent concentration of NO₃⁻-N was very low (0.33 mg NO₃⁻-N/L), suggesting that almost no NOB existed in influent to convert NO₂⁻-N into NO₃⁻-N. However, the NOB grew vigorously in the oxic FBR and converted NO₂⁻-N into NO₃⁻-N. In the first run, 5–32 days, the trend of the NO₃⁻-N curve was similar to that of the volumetric NH₄⁺-N loading rate. As the volumetric NH₄⁺-N loading rate increased, NH₄⁺-N removal efficiency increased and produced the more amount of NO₃⁻-N. Moreover, the biodegradation of COD in the influent by heterotrophic bacteria in nitrifying process was observed.

Figure 2e presents the variation of COD influent and effluent concentrations as well as COD removal efficiency with time in the first and second run. During the first run, 5–32 days, the volumetric COD loading rate was approximately 0.074 kg COD/m³·d in influent and the average COD removal efficiency was about 55%. In the second run from day 32 to 62, the COD removal increased up to 74% when the volumetric COD loading rate was approximately 0.271 kg COD/m³·d.
Figure 2 | Nitrification in nitrifying fixed-biofilm reactor (a) NH$_4^+$-N removal varied with time; (b) volumetric NH$_4^+$-N loading rate varied with time; (c) NO$_2^-$-N production and utilization by nitrifying bacteria varied with time; (d) NO$_3^-$-N production by nitrifying bacteria varied with time; and (e) COD removal varied with time.
Microscopic observation of biofilm in nitrifying FBR

Bacterial colonization attached on porous ceramic sticks in the nitrifying FBR proceeded in three distinct phases. In the first colonization phase (up to day 7), single bacteria were not noticeable and only small colonies formed due to acclimation (Figure 3a). The second intermediate phase (up to day 22) was characterized by increased bacterial colonization. Predominantly, caves and fissures in the porous ceramic sticks were colonized by rod- and coccus-shaped mixed-culture biomass (Figure 3b). In the final colonization phase of the steady state (up to day 53), the densely colonized areas expanded and joined together (Figure 3c). In addition to single attached bacteria, bacterial colonies, consisting of larger, rod- and coccus-shaped bacteria, were observed on day 53 (Figure 3d). Comparing Figure 3b with Figure 3c, the colonies of these rod- and coccus-shaped bacteria expanded, increasing in size and forming more biofilm on ceramic sticks in the final colonization phase. A significant increase in cell number from day 22 to day 53 was observed when volumetric NH$_4^+$-N loading rate increased from the first run to second run.

Denitrification

The denitrification process comprises biodegradation of organic carbon (as COD) and nitrate reduction by denitrifying bacteria. The denitrifying bacteria, in the presence of organic carbon and nitrate, use organic carbon as an electron donor and nitrate as the electron acceptor. When the anoxic FBR process was used to treat effluent from the oxic FBR, the performance of COD removal initially worsened, and then improved (Figure 4a). At the start of the test, the COD removal efficiency was high. However, the COD removal rate declined abruptly to roughly 55% due to startup and acclimation. The removal efficiency of COD was maintained at 80–89% when effluent concentration of COD was 30–70 mg/L after a stable condition from 20–62 days.

Figure 4b shows the variation in influent and effluent NO$_2^-$-N and NO$_3^-$-N concentrations versus time. The average influent concentrations for NO$_2^-$-N and NO$_3^-$-N were 1.49 mg/L and 33.71 mg/L, respectively, and average effluent concentrations of NO$_2^-$-N and NO$_3^-$-N were 2.38 mg/L and 5.91 mg/L, respectively. Approximately 82% of NO$_3^-$-N was converted into N$_2$ by denitrifying bacteria.
bacteria in the denitrifying FBR. A small amount of NO\textsubscript{2}-N and NO\textsubscript{3}-N accumulated in the denitrifying reactor. One explanation of NO\textsubscript{2}-N accumulation in denitrifying FBR was more rapid growth of nitrate respiring bacteria at the expense of true denitrifying bacteria, in the presence of nitrate (Glass & Silverstein 1998). The average influent and effluent concentrations of NO\textsubscript{x}-N (NO\textsubscript{2}-N + NO\textsubscript{3}-N) were 35.2 mg/L and 8.29 mg/L, respectively. Approximately 76% of NO\textsubscript{x}-N was removed by the denitrifying bacteria in the anoxic FBR.

Figure 4c presents the COD loading rate, COD and NO\textsubscript{x}-N removal efficiencies, each of which varied over time. The efficiency of NO\textsubscript{x}-N removal reached about 95% when COD loading rate was about 0.75 kg/m\textsuperscript{3}-d. The trend of the NO\textsubscript{x}-N removal curve was similar to that of the volumetric COD loading rate curve. The efficiency of NO\textsubscript{x}-N removal increased as the COD loading rate increased, demonstrating that a high COD loading rate facilitates the NO\textsubscript{x}-N removal by denitrifying bacteria. Moreover, COD removal became stable for 20–63 days as the COD loading rate varied, indicating that the effect of the COD loading rate on the COD removal rate was insignificant. The average COD removal efficiency was approximately 83% in denitrifying FBR.

Figure 4d plots the variation of NO\textsubscript{x}-N removal efficiency versus a ratio of CODr/NO\textsubscript{x}-N\textsubscript{r}. The CODr/NO\textsubscript{x}-N\textsubscript{r} ratio in the denitrifying FBR was 3.3–26. The average CODr/NO\textsubscript{x}-N\textsubscript{r} ratio was about 10, much higher than the theoretical value of 2.67. Approximately 98–100% high removal efficiencies of NO\textsubscript{x}-N could be reached, when the ratio of CODr/NO\textsubscript{x}-N\textsubscript{r} was controlled at 9–12 throughout the test.

The experimental results in this study indicated that the anoxic FBR was suitable for the treatment of low influent COD strength of swine wastewater. Sánchez et al. (2005) operated a down-flow anaerobic fixed bed reactor treating...
piggery wastewater at different hydraulic retention times (HRTs). They found that the removal efficiency of COD reached up to 89% when volumetric COD loading was up to 8 kg COD/m³-d. Their experimental results demonstrated that fixed-biofilm reactor could be applied in high influent COD strength of piggery wastewater.

**Microscopic observation of biofilm in denitrifying FBR**

The small bacterial rods observed during the initial phase (up to day 7) of colonization were roughly 0.45 μm wide and 1 μm long (Figure 5a). Their dimensions and shapes were similar to those of denitrifying bacterium isolated from a fluidized bed reactor (Van Rijn et al. 1996). The bacterial colonies that appeared during initial colonization were composed of thinner rod-shaped bacteria (Figure 5a). During the intermediate phase of biofilm formation (up to day 22), mixed-culture biomass proliferation was rapid, as indicated by the numerous cells colonies in the caves and fissures of the ceramic sticks (Figure 5b). Additionally, a dense accumulation of several layers of rod-shaped mixed-culture biomass attached and expanded on the ceramic sticks due to their growth until day 53 (Figure 5c). These bacteria had similar shapes and dimensions similar to those of the Pseudomonas stutzeri isolate (Sich & Van Rijn 1997). A dense rod-shaped bacteria was predominant in the final phase of colonization (Figure 5d). Comparing Figure 5c with Figure 5d, the cell number of biofilm in denitrifying FBR was greater than that of biofilm in nitrifying FBR on day 53.

**CONCLUSIONS**

Nitrification and denitrification in an oxic-anoxic fixed-biofilm process were feasible for removing nitrogen and organic carbon (as COD) from swine wastewater. Experimental results indicate that the removal efficiency of NH₄⁺-N increased to 86–92% from 52–98% as the volumetric ammonium-nitrogen loading rate increased to 0.25 kg NH₄⁺-N/m³-d from 0.082 kg NH₄⁺-N/m³-d. Moreover, the average removal efficiencies of COD and NOx-N by denitrification were 83 and 76%, respectively, when the average volumetric COD and NOx-N loading rates were 0.587 kg COD/m³-d and 0.07 kg N/m³-d. Microscopic observations show that cell number on the ceramic sticks in denitrifying FBR was greater than that in nitrifying FBR in the final phase of colonization. The approaches in this study

![Figure 5](https://iwaponline.com/wst/article-pdf/62/5/985/446578/985.pdf)
can be applied to design a large scale oxic-anoxic fixed-biofilm process using porous ceramic sticks and plastic rings as the supporting media for treating swine wastewater.

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