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Abstract The performance of nitrifying granules, which had been produced in an aerobic upflow fluidised bed (AUFB) reactor, was investigated in various types of ammonia-containing wastewaters. When pure oxygen was supplied to the AUFB reactor with a synthetic wastewater containing a high concentration of ammonia (500 g-N/m³), the ammonia removal rate reached 16.7 kg-N/m³/day with a sustained ammonia removal efficiency of more than 80%. The nitrifying granules possessing a high settling ability could be retained with a high density (approximately 10,000 g-MLSS/m³) in a continuous stirring tank reactor (CSTR) even under a short hydraulic retention time (44 min), which enabled a high-rate and stable nitrification for an inorganic wastewater containing low concentrations of ammonia (50 g-N/m³). Moreover, the nitrifying granules exhibited sufficient performance in the nitrification of real industrial wastewater containing high concentrations of ammonia (1,000–1,400 g-N/m³) and salinity (1.2–2.2%), which was discharged from metal-refinery processes. When the nitrifying granules were used in cooperation with activated sludge to treat domestic wastewater containing organic pollutants as well as ammonia, they fully contributed to nitrification even though a part of activated sludge adhered onto the granule surfaces to form biofilms. These results show the wide applicability of nitrifying granules to various cases in the nitrification step of wastewater treatment plants.

Keywords Aerobic upflow fluidised bed (AUFB); continuous stirring tank reactor (CSTR); high-rate nitrification; inorganic wastewater; metal-refinery wastewater; nitrifying granule; organic wastewater

Introduction

In biological wastewater treatment processes, separation of liquid and sludge is indispensable to maintaining the high quality of effluent water. Bacterial immobilization onto carriers that have a good settling ability is one of the effective techniques to meet a high flow-rate condition. On the other hand, if bacteria densely aggregate to form a so-called granule that exhibits an excellent settling ability, a large amount of bacteria can be retained in a reactor without any carrier materials under such a high flow-rate condition.

In the 1970s, the research on granules originated from the upflow anaerobic sludge blanket (UASB) reactor that enables a high-load operation in anaerobic wastewater treatment such as methane fermentation (Lettinga et al., 1980). In recent years, new techniques for producing aerobic granules of heterotrophic bacteria have been developed to remove effectively organic pollutants in wastewater using a column-type sequencing batch reactor (SBR) (Morgenroth et al., 1997; Beun et al., 1999; Etterer and Wilderer, 2001; Tay et al., 2001) or using an aerobic upflow sludge blanket (AUSB) with front aeration (Mishima and Nakamura, 1991). Furthermore, the techniques for producing granules of nitrifying bacteria using a conical-shaped AUSB with front aeration (de Beer et al., 1993; Schramm et al., 1998, 1999) or using an SBR (Tay et al., 2002) have been proposed.

Our group successfully produced nitrifying granules with a diameter of 200 µm within 100 days using an aerobic upflow fluidised bed (AUFB) reactor (Tsuneda et al., 2003). Furthermore, the time required for producing nitrifying granules could be greatly reduced by making seed sludge pre-aggregated with hematite (Tsuneda et al., 2004). Since nitrifying bacteria could be retained with a high density in the AUFB reactor due to the form of
granules, inorganic wastewater containing NH\textsubscript{4}\textsuperscript{+}-N 500 g/m\textsuperscript{3} was effectively and stably treated with an ammonia removal rate of 1.6 kg-N/m\textsuperscript{3}/day and ammonia removal efficiency of more than 90% throughout 500 days (Tsuneda et al., 2003).

In this study, the nitrification potential of these granules was tested by supplying a high concentration of oxygen gas to the AUFB reactor. Furthermore, in order to clarify the utility of the nitrifying granules produced in the AUFB reactor, they were transferred to a continuous stirring tank reactor (CSTR) and applied to the nitrification of various types of wastewaters such as inorganic wastewater containing a low concentration of ammonia, metal-refinery wastewater containing a high concentrations of saline and ammonia, and domestic wastewater containing both organic pollutants and ammonia. Then, the performance of the nitrifying granules was evaluated on the basis of the maximum nitrification rate, ammonia removal efficiency and stability through the long-term operation.

Materials and methods

Wastewater composition

In this study, two kinds of inorganic wastewaters (A, B) and one organic wastewater (C) were used for evaluating utility of the nitrifying granules. Synthetic inorganic wastewater A, which is the same wastewater as was used for granulation, contained 500 g/m\textsuperscript{3} of NH\textsubscript{4}\textsuperscript{+}-N and 5,800 g/m\textsuperscript{3} of SO\textsubscript{4}\textsuperscript{2-}. Moreover, FeSO\textsubscript{4}·7H\textsubscript{2}O and KH\textsubscript{2}PO\textsubscript{4} were added as a trace element. Table 1 shows the chemical composition of wastewater A. Industrial wastewater B, which was discharged from a metal-refinery process, contained a high concentration of NH\textsubscript{4}\textsuperscript{+}-N (5,500–6,000 g/m\textsuperscript{3}) and inorganic salts such as sodium chloride and sodium sulfate. The saline concentration by sodium sulfate conversion was 6–11% (based on electric conductivity). In this study, five-times diluted wastewater was used as wastewater B. Synthetic organic wastewater C, simulating domestic wastewater, contained dissolved organic carbon (DOC), NH\textsubscript{4}\textsuperscript{+}-N and T-N of 180, 60 and 100 g/m\textsuperscript{3}, respectively. The chemical composition of wastewater C is shown in Table 2.

Nitrifying granules

All the nitrifying granules used in this study were produced in an AUFB reactor with a diameter of 50 mm and a height of 3.2 m (effective volume: 6.3 L) where wastewater A (NH\textsubscript{4}\textsuperscript{+}-N: 500 g/m\textsuperscript{3}) had been continuously fed (Tsuneda et al., 2003) for approximately two years. The average diameter of the nitrifying granules was about 1,500 \(\mu\)m.

Reactor configuration and operating conditions (see Table 3)

Nitrification by aeration of high oxygen partial pressure gas (Run 1). The laboratory-scale AUFB reactor with a diameter of 31 mm and a height of 45 cm (effective volume: 0.34 L) was used. A solid–liquid separator was placed on the top of the reactor to prevent wash out of granules from the reactor. The nitrifying granules were inoculated to the AUFB reactor with an initial MLSS concentration of 15,100 g/m\textsuperscript{3}. From the bottom of the reactor, synthetic wastewater A (NH\textsubscript{4}\textsuperscript{+}-N: 500 g/m\textsuperscript{3}) was continuously fed.

Table 1 Chemical composition of synthetic inorganic wastewater A

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration [kg/m\textsuperscript{3}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}</td>
<td>2.357</td>
</tr>
<tr>
<td>Na\textsubscript{2}SO\textsubscript{4}</td>
<td>6.044</td>
</tr>
<tr>
<td>FeSO\textsubscript{4}·7H\textsubscript{2}O</td>
<td>0.005</td>
</tr>
<tr>
<td>KH\textsubscript{2}PO\textsubscript{4}</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Air (0–35.5 h), 40% O₂ gas (35.5–97.0 h), 60% O₂ gas (97.0–182 h), 80% O₂ gas (182–255 h) and 100% O₂ gas (255–336 h) were successively supplied to the reactor. The hydraulic retention time (HRT) was initially 4.9 h. When the effluent concentration reached a steady state, the HRT was reduced in a stepwise manner to elucidate the maximum nitrification rate under supply of each concentration of oxygen.

**Nitrification under short HRT conditions (Run 2)**. Nitrifying granules were inoculated to a continuous stirring tank reactor (CSTR) whose effective volume was 1.0 L. The initial MLSS concentration was 12,700 g/m³. The synthetic wastewater A was diluted 10 times and used as the initial influent solution (NH₄⁺-N: 50 g/m³). NaHCO₃ was used for the inorganic carbon source as well as pH control within the range of 7.0–7.3 throughout the experiment. The volumetric aeration rate was set to 1.0 L/min and the dissolved oxygen (DO) concentration kept between 4 and 6 g/m³. The HRT was initially 72 min, and was reduced in a stepwise manner when the effluent concentration reached a steady state.

**Nitrification of metal-refinery wastewater with high salinity (Run 3)**. Nitrifying granules were inoculated to the CSTR with an initial MLSS concentration of 12,100 g/m³. Wastewater B, which was discharged from metal-refinery processes, was used in the experiment. NaHCO₃ was used for the inorganic carbon source as well as pH control within the range of 7.0–7.5. The volumetric aeration rate and HRT were set to 0.5 L/min and 1.2 day, respectively, throughout the experiment.

**Nitrification of domestic wastewater containing both organic pollutants and ammonia (Run 4)**. The nitrifying granules (MLSS: 1,200 g/m³) and conventional activated sludge (MLSS: 1,500 g/m³) were inoculated to the CSTR. Wastewater C, which contained ammonia (NH₄⁺-N: 60 g/m³) and organic pollutants (DOC: 180 g/m³), was continuously supplied. NaHCO₃ was used for pH control within the range of 7.0–7.1. The volumetric aeration rate was initially set to 0.5 L/min and then increased to 1.0 L/min on day 65. The HRT was initially 9 h and reduced in a stepwise manner when the effluent concentration reached a steady state. For a comparative run, nitrifying sludge instead of nitrifying granules was mixed with activated sludge and inoculated to the reactor.

**Chemical analysis**

All samples obtained from the reactors were filtered with a glass filter (GF/F, Whatman, UK) and used in water quality measurement. NH₄⁺-N concentration was measured using an ion chromatograph (DX-120, Dionex, Japan). Both NO₂⁻-N and NO₃⁻-N concentrations were measured using an HPLC with a UV detector (column: IC-Anion-PW, Tosoh, Japan). Measurement of MLSS was followed by the standard method (APHA, 1995).

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**Table 2 Chemical composition of synthetic organic wastewater C**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration [g/m³]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrin</td>
<td>60.8</td>
</tr>
<tr>
<td>Meat extract</td>
<td>149.2</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>130.8</td>
</tr>
<tr>
<td>Peptone</td>
<td>130.8</td>
</tr>
<tr>
<td>NaCl</td>
<td>13.2</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>16.8</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>37.2</td>
</tr>
<tr>
<td>KCl</td>
<td>26.8</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>163.4</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>382.8</td>
</tr>
</tbody>
</table>
DOC concentration was measured using a total organic carbon analyser (TOC 5000-A, Shimadzu, Japan). DO concentration and pH were measured using an O₂ micro sensor (UC-12, Digital DO/O₂/Temp Meter, Central Kagaku, Japan) and a pH electrode (TPX-90 Toko, Japan), respectively.

Evaluation of granule size
The average diameter of granules was measured using an optical microscope (VH-Z450, Keyence, Japan) as a circle-equivalent diameter of 100 granules that were randomly obtained from the reactor.

Results and discussion
Application to oxygen-rich conditions
In order to demonstrate the potential of nitrifying granules, a high concentration of ammonia (600 g-N/m³) in the liquid and a high concentration of oxygen (20–100%) in the gas were supplied to the AUFB reactor. The time-course of the ammonia removal rate is shown in Figure 1. When the effluent concentration reached a steady state, the HRT was reduced in a stepwise manner to elucidate the maximum nitrification rate under supply of each concentration of oxygen. When supplying pure oxygen, the HRT could be reduced to 45 min and the ammonia removal rate reached 16.7 kg-N/m³/day with a sustained ammonia removal efficiency of more than 80%. This result indicates that retaining a high density of nitrifying bacteria in the reactor in the form of granules enables high-rate nitrification providing that sufficient oxygen is supplied.

Application to short HRT conditions
The nitrifying granules were applied to wastewater with a low concentration of ammonia (50 g-N/m³) under short HRT conditions. The time-courses of water quality data (the influent and effluent concentrations of NH₄⁺-N) and the ammonia removal rate are shown in Figure 2. Complete nitrification was stably attained even when the HRT was as short as 44 min. The ammonia removal rate reached 1.65 kg-N/m³/day on day 75. However, when the HRT was further shortened to 38 min, the ammonia removal efficiency suddenly decreased and ammonia of 5–30 g-N/m³ remained in the effluent. This result revealed that 44 min is the minimum HRT at which the nitrifying granules give complete nitrification in the CSTR. The DO concentration in the reactor was over 4.0 g/m³ throughout the experiment, indicating that ammonia removal efficiency was not limited by substrate but limited by each cell potential in the granule. Washout of biomass was hardly observed.

Table 3 Reactor configuration and operating condition of each experimental run in this study

<table>
<thead>
<tr>
<th>Run No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor type</td>
<td>AUFB</td>
<td>CSTR</td>
<td>CSTR</td>
<td>CSTR</td>
</tr>
<tr>
<td>Sludge type</td>
<td>Granule</td>
<td>Granule</td>
<td>Granule</td>
<td>Granule and activated sludge</td>
</tr>
<tr>
<td>Wastewater type</td>
<td>A</td>
<td>A (diluted)</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>NH₄⁺-N Conc.</td>
<td>600 g/m³</td>
<td>50 g/m³</td>
<td>1,000–1,400 g/m³</td>
<td>60 g/m³</td>
</tr>
<tr>
<td>TOC Conc.</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>180 g/m³</td>
</tr>
<tr>
<td>Supplied O₂ Conc.</td>
<td>20–100%</td>
<td>20% (air)</td>
<td>20% (air)</td>
<td>20% (air)</td>
</tr>
<tr>
<td>HRT</td>
<td>0.75–4.9 h</td>
<td>38–72 min</td>
<td>1.2 d</td>
<td>4.5–9.0 h</td>
</tr>
<tr>
<td>N loading rate</td>
<td>2.8–19 kg/m³/d</td>
<td>1.0–1.9 kg/m³/d</td>
<td>0.83–1.2 kg/m³/d</td>
<td>0.16–0.32 kg/m³/d</td>
</tr>
<tr>
<td>Initial MLSS</td>
<td>15,100 g/m³</td>
<td>12,700 g/m³</td>
<td>12,100 g/m³</td>
<td>Granule: 1,200 g/m³</td>
</tr>
<tr>
<td>Sludge: 1,500 g/m³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water quality data</td>
<td>Figure 1</td>
<td>Figure 2</td>
<td>Figure 3</td>
<td>Figure 4</td>
</tr>
</tbody>
</table>
except during the initial 50 days, indicating that nitrifying bacteria could be completely retained in the CSTR even if the HRT was as short as 44 min. Therefore, it was demonstrated that the nitrifying granules can be applied to high-rate nitrification in the CSTR under short HRT conditions.

Application to metal-refinery wastewater with high salinity

In order to demonstrate practical utility of the nitrifying granules in industrial wastewater processes, their nitrification performance in application to metal-refinery wastewater was examined. The time-courses of ammonia concentration, MLSS, ammonia removal efficiency and granule average diameter are shown in Figure 3. Since a large amount of biomass (>10,000 g-MLSS/m³) was retained in the CSTR with the form of granules, the ammonia removal rate and efficiency reached 1.0 kg-N/m³/day and 95%, respectively. In this experiment, granule average diameter slightly increased without any collapse and exfoliation of the granules. Generally, industrial wastewater has the feature that concentration of nitrogenous compounds and coexisting substances, such as heavy metals and inorganic saline, are various. The metal-refinery wastewater used in this experiment also has these features. The results obtained in this run indicate that the nitrifying granules are
also applicable to real industrial wastewater even though its composition is rather different from that of the solution used for the prior granulation process.

**Application to domestic wastewater containing organic pollutants**

In order to extend the scope of nitrifying granules, it is necessary to evaluate their applicability to wastewater containing both organic matters and ammonia. The time-courses of water quality data (concentrations of DOC, \( \text{NH}_4^+ \text{-N}, \text{NO}_2^- \text{-N} \) and \( \text{NO}_3^- \text{-N} \)) are shown in Figure 4. For comparison, an experimental run using nitrifying sludge instead of nitrifying granules was operated. Initially, in both runs, DOC was favourably reduced and nitrification was confirmed. Since almost all ammonia was oxidised to nitrate in the run of granules, the HRT was stepwisely shortened to 6.0, 5.5, 5.0 and 4.5 h on days 3, 6, 8 and 15, respectively. Even after raising loading rate in the run of granules, sufficient and stable nitrification was observed. In contrast, in the run of nitrifying sludge, ammonia oxidation deteriorated with shortening the HRT. This might be because nitrifying bacteria inoculated to the CSTR was washed out of the CSTR. In this experiment, sludge return...
was not performed. The nitrification performance never recovered, and thus the run of nitrifying sludge was stopped on day 20.

Afterwards, since nitrification became gradually unstable in the run of granules, the volumetric aeration rate was increased from 0.5 to 1.0 L/min on day 65. As a result, recovery of nitrification was confirmed, which indicated that DO permeation to the inside of the nitrifying granule was improved by sufficient supply of oxygen to bulk solution in the CSTR. After all, nitrification and TOC removal have stably progressed for 200 days.

During the experimental run, the granule diameter gradually increased. In addition, biofilm with a thickness of 100–200 μm adhered to the granule surface was confirmed by a light microscope as shown in Figure 5. From these results, although a considerable

**Figure 4** Time-courses of water quality in application to organic wastewater. Open and closed circles show the runs of suspended nitrifying sludge and nitrifying granules, respectively. (a) The run of suspended nitrifying sludge was stopped. (b) Volumetric aeration rate was increased from 0.5 to 1.0 L/min

**Figure 5** Photographs of nitrifying granules in the organic wastewater viewed by optical microscopy on (a) day 0; (b) day 114; (c) day 178. Bar = 500 μm
amount of heterotrophic bacteria adhered to the surface, the nitrifying granules were fully workable as nitrification assistance in organic wastewater.

**Conclusions**

In this study, in order to search for the utility of nitrifying granules produced in the AUFB reactor, their nitrifying performances in application to various wastewaters were evaluated. The experimental results are summarised as follows.

1. Under supply of pure oxygen gas, the ammonia removal rate reached 16.7 kg-N/m³/day with a sustained ammonia removal efficiency of more than 80%.
2. The nitrifying granules can be retained in a CSTR at a high density even under short HRT (44 min) conditions, and ammonia removal rate of 1.65 kg-N/m³/day was obtained even when applied to the wastewater with a low concentration of ammonia (50 g-N/m³).
3. The nitrifying granules exhibited sufficient performance when applied to metal-refinery wastewater containing high concentrations of ammonia (600 g-N/m³) and salinity.
4. When the nitrifying granules were applied to organic wastewater, they were fully workable as nitrification assistance in spite of adhesion of biofilms to their surfaces.

**References**


