High-rate nitrification of electronic industry wastewater by using nitrifying granules
Yoshiaki Hasebe, Hiroaki Meguro, Yuuki Kanai, Masahiro Eguchi, Toshifumi Osaka and Satoshi Tsuneda

ABSTRACT
Nitrifying granules have a high sedimentation property and an ability to maintain a large amount of nitrifying bacteria in a reaction tank. Our group has examined the formation process of nitrifying granules and achieved high-rate nitrification for an inorganic synthetic wastewater using these granules. In this research, a pilot-scale test plant with an 850-liter reaction tank was assembled in a semiconductor manufacturing factory in order to conduct a continuous water conduction test using real electronics industry wastewater. The aim was to observe the formation of nitrifying granules and determine the maximum ammonia removal rate. The average granule diameter formed during the experiment was 780 μm and the maximum ammonia removal rate was observed to be 1.5 kgN·m⁻³·day⁻¹ at 20°C, which is 2.5–5 times faster than traditional activated sludge methods. A fluorescence in situ hybridization analysis showed that β-proteobacterial ammonia oxidizing bacteria and the Nitrospira-like nitrite-oxidizing bacteria dominate the bacteria population in the granules, and their strong aggregation capacity might confer some benefits to the formation of these nitrifying granules.

Key words | aerobic granule, industrial wastewater, nitrification, nitrifying granule, Nitrospira

INTRODUCTION
In electronics production, for example semiconductor or liquid crystal manufacturing, nitrogen-containing compounds such as ammonium fluoride, ammonium peroxodisulfate, and tetramethylammonium hydroxide are used. In many cases, these nitrogen compounds are used in large amounts and the treatment of these compounds in wastewater has become a significant problem. In general, physicochemical treatments such as ammonia stripping are cost-effective methods employed for highly concentrated wastewater containing more than 1,000 mgN·L⁻¹ of the ammonium ion. On the other hand, biological treatment has economic advantages for wastewater with relatively low concentrations of the ammonium ion (<1,000 mgN·L⁻¹). Biological nitrification-denitrification systems are commonly used processes for nitrogen removal from ammonium-containing wastewater. The nitrification process, which is the biological oxidation of ammonium into nitrite and nitrate, tends to be the rate-limiting step in the biological nitrogen removal process because the nitrifying bacteria (i.e. ammonium-oxidizing bacteria and nitrite-oxidizing bacteria) responsible for the nitrification process have a low ammonia removal rate. Wastewater treatment systems using carrier materials have been utilized recently for accelerating the nitrification process (Levstek & Plazl 2009). These systems maintain a higher amount of sludge than conventional activated sludge systems. However, in a factory using large amounts of nitrogen-containing compounds, a nitrogen treatment facility usually requires a large footprint. A reduction in this footprint through high-rate nitrogen removal may be beneficial.

To increase the biological treatment rate, it is conceivable to increase the microbial concentration in the reactor. Using granular sludge is one possible method of achieving this. Generally, wastewater treatment systems using nitrifying granules can achieve high-rate treatment owing to the sedimentation properties of the granules and the ability to maintain a large number of microorganisms in the reaction tank. Initially, the nitrifying granule method was developed for anaerobic treatment and practically applied to upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactors (Seghezzo et al. 1998). However, it has been found that aerobic microorganisms can also form...
granules and this has been actively researched recently (Beun et al. 1999; Etterer & Wilderer 2001; Liu & Tay 2006). The majority of research regarding aerobic granules has focused on using sequencing batch reactors (SBR) (Beun et al. 1999; Etterer & Wilderer 2001; Liu & Tay 2006). An SBR requires batch processing in which the inflow of raw water and outflow from the reactor takes place within a short time frame. Large influent and effluent tanks are required for treatment of large volumes of wastewater in this case. Consequently, it is difficult to utilize SBRs for wastewater treatment in large factories.

Mishima & Nakamura (1991) reported the formation of aerobic granules using a preceding upflow aeration column under continuous flow conditions. In this process, granules are formed as a loosely assembled sludge, and are thus not as strong or dense as anaerobic granules. On the other hand, Tsuneda et al. (2005) demonstrated that it is possible to form nitrifying granules by treating ammonia-containing synthetic wastewater with a concentration of 500 mgN·L⁻¹ by utilizing a continuous flow, conduction type aerobic upflow fluidized bed (AUFB) reactor for 500 days. It was also demonstrated that high-rate treatment is possible using this approach with a nitrification ratio of over 90% (Tsuneda et al. 2005).

Our group has conducted various studies using synthetic wastewater that aim to use nitrifying granules for wastewater treatment. We have reported the influence of different operating conditions of reactors, such as the ammonia loading rate and the shape of the reactor, on the formation of nitrifying granules (Hasebe et al. 2009, 2011). We have proven that it is possible to form nitrifying granules and create high-rate wastewater treatment systems using a square-shaped reactor, with complete mixing that can easily be installed in existing industrial situations (Hasebe et al. 2011). However, an examination using actual electronic industry wastewater has not yet been conducted. Therefore, an understanding of the influence of water temperature fluctuations, the characteristics by which nitrifying granules are formed, and processing properties such as the quality of treated water and the ammonia removal rate are crucial in order to apply the nitrifying granule method to real electronics industry wastewater.

Unlike the treatment of synthetic wastewater, industrial wastewater has an existing ammonia removal rate and contains suspended solids (SS) and organic substances apart from ammonium and nitrogen. In this research, we have experimented with the treatment of electronics industry wastewater from a semiconductor factory over a period of about 200 days using a pilot-scale nitrification test plant. We have demonstrated the formation of nitrifying granules from the activated sludge and accomplished high-rate nitrification by using granular sludge. In addition, we have reported on our operating method for the formation of granules using seed sludge and analyzed the bacterial community living in the nitrifying granules that were produced.

### MATERIALS AND METHODS

#### Impact assessment of temperature

The impact of water temperature on the ammonia removal rate was assessed by a batch mode test using nitrifying granules. In this test, synthetic wastewater was used to eliminate the influence of inhibiting substances in industrial wastewater and to ensure that only the influence of water temperature was evaluated. The composition of the synthetic wastewater used in this test is shown in Table 1. Nitrifying granular sludge produced in a laboratory was added to 200 mL of synthetic wastewater in a 500 mL beaker and the mixed liquor suspended solids (MLSS) were adjusted to a concentration of approximately 2,500 mg·L⁻¹. This experiment was performed under aerated conditions and the water temperature was set in six phases: 5, 10, 15, 20, 25, and 30°C using a thermostatic tank. The temperature range for the experiment was selected to include temperatures of 15–25°C, which is assumed to be the operating temperature for electronics industry wastewater. Samples of the reaction solution were separated from the reactor at predetermined time intervals, and the NH₄-N concentration of each sample was measured. The ammonia removal rate was determined by an analysis of the changing NH₄-N concentration of the samples.

#### Comparison of nitrification activity in both synthetic and electronics industry wastewater

A nitrification activity test was conducted using nitrifying granules produced in a laboratory to assess the presence or absence of different substances in the industrial wastewater.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration [mg·L⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄-N</td>
<td>50</td>
</tr>
<tr>
<td>PO₄³⁻-P</td>
<td>2</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>720</td>
</tr>
</tbody>
</table>
absence of nitrification inhibitors in electronics industry wastewater. In order to assess the impact of other substances in the industrial wastewater, H₃PO₄ and NaHCO₃ were added to increase the concentration of these compounds to 10 mgP·L⁻¹ and 720 mg·L⁻¹, respectively. The amount of added NaHCO₃ was selected to maintain the pH of the reaction at 7.5–8.0. After 18 hours under aerated conditions, complete nitrification of NH₄⁺ in the wastewater to NO₃⁻ was achieved. Following this, NH₄⁺ was added to adjust the nitrogen concentration of the wastewater to 50 mgN·L⁻¹. Finally, the ammonia removal rate was assessed by calculating the rate of decline in the concentration of NH₄⁺-N in subsequent samples. All other conditions were the same as for the ‘Impact Assessment of Temperature’; however, the water temperature was maintained constant at 20 °C using a thermostatic tank. The presence or absence of nitrification inhibitors was assessed by comparing the results of this test with those from the synthetic wastewater test.

Continuous flow conduction test

Wastewater

Nitrogen-containing wastewater discharged from a semiconductor factory was used for a continuous flow conduction test. This wastewater is a mixture of several types of nitrogen-containing wastewaters discharged from different semiconductor manufacturing processes in the factory. The wastewater discharged from the factory contains both fluorine and organic matter and as such, needs to be treated by coagulation sedimentation to remove fluorine and aerobic biological treatment to reduce organic matter. The wastewater for the continuous flow conduction test was collected following these treatments. The typical composition of the wastewater included 300–600 mg·L⁻¹ of calcium, 30–110 mg·L⁻¹ of SS, and 10–50 mgC·L⁻¹ of dissolved organic carbon (DOC). The calcium in the wastewater is mainly added by fluorine treatment process. A representative composition of this raw wastewater is shown in Table 2.

Experimental set-up

The schematic design of the experimental apparatus for the continuous flow conduction test is shown in Figure 1. Electronics industry wastewater was collected in an inlet water tank and was continuously pumped from the top of the reactor tank. The effective volume of the reactor was 850 liters and a membrane diffuser (PMD-009, Daicen Membrane Systems Ltd, Japan) was installed at the bottom of the tank. Air was continuously supplied to the reactor by a blower and a pH sensor was installed. A 5% (w/w) solution of NaOH was added to maintain the pH during the experiment at 7.2. The effluent was removed through a trough at the top of the reactor and separated into a solid and liquid phase in a sedimentation tank. The liquid component was discharged as treated water. The sludge from the bottom of the sedimentation tank was piped to the reactor tank by an air-lift pump to prevent the nitrifying granules from being crushed.

Test conditions

As the semiconductor factory did not have an activated sludge process, activated sludge was collected from a food factory as seed sludge of continuous flow test in this study. This sludge was placed into the reactor as seed sludge at a concentration of 4,000 mg·L⁻¹. An inorganic sedimentation accelerator comprising mainly SiO₂ and Al₂O₃ was also added to the reactor until the sludge concentration had

Table 2 | Chemical compositions of wastewater discharged from an electronics manufacturing factory

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration [mg·L⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁻N</td>
<td>90–250</td>
</tr>
<tr>
<td>NO₃⁻N</td>
<td>40–70</td>
</tr>
<tr>
<td>PO₄³⁻-P</td>
<td>2–10</td>
</tr>
<tr>
<td>DOC</td>
<td>10–50</td>
</tr>
<tr>
<td>Ca</td>
<td>300–600</td>
</tr>
<tr>
<td>SS</td>
<td>30–110</td>
</tr>
<tr>
<td>pH</td>
<td>9.8–10.4</td>
</tr>
</tbody>
</table>

Figure 1 | Schematic illustration of the pilot test plant in this study.
increased to approximately 15,000 mg·L⁻¹. Throughout the operation of the experiment, the pH in the reactor tank was controlled at 7.2 ± 0.2 and the dissolved oxygen (DO) concentration was maintained above 5 mgO₂·L⁻¹. The temperature was not regulated. The returning rate of sludge was set between 0.5 and 1.0 times the inlet water flow, and output was discharged so that the sludge retention time (SRT) was approximately 20 days. The water temperature in the reactor ranged between 20 and 30 °C, except for a short period in summer when it exceeded this temperature.

Bacterial community analysis using the fluorescence in situ hybridization method

The granule samples were fixed in a 4% (w/v) solution of paraformaldehyde in phosphate-buffered saline (PBS; 8 g NaCl, 0.2 g KCl, 2.9 g Na₂HPO₄·12H₂O, and 0.2 g KH₂PO₄ per liter of distilled water at a pH of 7.2) and incubated for a period of 16 h at 4 °C. Afterward, the cells were washed three times with a PBS solution and resuspended in a 1:1 (v/v) mixture of PBS and ethanol, where they were stored at −20 °C. The fixed samples were embedded in Tissue-Tek optimum cutting temperature compound (Sakura Finetek, Tokyo, Japan), rapidly frozen to −20 °C, and then cut into 10 μm slices using a cryostat (CM1850; Leica Microsystems, Germany). The probes used in the fluorescence in situ hybridization (FISH) analysis are listed in Table 3. In order to detect all bacteria, an EUB338mix probe was utilized. The EUB338mix probe was labeled with fluorescein isothiocyanate (FITC). The probes for detecting each target bacterium were labeled with Cyanine Dye 3 (Cy3). Hybridization with fluorescent probes was performed as described in the previous literature (Amann et al. 1990; Mobarry et al. 1996; Wagner et al. 1996; Daims et al. 1999, 2001). Slides were mounted in SlowFade antifade reagent (Invitrogen, USA) and images were taken using a confocal laser scanning microscope (FV300; Olympus Corp., Japan). The FISH images were analyzed using the daime (Digital Image Analysis In Microbial Ecology) software package (Daims et al. 2006).

Analytical and evaluation method

The water sample for analysis was filtered through an industrial filter paper (No. 5C, Advantec Toyo Ltd, Japan) and sent to the laboratory using refrigerated transport. An analysis of the NH₄-N concentration was then conducted using indophenol blue absorption spectrophotometry and the analysis of NO₂-N and NO₃-N was completed using ion chromatography (ICS-1000, Dionex, USA). The sedimentation properties of the sludge from the reactor were assessed by measuring the SV₅ (the sludge volume measured at a settling time of 5 minutes) and SV₃₀ (the SV measured at a settling time of 30 minutes). Since the ammonia removal rate is heavily affected by water temperature, the ammonia removal rate (Kᵣ) at the actual water temperature (T) in °C was corrected to correspond to the rate at 20 °C (K₂₀) using the following equation.

\[
K_{20} = \frac{K_{T}}{e^{(20-T)}},
\]

An analysis of the MLSS and the mixed liquor volatile suspended solids (MLVSS), SV₅ and SV₃₀ was conducted according to Standard Methods (APHA 1998), and images of sludge were taken with a digital microscope (VH-8000, KEYENCE, Japan).

RESULTS AND DISCUSSION

Impact assessment of temperature on the nitrifying granule treatment

Ammonia removal rates recorded at various water temperatures are shown in Figure 2. It was assumed that the water temperature would significantly affect these rates; however, the change in the nitrification rate between 5 and 15 °C is almost linear when compared with the change in the nitrification rate between 15 and 30 °C and a rapid change was not identified. Tomizawa (1986) studied the impact of water temperature on nitrification activity using activated sludge and gel immobilized microorganisms. According to

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**Table 3** FISH probes used in this study

<table>
<thead>
<tr>
<th>Probe</th>
<th>Specificity</th>
<th>FA* [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB338 I</td>
<td>Most bacteria</td>
<td>–</td>
</tr>
<tr>
<td>EUB338 II</td>
<td>Planctomycetales</td>
<td>–</td>
</tr>
<tr>
<td>EUB338 III</td>
<td>Verucmicothiales</td>
<td>–</td>
</tr>
<tr>
<td>NSO190</td>
<td>Betaproteobacterial</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>ammonia-oxidizing bacteria</td>
<td></td>
</tr>
<tr>
<td>NIT3</td>
<td><em>Nitrobactor</em> spp.</td>
<td>40</td>
</tr>
<tr>
<td>Comp NT3</td>
<td>Competitor for NT3</td>
<td>–</td>
</tr>
<tr>
<td>Ntspa662</td>
<td><em>Nitrospira</em> spp.</td>
<td>35</td>
</tr>
<tr>
<td>Comp Ntspa662</td>
<td>Competitor for Ntspa662</td>
<td>–</td>
</tr>
</tbody>
</table>

*Formamide concentration.
his report the impact of water temperature on nitrification activity for normal activated sludge is more significant for low water temperatures (10–15 °C); however, this impact was significantly mitigated by immobilized microorganisms. These microorganisms are unlikely to be affected by temperature. At medium temperatures, where the activity of nitrifying bacteria is strong at the surface, the core of the carrier is low in DO; however, at low temperatures, the activity of nitrifying bacteria present on the surface of the carrier is reduced and DO can also reach the internal nitrifying bacteria. As a result, the number of nitrifying bacteria involved in the nitrification processes increases and a decrease in the nitrification rate does not occur overall. It is suggested that a similar phenomenon occurs for granular sludge.

A temperature correction coefficient (θ) was determined from the results shown in Figure 2. It was calculated on the basis of results in the range of 15–35 °C, representing assumed operating temperatures for electronics industry wastewater. In this case, θ was calculated to be 1.062. Salvetti et al. (2006) reports another study where θ was calculated to be 1.098 in the range of 13–22 °C as the result of temperature dependency tests on ammonia-oxidizing bacteria. The lower value for θ obtained in this study suggests that nitrifying granules are less susceptible to temperature effects normal activated sludge.

The nitrification activity test

The time course of the NH₄⁺-N concentration for the nitrification activity test is shown in Figure 3. The MLVSS in the synthetic wastewater test and real electronics industry wastewater test are shown to be 10,300 mg·L⁻¹ and 8,600 mg·L⁻¹, respectively. The ammonia removal rates were calculated to be 0.49 and 0.60 kgN·kgMLVSS⁻¹·day⁻¹, respectively, and no significant difference between the samples was identified. Jingru et al. (2015) also reported a specific ammonia-oxidizing activity of 0.44 kgN·kgMLVSS⁻¹·day⁻¹ in the batch test. Compared to this result from the literature, the granular sludge used in this study demonstrates a slightly higher nitrification activity. This result suggests that nitrification inhibitors are not present in the electronics industry wastewater sample used for this study and biological nitrification is a valid treatment method for electronics industry wastewater.

The continuous flow conduction test

Quality of raw water and treated water

The time courses of NH₄⁺-N inlet and treated waters in a continuous flow conduction test using electronics industry wastewater are shown in Figure 4. The NH₄⁺-N concentration of the inlet water changed between 100 and...
150 mg-N·L⁻¹ from the beginning of the experiment to day 110. After that, the concentration was observed to increase gradually before changing between 200 and 250 mg-N·L⁻¹ for 75 days until day 185. Although the cause of the rise in the NH₄⁺-N concentration is unknown, an increase in a number of chemicals used in the manufacturing process was considered as a likely possibility. During the test period, the NH₄⁺-N concentration of inlet water fluctuated widely but the NH₄⁺-N concentration of treated water was consistently less than 5 mg-N·L⁻¹ over the entire pilot. This suggests that the mean nitrification ratio is more than 99% and proves that the electronics industry water was treated satisfactorily using this process.

**Ammonia removal rate**

The time courses for the ammonia loading rate and the ammonia removal rate are shown in Figure 5, as well as the ammonia removal rate corrected for temperature as calculated by Equation (1). During the start-up process for the pilot, the ammonia loading rate was approximately 0.13 kgN·m⁻³·day⁻¹ at 20 °C. After increasing the ammonia loading rate by gradually increasing the inlet flow rate, the ammonia removal rate reached a peak of 0.68 kgN·m⁻³·day⁻¹ at 20 °C after 30 days. However, the concentration of the MLSS in the reactor rapidly decreased when the SS were removed from the sedimentation tank with the treated water (Figure 6). The linear velocity (LV) of the inlet of the sedimentation tank increased due to an increase in the volumetric flow rate, which exceeded the sedimentation velocity of sludge and resulted in the removal. To prevent a deterioration of the quality of the treated water the ammonia loading rate was decreased to approximately 0.3 kgN·m⁻³·day⁻¹, a reduction of about one-third, while the concentration of SS in the treated water was checked. As a result, the ammonia removal rate increased to a rate of 1.0 kgN·m⁻³·day⁻¹ at 20 °C and MLSS concentration began to increase again on day 75. After day 110, the flow rate of the inlet water was maintained at a constant level, while the ammonium concentration of inlet water increased. The loading rate increased along with the concentration until day 140. As a result, the ammonia removal rate at 20 °C increased to 1.5 kgN·m⁻³·day⁻¹ by day 140. Following this, the ammonia removal rate was maintained at 1.5–2.0 kgN·m⁻³·day⁻¹ for a period of more than 40 days. The ammonia removal rate of traditional activated sludge processes using a fluidized-bed and a carrier only achieved removal rates of 0.3–0.6 kgN·m⁻³·day⁻¹ (Nakamura & Fujita 1997). Levstek and Plazl investigated the nitrification performance of high-density, cylindrical, polyethylene ring-shaped carriers (AnoxKaldnes, K1 carrier; 335 m²/m³) and reported a maximum nitrification rate of 3.1 gN·m⁻²·day⁻¹ (Levstek & Plazl 2009; Shahot et al. 2014). As the K1 carrier is generally used with a packing ratio of 70% or less, the maximum nitrification rate can be calculated as 0.73 kgN·m⁻³·day⁻¹. This demonstrates that treatment can be achieved 2–5 times faster using nitrifying granules for electronics industry wastewater.

To date, our group has reported that the nitrifying granule process using 180 liters of synthetic wastewater (Hasebe et al. 2011) attained an ammonia removal rate of 1.8 kgN·m⁻³·day⁻¹ at 20 °C after 110 days. While the concentration of nitrogen (0.5 kgN·m⁻³) in the wastewater is slightly different from the electronics industry wastewater used in this study, the result was similar. Therefore, it was considered that if the LV of the sedimentation tank is
lower than the LV of the reactor, then it is necessary to maintain the sludge in the reactor, as the influence of the ammonium nitrogen concentration in inlet water is low. In the completely mixed reactor used in this study, the ammonium concentration in the reactor is always maintained at a low concentration, thus the influence of the inlet concentration on the nitrification rate is also small. Moreover, it is confirmed by this research that almost the same ammonia removal rate can be achieved for synthetic wastewater, even with the influence of organic compounds and SS components in the electronics industry wastewater. After day 180, the inlet flow rate was increased because the concentration of inlet water had returned to normal. Along with the increase in the flow rate, the LV of sedimentation tank increased significantly. On day 203, the LV was measured to be 3.6 m·h⁻¹ which is 40% higher than day 180. Despite this, a substantial decline in the MLSS concentration was not observed and it was shown that maintaining the volume of nitrifying granules in the reactor is possible by maintaining the LV of the sedimentation tank to less than 3.0 m·h⁻¹.

The formation of granules

The time courses of the MLSS and MLVSS in the reactor tank and the LV of sedimentation tank are shown in Figure 6, while the time course of sludge volume index (SVI) is shown in Figure 7. During the first 70 days, the MLSS concentration decreased by 85% from 22,000 to 3,500 mg·L⁻¹ and the MLVSS decreased 51% from 4,100 to 2,000 mg·L⁻¹. The rapid increase in the MLSS concentration during the first 70 days of the pilot is due to the removal of SS after use of the sedimentation accelerant during start-up. After 70 days, the ratio of the concentration of MLVSS to MLSS increased and reached 0.7–0.8. The MLVSS/MLSS ratio was approximately 0.5 on day 75 and about 0.7 on day 150. On the other hand, the inorganic matter concentration in the reactor calculated from MLVSS and MLSS concentrations remained fairly constant at about 2,500 mg·L⁻¹. However, considering that the SRT was under control after about 20 days, it is considered that the inorganic matter in the reactor changed gradually first from the added sedimentation accelerant and then from the calcium introduced in the inflow of the wastewater. The majority of sedimentation accelerant was discharged from the system along with the treated water and the sludge. As shown in Figure 7, both SVI₅ and SVI₃₀ remained at less than 50 during the first 30 days of the experiment. Although the use of sedimentation accelerant was considered effective in increasing the rate of sludge settling and preventing the wash-out of sludge in the early stages, further research is required to assess the necessity and the amount required as the majority of this compound was removed from the reactor with treated water as soon as the water experiment began.

The SVI of the sludge (both SVI₅ and SVI₃₀) rose significantly from day 35 to 50 during the time when the concentration of the MLSS dropped sharply. This phenomenon may have been caused by the heavy removal of sedimentation accelerant during this period. In this study, the ammonia loading rate was increased gradually through an increase in flow rate to keep the NH₄-N concentration in the effluent at 5 mgN·L⁻¹ or less. However, when the LV of the settling tank exceeded the settling velocity of the sludge with the increasing flow rate, the MLSS concentration in the reactor decreased because of the removal of the sludge. For the effective maintenance of the system, it is necessary to maintain the amount of MLSS in the reactor. In Figure 6, it can be shown that the MLSS concentration started to rise from around day 70. The SV₅ was at a value of 150 at this time. When it is considered that the first 5 minutes of the experiment was a free settling period for the measurement of SV, the settling rate of the sludge can be determined by measuring the settling distance after 5 minutes. Based on this assumption, when SV₅ is 50 in a graduated cylinder, the settling velocity was calculated to be about 1.7 m·h⁻¹, which is almost as fast as the LV of sedimentation tank (1.8 m·h⁻¹) at that time. Therefore it is concluded that the value for SV₅ is an effective indicator to determine the setting value of the loading rate. The relationship of the SVI and LV for the sedimentation tank is shown in Figure 8. From this figure, it can be seen that when the SVI₅ is about 130 mL·g⁻¹ or less, the LV of the sedimentation tank can be operated at 1.5 m·h⁻¹ or more. This provides important information...
regarding the design of the wastewater treatment plant. From day 150 both the SVI5 and SVI30 value declined and stabilized at 20 mL·g⁻¹. The picture of the nitrifying granules on day 170 is shown in Figure 9. The average diameter of the granules was 780 µm. These granules were not removed from the reactor even when the LV was greater than 3 m·h⁻¹ in the settling tank. The nitrifying granules were shown to have very strong sedimentation properties.

FISH analysis

The spatial distribution of ammonia-oxidizing and nitrite oxidizing bacteria was analyzed using the FISH method. FISH images using the NSO190 and EUB338 probes (Figure 10(a)) revealed that β-proteobacterial ammonia oxidizing bacteria (e.g. *Nitrosomonas, Nitrosospira*) existed near the granule surface. The FISH image using Ntspa662 and EUB338 probes (Figure 10(b)) demonstrated that a *Nitrospira*-like nitrite oxidizing bacteria was distributed throughout the entire granule. *Nitrobacter* spp. (detected with probe NIT3) was not detected in this study. The occupancy of each target bacteria determined by the analysis of FISH images is shown in Table 4. The FISH analysis revealed that 15% of the total bacteria within the granules was β-proteobacterial ammonia oxidizing bacteria (detected with probe NSO190) and 60% *Nitrospira*-like nitrite oxidizing bacteria (detected with probe Ntspa662). The total number of ammonia-oxidizing bacteria was lower than that for nitrite-oxidizing bacteria. Recently, some species of *Nitrospira* were found to be capable of both ammonia oxidation and nitrite oxidation (Daims et al. 2013). Thus, it is assumed that some of the *Nitrospira* detected in the granules are responsible for ammonia oxidation as well as nitrite oxidation.

As reported previously, β-proteobacterial ammonia-oxidizing bacteria (e.g. *Nitrosonomas, Nitrosospira*) and nitrite-oxidizing bacteria (e.g. *Nitrospira*) tend to display strong aggregation in nitrifying biofilms (Mobarry et al. 1996; Schramm et al. 1996, 1998). Furthermore, it has been reported that *Nitrospira* isolates itself from the rest of the sludge during wastewater treatment and is able to form a strong cluster or microcolony (Spieck et al. 2006; Fujitani et al. 2013; Ushiki et al. 2013). The capability for strong aggregation and microcolony formation observed in β-proteobacterial ammonia-oxidizing bacteria and *Nitrospira* and detected by the FISH analysis might confer some benefits to the improved formation of nitrifying granules.

CONCLUSIONS

In order to comprehend formation characteristics of nitrifying granules and assess the treatment of electronics industry wastewater, a continuous water conduction test was implemented using a pilot-scale test plant in an electronics manufacturing factory. Furthermore, a microbial community analysis employing the FISH method was conducted regarding the formation of nitrifying granules. The following major conclusions were reached through this research.

(1) Through a continuous flow conduction test for 200 days using electronics industry wastewater, an average grain diameter 780 µm was formed for nitrifying granules with good settling properties.
By using granulated sludge, it was possible to maintain the MLSS concentration as high as 10,000–13,000 mg·L⁻¹. As a result, the ammonia removal rate reached 1.5–2.0 kgN·m⁻³·day⁻¹ at 20 °C, which is 2.5–5 times faster than the rate attained with traditional activated sludge processes. Additionally, the overall nitrification performance for real electronics industry wastewater was almost as effective as that for synthetic wastewater.

The results of an analysis based on FISH method showed that 15% of bacteria forming granules were ammonia-oxidizing bacteria and 60% were nitrite-oxidizing bacteria. Only bacteria of the genus *Nitrospira* were detected as nitrite-oxidizing bacteria and not bacteria of the genus *Nitrobacter*. This suggests that that bacteria of the genus *Nitrospira* contribute to granule formation.

### REFERENCES


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