Removal of nitrogenous and carbonaceous substances by a porous carrier–membrane hybrid process for wastewater treatment

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Abstract

Porous carriers in fluidized bed have been used for microbial immobilization in order to simultaneously remove organic carbon and nitrogen in wastewater. In particular, multifunctional microbial reactions in the carrier, such as simultaneous nitrification/denitrification, play important roles in nitrogen removal. To enhance these reactions the substrates should be supplied into the carrier with appropriate rates. This is because the denitrification reaction was often limited by the supply of organic substances, due to overgrowth of heterotrophs in the region near the carrier surface. A porous carrier–membrane hybrid process was found to have improved nitrogen removal efficiency, due to stimulated denitrification as well as nitrification. The hybrid system achieved a 30% higher nitrogen removal ratio than that in the fluidized porous carrier system. Microelectrode studies revealed that although intracarrier denitrification rate in a conventional fluidized bed was limited by organic carbon, this limitation was reduced in the hybrid process, resulting in the increased intracarrier denitrification rate. These effects were due to suppressed glucose oxidation in aerobic region in the hybrid process.

Keywords: Carrier–membrane hybrid process; Dissolved oxygen; Fluidized bed bioreactors; Membrane bioreactor; Nitrification; Wastewater treatment

1. Introduction

Both fluidized carrier and membrane processes have been extensively studied for biological wastewater treatment. Retained microbial processes using porous carrier has been applied in terms of high concentration culture of microbes [1] or multifunctional microbial reaction such as simultaneous removal of carbonaceous and nitrogenous substances from wastewater [2,3]. The influence of substrate concentration on simultaneous nitrification and denitrification using fluidized bed reactors has been investigated for the wastewater containing inorganic carbon [4,5] and organic substance [6–9]. Inorganic carbon supplements accelerated the nitrifying reaction, while a higher concentration of organic substance suppressed it. Because the organic substances are dominantly utilized by heterotrophic bacteria as carbon source, there is a competition between nitrifiers and fast-growing heterotrophic bacteria for available dissolved oxygen (DO) in the surface region of the carrier [10]. Consequently, little organic substance can diffuse into the anaerobic intracarrier region, where it provides electrons for denitrification. Therefore, the investigation on the consumption of organic substances together with its diffusion into the carrier is indispensable for clarifying the denitrification behavior in fluidized bed reactors.

Membrane processes have been applied to two objectives, removal of particulate substances and retainment of low-growth microbes in the reactor for high cell density operation [11]. Biological nitrogen removal has been done by using membrane systems to concentrate the nitrifier in a reactor [12,13]. Making the microbial flocs small increased the oxygen supply rate and improved the nitrification rate [14,15]. In spite of such improved nitrification capability in membrane processes, an anaerobic denitrification reactor is still needed for achieving complete nitrogen removal.

By microelectrode techniques, the oxygen distributions in autotrophic and auto/heterotrophic biofilms have been studied [16]. Some studies concerning glucose diffusion in a biofilm [17,18] revealed that increased glucose loading stimulated heterotrophic organic oxidation due to increased oxygen supply. On the other hand, the availability of organic substances also limits the denitrification rate in suspension culture [19] and biofilms [20,21]. As a result, the supply rate
of organic substances plays an important role in denitrifying reactions not only as an electron donor but also as a way to increase the anaerobic region in biofilm.

According to our previous study [6], the concentration of suspended microbes in the fluidized porous carrier reactor was around 100–200 mg l$^{-1}$. The combination of membrane with this reactor is expected to effect a high concentration of suspended microbes due to the blocking of microbes in the reactor by membrane. Based on this idea, a hybrid reactor system composed of suspended porous carriers and membrane is proposed in the present research for an efficient removal of nitrogen together with carbonaceous substances. The results are compared with the conventional carrier fluidized bed reactor, and also by taking the intracarrier concentration distribution of substrate and oxygen into consideration.

2. Materials and methods

2.1. Apparatus and procedure

Arrangement of the apparatuses for the carrier suspending system (CS) without membrane and the hybrid system (HS) of porous carrier and membrane is shown in Fig. 1.

Fig. 1(A) shows the apparatus for CS made of acrylic resin containing suspending porous carriers of polyurethane. Fig. 1(B) shows the apparatus for HS, where membrane modules were installed into the apparatus of Fig. 1(A). The liquid volume in the reactor was 10 l for both systems. Physical characteristics of the carrier and membrane are summarized in Table 1. The components of synthetic wastewater are shown in Table 2. The feed rate of wastewater and the aeration rate were respectively 1.25 l h$^{-1}$, 4.001 min$^{-1}$ in CS and 2.08–0.83 l h$^{-1}$, 25.0 l min$^{-1}$ in HS. The influent and effluent were controlled by using a suction pump as shown in Fig. 1(B). The liquid permeation rate was monitored by effluent flow measurement and the transmembrane pressure was also measured by a pressure gauge.

2.2. Measurement of intracarrier oxygen and glucose concentration

Oxygen concentration in carrier was measured by a Clark-type oxygen microelectrode. The microelectrode was fabricated as described by Revsbech and Jørgensen [22]. The microelectrode was calibrated in air-saturated water and 7% sodium sulfite solution. Relatively thick and solid electrodes with tip diameter of about 200 μm were used. The reason was that the tip should be strong enough to avoid a breaking when contacting the polyurethane matrix of the carrier. The time constant for 90% response of the microelectrode was about 3 s. A glucose microelectrode was fabricated as described by Cronenberg and van den Heuvel [17]. A carrier from the reactor was set in a measurement vessel with 200 ml working volume as described previously [10]. The position of the electrode tip was adjusted by a three-dimensional micromanipulator (MMS-77; Shimadzu).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of carrier and membrane</th>
</tr>
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<tbody>
<tr>
<td>Material</td>
<td>Pore size (μm)</td>
</tr>
<tr>
<td>Porous carrier</td>
<td>Polyurethane</td>
</tr>
<tr>
<td>Membrane</td>
<td>Polyethylene</td>
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</tbody>
</table>
Table 2

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mg l(^{-1}))</th>
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<tbody>
<tr>
<td>(NH(_4))(_2)SO(_4)</td>
<td>20–40</td>
</tr>
<tr>
<td>Glucose</td>
<td>100–400</td>
</tr>
<tr>
<td>NaHCO(_3) (mg CaCO(_3) l(^{-1}))</td>
<td>0–400</td>
</tr>
<tr>
<td>Inorganic salts</td>
<td></td>
</tr>
<tr>
<td>KH(_2)PO(_4)</td>
<td>12.6</td>
</tr>
<tr>
<td>MgSO(_4)</td>
<td>9.5</td>
</tr>
<tr>
<td>CaCl(_2)·2H(_2)O</td>
<td>1.2</td>
</tr>
<tr>
<td>FeCl(_3)·6H(_2)O</td>
<td>0.1</td>
</tr>
</tbody>
</table>

2.3. Analytical method

Total organic carbon (TOC) concentration was determined by a TOC analyzer (TOC-5000A; Shimadzu). Ammoniacal nitrogen (NH\(_4\)+-N), nitrite nitrogen (NO\(_2\)-N) and nitrate nitrogen (NO\(_3\)-N) were analyzed by ion chromatography (SCL-10A; Shimadzu). DO in the suspension was measured by a DO meter (MK-250; Automatic Systems Research, Saitama, Japan). The effect of DO on denitrification activity was investigated by measuring the decrease of NO\(_3\)-N concentration in the 500 ml glass vessel. Biomass was measured by methods described previously [6]. Chemical components of microbes were determined by element analysis.

3. Results and discussion

3.1. Carrier system

In CS, each continuous operation was carried out under the various conditions for more than 30 days. After about 20 days, the condition of the reactor was stable. The DO concentration and pH in the suspension were about 5 mg l\(^{-1}\) and 7.4, respectively (data not shown). Fig. 2(A) shows the average microbial concentration under steady state conditions.
The suspended microbial concentration and retained microbial concentration were approximately 200 mg (l medium\(^{-1}\)) and 20000 mg (l carrier\(^{-1}\)), respectively. These values were extremely low compared with those of conventional suspended microbial reactor system such as activated sludge or other similar systems [23,24]. Suspended and retained microbial concentrations slightly increased with increases in the influent TOC concentration, while they scarcely depended on the influent nitrogen concentration. The average microbial concentration based on the reactor working volume was the same order of a conventional activated sludge system.

Fig. 2(B) and (C) show effluent nitrogen concentration and TOC concentration in CS, respectively. The dominant form of nitrogen in the effluent was affected by the influent TOC and nitrogen concentrations. At high TOC loading concentration, the main nitrogen component in the effluent was ammonia, although small amount of nitrate was detected. This is due to the inhibition of nitrification by a competition for oxygen between heterotrophs and nitrifiers. Moreover, at the high nitrogenous concentrations (40 mg N l\(^{-1}\)), the remaining nitrogen concentration was high, as shown in Fig. 2(B). This result means the decline of nitrogenous removal ratio and seems to be caused by the lack of organic compounds for use as an electron donor in denitrification. On the other hand, at more than 300 mg C l\(^{-1}\) of TOC concentration, the effluent TOC concentration still remained high (Fig. 2(C)).

Fig. 3 shows the efficiency of nitrogen removal in CS in terms of the ratio between carbonaceous concentration and nitrogenous concentration (C/N ratio (mg l\(^{-1}\)), (mg l\(^{-1}\))\(^{-1}\)), which was calculated by using both the concentration of unremoved nitrogen is shown in Fig. 2(B) and that of assimilated nitrogen estimated from cell components [6]. Except for assimilation, nitrogen removal was presumed to be due to denitrification. At lower nitrogen loading (20 mg N l\(^{-1}\)), the nitrogen removal rate (the sum of denitrified and assimilated nitrogen rate) increased from 60 to 90% on increasing of TOC concentration from 100 to 200 mg C l\(^{-1}\). This mainly resulted from increased assimilation due to increased cell growth. On the other hand, at higher nitrogen loading (40 mg N l\(^{-1}\)), the nitrogen removal rates were approximately less than 50%. These phenomena were caused by accumulation of the remaining ammonia or inhibition of denitrification. Higher TOC loading (more than 300 mg C l\(^{-1}\)) suppressed the nitrification and/or denitrification rate as shown in Figs. 2(B) and 3, although they did not affect the nitrogen removal rate so much.

3.2. Hybrid system

By introducing membrane modules into the CS, the suspended microbial concentration increased 10 times or more relative to that observed in CS. The reason for the increase of suspended microbe concentration was the blocking of microbe washout by the membrane. None of the microbes permeated through the membrane because the mean pore size of membrane is 0.4 \(\mu\text{m}\). During the continuous operation, the flux of effluent decreased gradually because of membrane fouling, which can be caused by such things as extracellular polymer substrate (EPS). The membrane flux of the effluent changed from 0.4 to 0.2 m per day for a few days. These attached EPS were gelatinous materials, although the components of them have not been clarified. However, the fouling could be overcome by the chemical washing by 70% ethanol or 0.1% sodium hypochlorite and direct aeration from the bottom of membrane module. As a result, the hydraulic retention time could be kept at 8 h\(^{-1}\) during the operation.

Effluent TOC, nitrogen and microbial concentrations in the HS at steady state are shown in Table 3 together with the concentrations of both suspended and retained microbes. Based on the effect of inorganic carbon on stimulated denitrification by supplying electron donor as reported elsewhere for CS [10], the effect of alkalinity was also examined.

TOC removal ratio was more than 90% in all experiments in HS, while that was affected by influent conditions in CS. This is because the increase in microbial concentration was due to not only heterotrophs but also other bacteria such as nitrifying or denitrifying bacteria, in HS. Moreover, the total nitrogen (T-N) removal ratio was influenced by alkalinity and the concentration of influent carbon and nitrogen. This is because the T-N removal ratio related to both nitrification (ammonia-oxidation and nitrite oxidation) and denitrification, which implies that the alkalinity assisted first nitrification, not denitrification. Therefore, the alkalinity does not always enhance the T-N removal ratio.

The influence of influent alkalinity concentration on the oxidized ammonia concentration was presented in Fig. 4. At a given alkalinity, the oxidized ammonia concentration in HS was much higher than that in CS. The higher ammonia-oxidation capability in HS might be because of
Table 3
Water quality in HS of carrier and membrane system

<table>
<thead>
<tr>
<th>Run no.</th>
<th>Influent concentration (mg l(^{-1}))</th>
<th>Removal ratio (%)</th>
<th>Microbial concentration (mg l(^{-1})) retained (mg l(^{-1}) carrier)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 50 0</td>
<td>95 3</td>
<td>3190 12000</td>
</tr>
<tr>
<td>2</td>
<td>100 250 250</td>
<td>95 21</td>
<td>3680 17300</td>
</tr>
<tr>
<td>3</td>
<td>100 100 500</td>
<td>96 9</td>
<td>3410 15900</td>
</tr>
<tr>
<td>4</td>
<td>100 40 200</td>
<td>95 40</td>
<td>7130 15500</td>
</tr>
<tr>
<td>5</td>
<td>300 40 200</td>
<td>97 69</td>
<td>8120 22500</td>
</tr>
</tbody>
</table>

a Operation period.
b 40 days.
c 120 days.
d 150 days.
e 80 days.

retainment of nitrifiers, whose specific growth rate was relatively small, at higher concentration in the reactor.

Nitrogen removal ratio in CS and HS is shown in Fig. 5(A). When the influent carbon concentration was 300 mg C l\(^{-1}\), the nitrogen removal ratio in HS was about 30% higher than that in CS. Effluent ammonia concentration in CS increased with increase of carbon concentration even at a constant influent nitrogen concentration, which limited the T-N removal efficiency. While this inhibition was not observed in HS as shown in Fig. 5(B). Considering the results of Fig. 3, nitrogen removal through denitrification was reduced by suppressed nitrification in CS. Because the nitrogen removal by the cell growth was negligibly small in HS (below 0.5 mg N l\(^{-1}\)), the removal was mostly due to denitrification.

3.3. Denitrification activity of microbes in HS and CS

Denitrification activity of microbial community cultured in CS or HS was measured using the carriers and medium sampled from each reactor. The sample of carriers and medium was cultivated batch-wise in a 500 ml glass vessel. Nitrate was used as an initial substrate for the measurement of denitrification activity. During the culture, periodic sampling and measurement of the nitrogen compounds (NH\(_4^+\)-N, NO\(_2^-\)-N and NO\(_3^-\)-N) were carried out under
Fig. 6. Time courses of nitrogen concentration under different DO concentrations. (A) CS: (□) 8.0 mg l\(^{-1}\); (○) 0.0 mg l\(^{-1}\); (■) HS: (□) 8.0 mg l\(^{-1}\); (△) 1.5 mg l\(^{-1}\); (○) 0.5 mg l\(^{-1}\); (☐) 0.1 mg l\(^{-1}\); (□) 0.0 mg l\(^{-1}\). All experiments were carried out under 100 mg Cl\(^{-1}\) TOC concentration.

Fig. 7. Intracarrier distribution of glucose concentration, DO concentration and estimated glucose flux in CS and HS. (□) glucose concentration; (○) DO concentration. Solid lines show the estimated glucose diffusive flux.

the control of DO concentration. Fig. 6 shows time courses of nitrate concentration under the various DO concentrations in CS and HS. All measurements were conducted under the initial TOC concentration of 100 mg Cl\(^{-1}\) and the ammonium and nitrite were not detected during the measurement. The decrease of NO\(_3^-\)-N concentration in CS was small regardless of bulk DO concentration as shown in Fig. 6(A). Even though the DO concentration was 0 mg l\(^{-1}\), where the internal region of carrier would be completely anaerobic, the nitrogen removal rate was not more than 0.6 mg N l\(^{-1}\) h\(^{-1}\). On the contrary, the decrease of nitrogen concentration in HS was remarkably influenced by the DO concentrations less than 0.5 mg l\(^{-1}\) as shown in Fig. 6(B). Under the high DO concentration of 8 mg l\(^{-1}\), denitrification was observed and the denitrification rate was about 2.5 mg N l\(^{-1}\) h\(^{-1}\). On the other hand, under the reduced DO concentrations less than 1.5 mg l\(^{-1}\), remarkable denitrification was observed and the denitrification rate was about 2.5 mg N l\(^{-1}\) h\(^{-1}\) at 0 mg l\(^{-1}\), which is four times higher than that in CS. It seems that the intracarrier denitrification rate in the HS increased with decrease of DO concentration in the bulk. Actually, in HS, the DO concentration in the bulk in HS was relatively lower than that in CS (data not shown), because the suspended microbial concentration in HS was about 10 times higher than that in CS.

The distribution of the oxygen and glucose, and their apparent diffusion fluxes are shown in Fig. 7. The depth of DO penetration into the carrier in CS was smaller compared with that of HS and they were evaluated to be 0.6 and 1.0 mm, respectively. The difference in the DO penetration thickness is considered to be due to the difference in the surface microbial activities and substance transfer conditions. The decrease in glucose in the aerobic region was due to aerobic oxidation, which can be ascribed to glucose oxidation. The residual glucose in the anaerobic region decreased to zero, which can be ascribed to the denitrification. The thickness of anaerobic regions with glucose were about 0.4 mm (CS) and 0.8 mm (HS), respectively. The low denitrification rate in CS shown in Fig. 5 seems to have been due to a small glucose penetration rate into the anaerobic region, which might
have resulted from high concentration of retained microbes. On the other hand, the glucose penetration was remarkably deeper in HS compared with that in CS.

Apparent diffusion fluxes of DO and glucose were calculated with Eq. (A.5) in Appendix A. From the flux distribution, it is concluded that the rate limiting substrate in HS was not glucose but oxygen, which reflected the effect of the denitrification rate on the DO concentration is shown in Fig. 6. From these results, the overall denitrification rate was not glucose but oxygen, which reflected the effect of the denitrification rate on the DO concentration.

On the other hand, the glucose penetration was remarkably high concentration of retained microbes. Assuming that the overall denitrification rate is proportional to the depth of the region with oxygen depleted but glucose present, the intracarrier denitrification rate in HS was twice faster than that in CS.

The ratio between intracarrier denitrification rate and an apparent diffusion coefficient $D$ calculated by using Eq. (A.4) were calculated as 11.5 and 4.6 mg cm$^{-1}$ s$^{-1}$ cm$^2$ s$^{-1}$ for CS and HS, respectively. Combining these calculations, the apparent diffusion coefficient $D$ in HS was estimated to be about five times larger than that in CS. These results reflected the fact that CS had a smaller glucose diffusion coefficient than HS, which resulted in the limited denitrification rate in anaerobic region.

As shown in Fig. 6, the aerobic oxidation and decreased glucose concentration in CS lead to the depletion of electron donor, which might have resulted in low denitrification rate. Therefore, due to remaining electron donor, the denitrification rate in HS seems to be much higher than that in CS.

The feasibility of hybridizing a fluidized porous CS with a membrane system to obtain an advanced process for removal of carbonaceous and nitrogenous substances was studied. This carrier–membrane HS showed a high ability to simultaneously remove carbonaceous and nitrogenous substances under the higher influent carbon concentration. This is especially due to the denitrification enhancement under conditions of Eq. (A.1) and distance from the carrier surface (cm), respectively.

Microbial distribution in the anaerobic region can be assumed linearly perpendicular to the carrier surface since the observed glucose penetration thickness (Fig. 7) was very small compared with the size of the carrier. Under this assumption and the constant reaction rate assumption, the steady state distribution of glucose concentration $C(x)$ can be described as

$$D \frac{d^2C(x)}{dx^2} - R = 0$$

where $D$, $R$ and $x$ are the effective diffusion coefficient (cm$^2$ s$^{-1}$), the rate of substrate consumption rate by microbes (g l$^{-1}$ s$^{-1}$) and distance from the carrier surface (cm), respectively.

4. Conclusions

The idea intending to obtain an advanced process for removal of carbonaceous and nitrogenous substances by hybridizing a fluidized porous CS with a membrane system (HS) was studied. The nitrogen removal ratio in the HS achieved about 30% higher compared with that in the fluidized porous CS. The nitrogen removal was not solely due to microbial nitrification/denitrification but also assimilation derived from microbial growth. The apparent diffusion coefficient in the carrier of HS was about five times larger than that of CS and the intracarrier denitrification rate was twice as fast. The HS examined in the present study will be effective for the improved treatment of wastewater.

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Appendix A. Evaluation of apparent diffusion flux of glucose

Although the carrier size was finite, substrate penetration can be assumed linearly perpendicular to the carrier surface with oxygen depleted but glucose present. According to Eq. (A.4) the second order regression of observed glucose consumption per unit area, the ratio between intracarrier denitrification rate and the membrane system, respectively. According to Eq. (A.4) the second order regression of observed glucose concentration in the anaerobic region of carrier was used to determine the glucose diffusion coefficient $D$ and reaction rate $R$. By using this evaluated
diffusion coefficient, the effective diffusion flux $J(x)$ is calculated by Eq. (A.5)

$$J(x) = -D \frac{dC(x)}{dx} = -R \left( x - \frac{X_A + X_B}{2} \right) + D \frac{C_A - C_B}{X_B - X_A} \quad \text{(A.5)}$$

References


