Kinetic analysis of nitrifying biofilm growing on the rotating membrane disk

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Abstract: The authors have proposed a novel water treatment process in which nitrifying bacteria are fixed on the surface of rotating membrane disks. This biofilm-membrane process can perform strict solid-liquid separation and oxidation of ammonia nitrogen simultaneously. In this research, applicability of the conventional biofilm model (assuming the biofilm structure to be flat, homogeneous and continuous) to analysis of the biofilm developing in the proposed process was examined. A long-term operation for culturing the active nitrifying biofilm was carried out prior to kinetic investigation. By cryosectioning of the biofilm and image analysis, the thickness of the biofilm was determined to be 87 µm. From this biofilm thickness and the result of the batch ammonia consumption test, the intrinsic zero-order ammonia consumption rate of the biofilm was estimated precisely to be 930 g/m³/h. Using these parameters, the ammonia concentration profile in the biofilm was calculated by the conventional model, and the applicability of the model was examined by comparing the calculated profile with the ones measured with a microelectrode. The calculated profile was very close to the measured ones, which indicated feasibility of the conventional model to the analysis of the biofilm grown in the proposed process. The studied biofilm actually had a simple, i.e. flat, homogeneous and continuous, structure due to membrane filtration. This was the reason why the conventional model could still be employed. In the analysis of the data dealing with low concentrations of ammonia, however, first-order kinetics should be used. The first-order ammonia consumption rate constant of the studied biofilm was estimated to be 808 h⁻¹.

Keywords: Biofilm; biofilm structure; conventional biofilm model; drinking water treatment; membrane filtration; microelectrode

Introduction

Rapid sand filtration systems (coagulation, sedimentation, sand filtration and disinfection) have been used widely in Japan. However, they cannot remove ammonia nitrogen (NH₄⁺–N) which increases chlorine demand and brings about the production of undesirable disinfection by-products (DBPs) such as trihalomethane. In addition, chlorine reacts with NH₄⁺–N to produce chloramines which deteriorate the taste of tap water (Nabeta and Nishikawa, 1997). Since the breakpoint dose is accompanied by the increase of DBPs or the deterioration of taste, a biological process such as biological activated carbon (BAC) will be preferred in the future. However, detachment and leakage of bacteria from the activated carbon surface may cause other problems. On the other hand, membrane process has received a lot of attention recently because of its extremely high solid-liquid separation efficiency, which can remove chlorine resisting pathogenic microorganisms such as Cryptosporidium or Giardia (Jacangelo et al., 1995). However, the membranes cannot remove NH₄⁺–N efficiently.

In order to solve the problems described above, authors have proposed a novel water purification process in which nitrifying bacteria are fixed on the surface of rotating membrane disk (Watanabe et al., 1997). Figure 1 shows the concept of the proposed process. A type of biofilm is fixed on the membrane surface and raw water passes through the biofilm and the membrane as a result of the advection flow produced by the suction pressure. With the proposed process, oxidation of NH₄⁺–N and strict solid-liquid separation can be
performed simultaneously. This process is a type of biofilm process. Therefore, it is expected to express difficulty in oxidation of low concentrations of NH$_4^+$-N encountered in water purification because mass transfer limitation occurs. The authors carried out a kinetic analysis of the nitrifying biofilm formed in the proposed process using a “conventional” biofilm model, i.e. one dimension, zero-order kinetics, assuming flat, homogeneous and continuous biofilm, and concluded that nitrification efficiency of the proposed process was high even in treatment of low concentrations of NH$_4^+$-N. The reasons of the high efficiency were, firstly, enhancement of the NH$_4^+$-N transport toward the biofilm due to the advection flow and, secondly, collection of the treated water from the bottom of the biofilm where the concentration of NH$_4^+$-N was the lowest in the process (Watanabe et al., 1997).

In the last decade, some new techniques which provided a lot of interesting information on the biofilm structure were developed. Especially, application of confocal scanning laser microscopy (CSLM) has shown that structure of the actual biofilm was quite heterogeneous and not continuous (Massol-Deya et al., 1995, Okabe et al., 1998). In addition, the presence of the highly permeable water channels within the biofilm which is supposed to influence the mass transport was clearly demonstrated (de Beer et al., 1994). Therefore, the conventional biofilm model seems to be inadequate for describing the phenomenon occurring in biofilms. As described above, the previous kinetic analysis by the authors was carried out using the “conventional” biofilm model, and therefore, validity of the conclusions derived from the previous analysis could not be ensured immediately. In the proposed process, however, the advection flow caused by membrane filtration artificially holds the biofilm on the membrane surface, and its structure may be homogeneous and continuous, which differs from the one seen in naturally formed biofilms.

In this study, feasibility of applying the conventional biofilm model to the analysis of the proposed biofilm-membrane process was examined. Firstly, the structure of the biofilm developing in the proposed process was investigated by cryosectioning the biofilm. Secondly, the NH$_4^+$-N concentration profile within the biofilm was measured with a micro-electrode in order to compare it with the calculated profile. Finally, a comparison between the model calculations and the experimental results obtained in the long-term operation was carried out and some discussion about reaction kinetics was made.

**Experimental methods**

The rotating membrane disk module was used in this study. The water collection mechanism of the disk is shown in Figure 2 (Ohkuma et al., 1994). Two membranes...
covered the surface of a disk and the center of the disk was fastened to a rotating shaft. The material and molecular weight cut-off level of the employed membrane were polysulfone and 750,000 (daltons), respectively. The diameter of the membrane disk was 210 mm and the effective membrane surface area of a disk was 0.05 m$^2$. There were three disks in the experimental apparatus used in this study, and the total effective membrane surface area was 0.15 m$^2$. The disk rotational speed and the membrane permeate flux were fixed at 50 rpm and 0.8 m$^3$/m$^2$/d, respectively. The flow chart of the experiment is shown in Figure 3. Tap water was supplemented with ammonia (NH$_4$Cl), inorganic carbon (NaHCO$_3$) and basic minerals (KH$_2$PO$_4$, MgSO$_4$7H$_2$O and NaCl). Concentrations of NH$_4^+$–N in the feed water were maintained around 1.0 mg/l which is the typical concentration seen in the polluted drinking water source in Japan. The feed water flow rate ($Q_{in}$) was always greater than the membrane permeate flow rate ($Q_p$) and the excess volume of water was discarded from the membrane chamber as overflow. The water recovery rate defined as ($Q_p / Q_{in}$) was in the range of 0.9–0.95. Water temperature and pH were not controlled and they changed between 15.2–23.0ºC and 7.0–7.5 in the membrane chamber, respectively. No aeration was attempted in this experiment, however, dissolved oxygen (DO) concentrations in the membrane chamber were always above 7 mg/l.

Employing the experimental conditions described above, a long-term membrane operation (about 850 hours) was carried out in order to grow active nitrifying biofilm. Prior to the continuous operation, a small quantity of the activated sludge previously acclimated to the autotrophic environment was fixed on the membrane surface. Fixation of the microorganisms was implemented by the two hours dead-end filtration, i.e. no overflow from the membrane chamber, of the microorganisms suspension. The density of the fixed micro-organisms was 0.61 g/m$^2$.

After finishing the continuous operation, several biofilm samples were cut out with the membrane for cryosectioning and microelectrode measurement. The preparation of the specimen for cryosectioning was implemented following the procedure described by Okabe et al. (1998). The cut out biofilm samples (attached on the membrane) were embedded with Tissue-Tek® OCT-compound (Miles, Elkhart, IN) and rapidly frozen. The frozen samples were cut into 30 µm-thick vertical sections with a cryostat (Reichert-Jung Cryocut 1800, LEICA) at –20ºC. The sectioned specimen was fixed on a glass slide (Cell-line, USA), and allowed to air dry. The specimen was examined with a normal light microscopy. Determination of the biofilm thickness was carried out with an image analysis software provided by Zeiss. The microelectrode was used to determine the NH$_4^+$–N concentration profile in the biofilm. Measurement with the microelectrode was implemented just after finishing the continuous operation. The protocol of the preparation of the microelectrode for NH$_4^+$–N measurement was obtained from de Beer et al. (1997).
In the determination of \( \text{NH}_4^+ - \text{N} \) concentration, the indophenol method (Scheiner, 1976) was used for low concentration range and an ion chromatograph (Dionex DX-100, column; Dionex CS3) was used for high concentration range.

Results and discussion
The comparison between the model calculation and the microelectrode measurement
Changes in \( \text{NH}_4^+ - \text{N} \) concentration and suction pressure in the continuous operation are shown in Figure 4. Since the constant flow rate operation was employed, needed suction pressure increased slowly. Sufficient \( \text{NH}_4^+ - \text{N} \) oxidation was observed after 300 hours of the duration. At the end of the continuous operation, a cross-section view of the biofilm attached on the membrane (Figure 5) was obtained by the means described above. From Figure 5, it was shown that the biofilm formed in this study had a very dense and homogeneous structure, which was quite different from the one seen in the biofilm grown in wastewater (Okabe et al., 1998). This dense structure was probably caused by the fact that the studied biofilm had been under the influence of suction pressure. For the analysis of this type of biofilm, it seemed to be reasonable to use the “conventional” biofilm model assuming a flat, continuous and homogeneous structure.

In the analysis using biofilm models, obviously, the reaction rate within the biofilm has great influences on the result of the calculation. In the previous study (Watanabe et al., 1997), a batch \( \text{NH}_4^+ - \text{N} \) consumption test was carried out in order to determine the intrinsic reaction rate, and it was “graphically” determined from the figure obtained in the batch test (Watanabe and Nishidome, 1989). However, in this method to determine the reaction rate, some erroneousness is supposed to exist due to the researcher’s subject. An improvement in determining the reaction rate was needed for a more accurate biofilm analysis.

In order to determine the reaction rate more objectively, the universal relationship expressed as Eq. (1) was considered:

\[
F^0 = rL
\]

where \( F^0 \) is the zero-order \( \text{NH}_4^+ - \text{N} \) flux to the biofilm \( (\text{ML}^{-2}\text{T}^{-1}) \), \( r \) is the volumetric zero-order \( \text{NH}_4^+ - \text{N} \) consumption rate within the biofilm \( (\text{ML}^{-3}\text{T}^{-1}) \) and \( L \) is the biofilm thickness \( (\text{L}) \). The image analysis of Figure 5 determined the value of \( L \) to be 87 \( \mu \text{m} \). Figure 6 shows the results of the batch \( \text{NH}_4^+ - \text{N} \) consumption experiment carried out just before the end of the continuous operation. The suction pump was stopped and the disk rotational speed was decreased to 15 rpm in the batch experiment. Also, the aeration in the chamber with the air pump was implemented in order to ensure the sufficient DO level. From the linear slope drawn in Figure 6, the value of \( F^0 \) was determined to be 0.081 g/\( \text{NH}_4^+ - \text{N}/\text{m}^2/\text{h} \) and
consequently the value of $r$ was determined to be 930 g/NH$_4^+$N/m$^3$h. This value of $r$ was supposed to be very objective and accurate in analyzing the biofilm formed in this study.

Utilizing this value of $r$, the NH$_4^+$N concentration profile within the biofilm attached on the membrane can be derived from a simple mass balance equation described below (Watanabe and Nishidome, 1989, Watanabe et al., 1990):

$$D_f \frac{d^2C}{dz^2} = r$$

where $D_f$ is the NH$_4^+$N diffusivity in the biofilm (L$^2$T$^{-1}$), $C$ is the NH$_4^+$N concentration in biofilm (ML$^{-3}$) and $z$ is the the biofilm depth measured from the interface between the liquid and biofilm (L). The boundary conditions assumed for the biofilm are that $C = C_s$ (NH$_4^+$N concentration at the surface of the biofilm) at $z = 0$ and $dC/dz = 0$ at $z = L$. Solving Eq. (2) with the previously stated boundary conditions yields:

$$C = \frac{r}{2D_f}z^2 - \frac{rL}{D_f}z + C_s$$

NH$_4^+$N concentration profile in the biofilm can be calculated by using Eq. (3). In this study, the actual NH$_4^+$N concentration profiles in the biofilm were also obtained by the microelectrode measurement. Then, a comparison between the calculated profile and the measured ones was examined. This comparison would be expected to give information on the applicability of the conventional biofilm model. Figure 7 shows both the results of the calculation and the microelectrode measurements. The microelectrode measurements were implemented in the bulk concentration of 6.1 mg–NH$_4^+$N/l, which was similar to the one in the batch experiment (Figure 6). According to Williamson and McCarty (1976) and Horn and Hempel (1997), $D_f$ was considered as 0.8 $D_w$ (NH$_4^+$N diffusivity in water) in this calculation. 1.53 cm$^2$/d was used for $D_w$ (Lide, 1998) and the mean value of microelectrode measurements was used for $C_s$. The values of $r$ and $L$ were determined previously. The model calculation proved to be very close to the measured profile.

There are some researches that noticed the importance of the heterogeneity of the biofilm structure in the model calculation. For instance, Horn and Hempel (1997) reported that only taking into account the porosity inside the biofilm could explain the experimental results. However, considering the accuracy of the value of $r$ determined in this study, the conventional and simple biofilm model (assuming flat, homogeneous and continuous structure) was still useful and accurate in the analysis of the biofilm grown in the proposed process. This was because the studied biofilm actually had a simple structure.
The analysis of data obtained in the continuous operation and the NH$_4^+$–N flux through the diffusion layer at steady state and the NH$_4^+$–N concentration at the biofilm surface are given as follows (Watanabe and Nishidome, 1989; Watanabe et al., 1990):

\[ F_b = \frac{D_w}{L_d} (C_b - C_s) \]  
\[ C_s = C_b + \lambda - (\lambda^2 + 2\lambda C_b)^{1/2} \]  
\[ \lambda = 0.8rL_d^2 / D_w \]

where \( F_b \) is the NH$_4^+$–N flux through the diffusion layer at steady state (ML$^{-2}$T$^{-1}$), \( D_w \) is the NH$_4^+$–N diffusion coefficient in the water (L$^2$T$^{-1}$), \( C_b \) is the NH$_4^+$–N concentration in the bulk water (ML$^{-3}$) and \( L_d \) is the thickness of the external diffusion layer (L). The NH$_4^+$–N flux calculated with these equations was compared with the fluxes observed in the continuous operation and the latter was expected to be higher, because the advection flow toward the biofilm caused by the membrane filtration was supposed to increase the NH$_4^+$–N flux (Watanabe et al., 1997). The value of \( r \) was determined in the previous section (930 g/NH$_4^+$–N/m$^2$/h) and used in this calculation again. \( L_d \) was calculated by the equation that Levich (1962) showed for the submerged rotating disk. The calculation determined the \( L_d \) to be 67 µm when the disk rotates at 50 rpm.

Figure 8 shows the comparison of the observed NH$_4^+$–N fluxes in the continuous operation and the calculated NH$_4^+$–N flux. The data observed after 300 hours when the steady-state operation was reached, were compared with the calculated values. The observed data were plotted against the calculated values and the agreement was satisfactory.

Figure 9 shows the batch experiment with suspended biomass prepared by collapsing the biofilm. The reaction rate was determined by the zero-order reaction rate equation:

\[ \text{ammonia flux} = 1.62 \times 10^{-3} \text{gN/m}^2\text{h} \]

Figure 10 shows the comparison between the calculated ammonia fluxes and the observed ones for both zero- and first-order reactions.

\[ \text{ammonia flux} = \text{Equation 12} \]
\[ \text{ammonia flux} = \text{Equation 13} \]
\[ \text{ammonia flux} = \text{observed} \]
state was achieved are plotted in Figure 8. Differing from the expectation, the observed
fluxes were almost the same as the calculated one. However, this result does not mean that
the employed model is inadequate for the analysis or that the determination of the magni-
tude of \( r \) was a default. This discrepancy seemed to reflect that \( \text{NH}_4^+ - \text{N} \) oxidation does not
proceed in zero-order kinetics in the low concentration range such as below 1.0 mg/l.

A portion of the biofilm was removed and collected at the end of the continuous opera-
tion. The collected biomass was homogenized in the substrate solution and then a batch
\( \text{NH}_4^+ - \text{N} \) consumption experiment was carried out with the suspended biomass. Figure 9
shows the result of the batch experiment. Obviously, zero-order kinetics did not apply in the
concentration range which was examined in the continuous operation. In the low concentra-
tion range, \( \text{NH}_4^+ - \text{N} \) oxidation was found to proceed in first-order kinetics, and the first-order
reaction constant in the batch experiment was determined as 1.15 (l/h). This reaction
constant, however, cannot immediately be used for the analysis of the continuous operation.

Usually, reaction rate increases with increase of biomass. Therefore, the first-order reaction
constant in the continuous operation was estimated from the difference in biomass density
between the batch experiment and the continuous operation. In the batch experiment, the bio-
mass density was 133 g/SS/m³. At the end of the continuous operation, the biomass density in the
biofilm was 93.4 kg/SS/m³. Compared to the batch experiment condition, the biomass den-
sity was about 700 times more in the biofilm of the continuous operation. Consequently, the
first-order reaction constant in the continuous operation \( (k) \) was determined to be 808 h⁻¹. This
determination of \( k \) was reasonable because the zero-order reaction rate obtained from Figure 9
\((=1.62 \ g/\text{NH}_4^+ - \text{N}/\text{m}^3/\text{h})\) was fairly close to \( r/700 \ (=1.33 \ g/\text{NH}_4^+ - \text{N}/\text{m}^3/\text{h})\).

First-order reaction model is described by:

\[
D_f \frac{d^2 C}{dz^2} = kC \quad \text{(7)}
\]

The boundary conditions are that \( C=C_s \) at \( z=0 \), and \( dC/dz = 0 \) at \( z = L \). Solving Eq. (8) with
the boundary conditions yields (Melcer et al., 1995):

\[
C = C_s \frac{\cosh(\eta(L-z))}{\cosh(\eta L)} \quad \text{(8)}
\]

\[
\cosh(u) = 0.5(e^u + e^{-u}) \quad \text{(9)}
\]

\[
\eta = \sqrt{\frac{k}{D_f}} \quad \text{(10)}
\]

Similar to the zero-order analysis, the \( \text{NH}_4^+ - \text{N} \) flux through the diffusion layer at steady
state can be calculated as follows:

\[
F_b = \frac{D_w}{L_d} \frac{D_f \eta \tanh(\eta L)}{D_f \eta \tanh(\eta L) + (D_w / L_d) C_b} \quad \text{(11)}
\]

Figure 10 shows the comparison of the observed \( \text{NH}_4^+ - \text{N} \) fluxes in the continuous opera-
tion and the calculated \( \text{NH}_4^+ - \text{N} \) flux using Eq. (12). Differing from the zero-order analysis (Figure 8),
observed fluxes were higher than the calculated one, which shows the enhancement of the
mass transport toward the biofilm due to the advection flow. Considering the effect of the
advection flow, the \( \text{NH}_4^+ - \text{N} \) flux through the diffusion layer can be given as follows:

\[
F_b = \frac{D_w}{L_d} (C_b - C_s) + u C_b \quad \text{(12)}
\]

\[
C_s = \frac{D_w}{L_d + u} \frac{C_b}{\left( \frac{D_w}{L_d + u} - \frac{e^{-\lambda_1 L} - e^{-\lambda_2 L}}{\lambda_1 e^{-\lambda_2 L} - \lambda_2 e^{-\lambda_1 L}} \lambda_1 \lambda_2 D_f \right)} \quad \text{(13)}
\]
where \( u \) is the advection flow rate to the biofilm (LT\(^{-1}\)). \( u \) was equal to the membrane permeate flux (0.8 m\(^3\)/m\(^2\)/d). The calculated flux considering the advection flow is also drawn in Figure 10 and was found to correspond well with the measured data.

### Conclusion

The authors have proposed a novel water purification process in which nitrifying bacteria are fixed on the surface of the rotating membrane disks. This biofilm-membrane process can perform strict solid-liquid separation and oxidation of NH\(_4\)\(^+\)-N simultaneously. In this research, applicability of the conventional biofilm model to analysis of the performance of the biofilm developing in the proposed process was examined. Although the conventional model was formulated assuming the simple, i.e. flat, homogeneous and continuous, biofilm structure, it was found to be appropriate for the analysis of the biofilm developing in the proposed process. This was because the biofilm actually had a simple structure, which was confirmed by cryosectioning. When the model is applied to the case dealing with low concentrations of NH\(_4\)\(^+\)-N, first-order kinetics should be used in the analysis of the datum, and then the model can predict the performance of the proposed process well.

### References