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<td>Satoh, Hisashi; Ono, Hideki; Rulin, Bian; Kamo, Jyn; Okabe, Satoshi; Fukushi, Ken-Ichi</td>
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Macroscale and microscale analyses of nitrification and
denitrification in biofilms attached on membrane aerated biofilm
reactors

Short running title: Nitrification in MABRs

By

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Abstract

A membrane aerated biofilm reactor (MABR), in which O$_2$ was supplied from the bottom of the biofilm and NH$_4^+$ and organic carbon were supplied from the biofilm surface, was operated at different organic carbon loading rates and intra-membrane air pressures to investigate the occurrence of simultaneous COD removal, nitrification and denitrification. The spatial distribution of nitrification and denitrification zones in the biofilms was measured with microelectrodes for O$_2$, NH$_4^+$, NO$_2^-$, NO$_3^-$ and pH. When the MABR was operated at approximately 1.0 g-COD/m$^2$/day of COD loading rate, simultaneous COD removal, nitrification and denitrification could be achieved. The COD loading rates and the intra-membrane air pressures applied in this study had no effect on the start-up and the maximum rates of NH$_4^+$ oxidation in the MABRs. Microelectrode measurements showed that O$_2$ was supplied from the bottom of the MABR biofilm and penetrated the whole biofilm. Because the biofilm thickness increased during the operations, an anoxic layer developed in the upper parts of the mature biofilms while an oxic layer was restricted to the deeper parts of the biofilms. The development of the anoxic zones in the biofilms coincided with increase in the denitrification rates. Nitrification occurred in the zones from membrane surface to a point of ca. 60 µm. Denitrification mainly occurred just above the nitrification zones. The COD loading rates and the intra-membrane air pressures applied in this study had no effect on location of the nitrification and denitrification zones.

Key words

Membrane aerated biofilm reactors; COD removal; Nitrification; Organic carbon loading rate; Intra-membrane air pressure; Microelectrodes
Introduction

Microbial nitrification is a key process in the removal of ammonia nitrogen from wastewater. The process of nitrification is carried out by ammonia-oxidizing and nitrite-oxidizing bacteria. Due to slow growth rates and the relatively high $K_m$ values to $O_2$ of nitrifying bacteria, nitrifying bacteria are generally outcompeted by heterotrophic bacteria for $O_2$ (Satoh et al., 2000). As a result, nitrification only occurs in conventional biofilm reactors when the organic carbon concentration is low.

A membrane aerated biofilm reactor (MABR) has been proposed as a promising alternative to the conventional biofilm reactors (Timberlake et al., 1988; Hibiya et al., 2003; Yamagiwa et al., 1994; Pankhania et al., 1994). In the MABR system, the biofilm develops on the outside surface of a hollow fiber membrane. $O_2$ is supplied from the inner side of the membrane to the bottom of the biofilm, whereas organic carbon in wastewater is supplied from the biofilm surface. Consequently, nitrification can occur at the bottom of the biofilm, where $O_2$ concentration is high and the organic carbon concentration is low due to diffusion limitation, resulting in the stable nitrification capacity of the reactor. Timberlake et al. (1988) reported that with combined organic carbon removal, nitrification and denitrification occurred simultaneously in the single MABR. They suggested that the occurrence of a vertical stratification of nitrification, aerobic heterotrophic oxidation, denitrification and anaerobic fermentation zones from the bottom to the surface of the biofilm. In practice, Hibiya et al. (2003) verified that ammonia-oxidizing bacteria were mainly distributed inside the biofilm and denitrifying bacteria were mainly distributed outside the biofilm in a MABR using the fluorescence in situ hybridization method. Furthermore, de Beer et al. (1997) was first to directly measure the nitrification and denitrification zones in the biofilm of a pilot-scale membrane reactor using microelectrodes. Such microscale information could be highly valuable when designing and operating MABRs. However, they did not investigate the effect of operating conditions of the MABR on the stratification of the microbial activity in the biofilm. Nitrogen and organic carbon loading rates, an intra-membrane air pressure, biomass retention time, and so on, should have significant effects of development of the stratification of the microbial activities in the MABR biofilm.

In this study, five types of a bench-scale MABR were operated at different chemical
oxygen demand (COD) loading rates and intra-membrane air pressures, and the performances of the MABRs were compared. The reaction rates of the MABR (i.e. macroscale activity) were monitored. Successive developments of oxic zones and spatial distributions of nitrification and denitrification zones in the biofilms (i.e. microscale activity) were investigated using O$_2$, NH$_4^+$, NO$_2^-$, NO$_3^-$ and pH microelectrodes. The objectives of this study were to examine where nitrification occurred in the MABR biofilms achieving simultaneous COD removal, nitrification and denitrification, and the effects of operating conditions on the nitrification process in the biofilm on a macro- and microscale.

2. Materials and methods

2.1. Reactor operation

A MABR setup is presented schematically in Fig. 1. The reactor, which houses a membrane module, was constructed using an acrylic board. The cover of the reactor had openings for sample collection. The reactor volume including the recirculation line was 4.5 L. The membrane module consisted of gas permeable, polyurethane hollow fiber membranes. The inner and outer diameters of the hollow fiber are 237 µm and 275 µm, respectively. O$_2$ flux of the hollow fiber is 0.28 m$^3$/m$^2$/h/MPa. The membranes were arranged in parallel as a sheet (ca. 250 mm long and 300 mm wide) in the reactor with O$_2$ supplied via a manifold at the end of the membrane sheet. The other end of the membrane sheet was also connected to a manifold and the end of the manifold was open. The membrane surface area available for the biofilm attachment and oxygenation in the MABR was 0.25 m$^2$. The MABRs were each operated at 20 ± 1°C, a constant dilution rate of 0.45 day$^{-1}$ and a recirculation rate of 170 ml/s.

Five different types of biofilms were cultured in the bench scale MABRs. All biofilms were initially cultured in the batch-fed mode for three days with primary settling tank effluent (PSTE) provided by the Asahigaoka Domestic Wastewater Treatment Plant, Hachinohe, Japan. During the batch-fed cultivation period, the bulk liquid was completely withdrawn, and a fresh PSTE was filled daily. After initial cultivation with the PSTE, the MABR was operated in continuous flow mode with a synthetic medium. The medium
composition and operating conditions for each MABR are summarized in Table 1 and Table 2. The pH values in the influents were adjusted to 7.0 ± 0.1 for all the runs. O₂ concentrations in the medium were kept at 0 mg/L in the influent reservoir by purging with N₂ gas. Air was pumped under a pressure of 0.01 or 0.04 MPa. The reaction rate of MABR was calculated using the following equation, \( (C_{\text{in}} - C_{\text{out}}) \times Q / A \), where \( C_{\text{in}} \) is solute concentration in the influent, \( C_{\text{out}} \) is solute concentration in the effluent, \( Q \) is volumetric flow rate of the reactor and \( A \) is area of the membrane surface.

2.2. Microelectrode measurements and rate calculations

Clark-type microelectrodes for O₂ with tip diameters of approximately 15 µm were prepared and calibrated as described by Revsbech (1989). LIX-type microelectrodes for NH₄⁺, NO₂⁻, NO₃⁻ and pH with tip diameters of approximately 15 µm (de Beer et al., 1997) were constructed, calibrated, and used according to the reported protocol (Okabe et al., 1999). Microelectrode measurements of O₂ for in situ conditions were directly performed in the MABRs at regular time intervals under actual growth conditions during operations. The microelectrode was introduced into the MABR through the sampling opening. When the microprofiles of O₂, NH₄⁺, NO₂⁻, NO₃⁻ and pH in the biofilm were measured, the bulk liquid in the MABR was exchanged for a synthetic medium. The medium that was used to monitor the concentration profiles consisted of NH₄Cl (16 mg-N/L), NaNO₂ (7 mg-N/L), NaNO₃ (25 mg-N/L), Na₂HPO₄ (81 mg/L), MgCl₂·6H₂O (17 mg/L), CaCl₂ (22 mg/L), and EDTA (100 mg/L). The biofilm was acclimatized in the medium for microelectrode measurements with less than 1 mg/L of O₂ and at 20°C for at least two hours before measurement, to ensure that steady-state profiles were obtained. The concentration profiles in the biofilm were recorded using a motor-driven micromanipulator at intervals of 20 to 100 µm from the bulk liquid into the biofilm (Satoh et al., 2003). The membrane surface was considered as the point where solute concentrations were unchanged in the biofilm.

Net specific consumption rates of NH₄⁺, NO₂⁻ and NO₃⁻ were estimated from the mean concentration profiles by using Fick’s second law of diffusion, \( \partial C(z,t)/\partial t = D \times \partial^2 C(z,t)/\partial z^2 - R(z) \), where \( C(z,t) \) is the concentration at time \( t \) and depth \( z \), \( D \) is the molecular diffusion coefficient in the liquid phase, \( R \) is the net specific consumption rate. After integration of
this equation, we have $C_{n-1} = C_n + h \times [dC/dz_{n-1} + h \times A_{n-1}]$, where $C_n$ is the concentration measured with a microelectrode at n time, $h$ is the step size of microelectrode measurement and $A_n = R_n/D$. Using this equation, we can calculate concentration profiles by altering these net activities and minimizing the sum of squared deviations of the calculated profile from the measured profile. We chose to use Microsoft EXCEL Solver to achieve this goal. The details of this method have been described previously by Lorenzen et al. (1998).

Furthermore, the total nitrification rate [$J; \text{g-N/m}^2/\text{day}$] of the biofilm was calculated using Fick's first law of diffusion, $J = -D_s (dC_s/dz)$, where $dC_s/dz$ is the measured concentration gradient of each solute in the boundary layer at the biofilm-liquid interface. Molecular diffusion coefficients of $1.38 \times 10^{-5} \text{ cm}^2/\text{s}$ for $\text{NH}_4^+$, $1.23 \times 10^{-5} \text{ cm}^2/\text{s}$ for $\text{NO}_2^-$ and $1.23 \times 10^{-5} \text{ cm}^2/\text{s}$ for $\text{NO}_3^-$ at 20°C were used for the calculations, respectively (Andrusow, 1969).

2.3. Analytical methods

The $\text{NH}_4^+$ and $\text{NO}_2^-$ concentrations were determined colorimetrically. The $\text{NO}_3^-$ concentration was determined using an ion chromatograph (HIC-6A; Shimadzu) equipped with a Shim-pack IC-A1 column. The samples for $\text{NH}_4^+$, $\text{NO}_2^-$ and $\text{NO}_3^-$ were filtered with 0.45 µm membrane filters before analysis. COD was analyzed using $\text{KMnO}_4$ according to the standard methods (APHA, 1998). Total nitrogen (TN) was analyzed colorimetrically with potassium peroxodisulfate. The $\text{O}_2$ concentration and pH were determined using an $\text{O}_2$ electrode and a pH electrode, respectively.

3. Results and discussion

3.1. Reactor performance

When the MABR was operated at a high COD loading rate (run-1), a thick and brownish biofilm developed. Microelectrode measurements revealed that biofilm thickness was ca. 3 mm. $\text{NH}_4^+$ concentrations in the effluent were higher than in the influent during the more than 60 days of operation (data not shown). This was probably explained by the degradation of yeast extract to $\text{NH}_4^+$ and low $\text{NH}_4^+$ oxidation rate due to a low
intra-membrane air pressure. Fig. 2 shows the influent and effluent concentrations of NH$_4^+$, NO$_2^-$, NO$_3^-$ and COD, and O$_2$ concentrations in the MABRs in run-2 to run-5. Fig. 3 shows development of NH$_4^+$, NO$_2^-$, COD and total nitrogen removal rates of the MABRs in run-2 to run-5. When the MABRs were operated at lower COD loading rates (run-2 to run-5), the biofilms had a fluffy structure and were brownish in color. The thickness of the biofilms was less than 2.1 mm (Fig. 4). Visual observations indicated that the biofilm attachment areas accounted for about half of the total membrane surface area on day 40 during run-2 to run-5. Therefore, O$_2$ diffused directly into the bulk liquid without utilization by microorganisms in the biofilm. O$_2$ concentrations in the bulk liquid fluctuated between 0 and 2 mg/L (Fig. 2I). The partial attachment of the biofilm might be explained by low organic carbon loading rate and sloughing of the biofilm due to liquid flow in the reactor and air pressure from the bottom of the biofilm. NH$_4^+$ oxidation occurred immediately after switching to the continuous-feed operation on day 0 and reached maximum rates within 10 days during run-2 to run-5 (Fig. 3A). NO$_2^-$ oxidation was achieved within 30 days (Fig. 3B). Consequently, we can conclude that the intra-membrane air pressures applied in this study had no effect on the start-up and the maximum rates of nitrification in the MABRs at a low COD loading rate, whereas at a high COD loading rate the MABR had to be operated with a high intra-membrane air pressure for stable nitrification. A possible explanation for the complete nitrification at the low intra-membrane air pressure is that nitrification occurred in the deeper parts of the biofilms, as discussed below.

COD removal rates were less than 0.15 g-COD/m$^2$/day (corresponding to ca. 60% COD removal) in run-1 and run-2 (Fig. 3C). These low rates were due to fluctuations in the COD loading rate. In contrast, the COD removal rates increased during start-up periods and reached rates of ca. 1.0 g-COD/m$^2$/day (corresponding to ca. 90% COD removal) after 17 days in run-4 and run-5. Consequently, simultaneous organic carbon removal and nitrification were achieved in run-4 and run-5.

The rates of organic carbon removal and nitrification for the various types of MABRs are summarized in Table 3. Yamagiwa et al. (1994) reported the simultaneous occurrence of organic carbon removal and nitrification by the biofilm that was formed on an O$_2$ enrichment-type support. The higher rates of organic carbon removal and nitrification could be attributed to accumulation of microorganisms in a fibrous support woven around the fiber.
In contrast, nitrification rates were lower with the laboratory scale permeable-support biofilm reactor (Timberlake et al., 1988) and the laboratory scale hollow fiber membrane bioreactor (Pankhania et al., 1994), although organic carbon removal was achieved. This was attributed to either a low pH, a short retention time of biomass by backwashing, a mass transfer limitation due to a thicker biofilm, or the lack of fully developed nitrification zones. Therefore, simultaneous occurrence of organic carbon removal and nitrification in this study could be a result of lower organic carbon loading rates and operation without backwashing.

In addition to organic carbon removal and nitrification, total nitrogen (TN) removal occurred, although the rates were lower than the nitrification rates (Fig. 3D). TN removal rates on day 40 were ranged from 0.12 to 0.33 g-N/m²/day for run-2 through run-5. TN removal is usually attributed to denitrification and bacterial assimilation. It was possible that denitrification occurred in the floc present in the bulk liquid.

3.2. $O_2$ microprofiles in the biofilms under in situ conditions

$O_2$ microprofiles in the biofilms were directly measured under in situ conditions at different stages of biofilm development in run-4 and run-5. Fig. 4 shows the mean values of more than three $O_2$ profiles measured at different positions and average biofilm thickness, except that at day 7, at which a typical profile is displayed. The biofilm thickness increased from ca. 100 µm on day 7 to ca. 1,200 µm at day 51 in run-4. During the operation at a high intra-membrane air pressure (i.e. run-5) the biofilm thickness increased from ca. 200 µm on day 7 to ca. 2,100 µm on day 51.

$O_2$ concentration was highest at the membrane surface in all stages of biofilm development, indicating that $O_2$ was diffused through the membrane. $O_2$ concentrations decreased in the direction from the membrane to the bulk liquid. $O_2$ penetrated the whole biofilm and diffused out of the biofilms until day 23 in run-4. On day 29, the anoxic zones were first detected in the upper parts of the biofilm. In this case, a trace amount of $O_2$ present in the bulk liquid as well as the $O_2$ supplied from the membrane was utilized. In run-5, the anoxic zones were first detected on day 37. Since the biofilm thickness increased thereafter without obvious sloughing, the anoxic zones expanded. The development of anoxic zones in
The biofilms coincided with the increase in the TN removal rates (i.e. denitrification rates) after day 37 and day 41 in run-4 and run-5, respectively (Fig. 3D).

The O$_2$ concentrations at the membrane surface ranged between 1.7 - 4.7 and 1.3 - 4.8 mg/L in run-4 and run-5, respectively. The O$_2$ concentrations at 20°C and air pressure below 0.01 and 0.04 MPa are theoretically 0.9 and 3.5 mg/L, respectively. The O$_2$ concentrations at the membrane surface were not constant during operations of both runs, and the higher intra-membrane air pressure did not result in the higher O$_2$ concentration at the membrane surface. The fluctuation of intra-membrane air pressure was in the range of 0.005 MPa. The fluctuation of O$_2$ concentrations might result from clogging of the membrane during operation (Timberlake et al., 1988), spatial heterogeneity of pore size throughout the membrane (Kosutic et al., 2000), and the partial attachment of the biofilm onto the membrane surface.

3.3. Microprofiles of O$_2$, NH$_4^+$, NO$_2^-$, NO$_3^-$ and pH in the biofilms measured in the medium for microelectrode measurements

O$_2$, NH$_4^+$, NO$_2^-$, NO$_3^-$ and pH microprofiles in the biofilms in run-2, run-4 and run-5 were measured at 30, 32 and 38 days, respectively (Fig. 5), and the net specific consumption rates of NH$_4^+$, NO$_2^-$ and NO$_3^-$ were estimated on the basis of the measured profiles (Fig. 6). These results represent the potential nitrification capacity of the biofilms in the MABRs because no organic carbon was added to the medium for microelectrode measurements. In contrast to the measurements under *in situ* conditions (Fig 4), O$_2$ was not depleted in all biofilms (Fig. 5). This was probably due to the absence of organic carbon in the medium for microelectrode measurements. Nitrification was detected in the zones from membrane surface to a point of ca. 60 µm in run-2, run-4 and run-5. In contrast, since O$_2$, NH$_4^+$ and organic carbon are supplied from one side of the biofilm in the conventional biofilm reactors, the oxic zones are restricted to the upper parts of the biofilm (Okabe et al., 1999; Schramm et al., 1996). From these results, we can conclude that O$_2$ supplied from the bottom of the biofilm using a gas permeable membrane results in the development of a nitrifying layer in the deeper parts of the biofilm. This unique distribution of an active nitrifying layer in the MABR biofilm has several advantages over the conventional biofilm reactors (Timberlake
et al., 1988): (i) nitrifying bacteria in the MABR were present under the favorable condition in which O₂ concentrations were high while organic carbon concentration was low. In contrast, in the conventional biofilm reactor autotrophic nitrifying bacteria are excluded from the upper oxic layer of the biofilm due to the faster growth of heterotrophic bacteria, which often leads to deterioration of nitrification (Satoh et al., 2000); (ii) nitrifying bacteria, which usually showed slow growth rates and high sensitivity to several environmental factors, were immobilized in the deeper parts of the biofilm, and they were thus protected from toxic shocks and detachment losses by sloughing.

Denitrification mainly occurred just above the nitrification zones. The close vicinity of nitrification and denitrification zones enhanced denitrification, because NO₃⁻, produced in the deeper parts of the biofilm, diffused through the anoxic parts of the biofilm that had high organic carbon content. The COD loading rates and the intra-membrane air pressures under the conditions of run-2, run-4 and run-5 had no effect on the location of the nitrification and denitrification zones. This result might explain that the intra-membrane air pressures applied in this study had no effect on the NH₄⁺ oxidation rates in the MABRs at a low COD loading rate (Fig. 3).

Conclusions

(1) Simultaneous COD removal, nitrification and denitrification could be achieved in the MABRs. The efficiencies of nitrification and COD removal were 95% and 90%, respectively. The intra-membrane air pressures applied in this study had no effect on the start-up and the maximum rates of NH₄⁺ oxidation in the MABRs at a low COD loading rate, whereas at a high COD loading rate the MABR had to be operated with a high intra-membrane air pressure for stable nitrification.

(2) Microelectrode measurements directly revealed O₂ profiles in the MABR biofilms. O₂ was diffused through the membrane and utilized by microorganisms in the biofilm. The development of anoxic zones in the biofilms coincided with the increase in the denitrification rates of the MABRs.

(3) Microelectrode measurements of O₂, NH₄⁺, NO₂⁻, NO₃⁻ and pH in the biofilms demonstrated that nitrification occurred in the zones from membrane surface to a point of ca.
60 µm and denitrification mainly occurred just above the nitrification zones. The low COD loading rates (<1.1 g-COD/m²/day) and the intra-membrane air pressures (0.01 and 0.04 MPa) had no effect on the location of the nitrification and denitrification zones.

References


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Table 1. Summary of synthetic media composition for biofilm cultivation in run-1 to run-5.

Table 2. Summary of operating conditions for the MABRs in run-1 to run-5. Values indicate the mean rates ± standard deviations.

Table 3. Summary of the rates of organic carbon removal and nitrification in the MABRs under different operation conditions.

Fig. 1. Scheme of the MABR setup: 1, MABR; 2, membrane module; 3, openings for sample collection; 4, recirculation loop; 5, air pump; 6, pressure gauge; 7, valve; 8, synthetic medium; 9, pump; 10, effluent.

Fig. 2. Influent and effluent concentrations of NH$_4^+$ (●), NO$_2^-$ (△), NO$_3^-$ (■) and COD (×) in run-2 to run-5: A and B, run-1; C and D, run-2; E and F, run-3; G and H, run-4; A, C, E and G, influent; B, D, F and H, effluent; I, O$_2$ concentrations in the MABRs in run-2 (●), run-3 (○), run-4 (■), and run-5 (□), respectively.

Fig. 3. Development of NH$_4^+$ oxidation rates (A), NO$_2^-$ oxidation rates (B), COD removal rates (C), and total nitrogen removal rates (D) of the MABRs in run-2 (●), run-3 (○), run-4 (■), and run-5 (□), respectively.

Fig. 4. Mean concentration profiles of O$_2$ in the MABR biofilms in run-4 and run-5 at 7 days, 23 days, 29 days, 37 days, 43 days and 51 days, respectively. Error bars represent standard deviations of measurements. The membrane surface is at a depth of 0 µm. The biofilm is indicated by the gray area.

Fig. 5. Mean concentration profiles of O$_2$, NH$_4^+$, NO$_2^-$, NO$_3^-$ and pH in the MABR biofilms in run-2, run-4 and run-5 at 30 days, 32 days and 38 days, respectively. Error bars represent standard deviations of measurements. The membrane surface is at a depth of 0 µm. The
biofilm is indicated by the gray area.

Fig. 6. The spatial distribution of the net specific consumption rates of NH$_4^+$ ($\bullet$), NO$_2^-$ (+) and NO$_3^-$ (□) in the MABR biofilms in run-2, run-4 and run-5 at 30 days, 32 days and 38 days, respectively. The membrane surface is at a depth of 0 µm. The biofilm is indicated by the gray area.
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<th>KHCO₃ (g/L)</th>
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Table 1
Macro-scale and micro-scale analyses of nitrification and denitrification in biofilms attached on membrane aerated biofilm reactors.
Hisashi Satoh et al.
<table>
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<th>Run</th>
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<th>Total nitrogen loading rate (g-N/m²/day)</th>
<th>NH₄⁺ loading rate (g-N/m²/day)</th>
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⁴Not determined.

Table 2
Macroscale and microscale analyses of nitrification and denitrification in biofilms attached on membrane aerated biofilm reactors.
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<tbody>
<tr>
<td>1.2 - 1.5 (as COD)</td>
<td>1.0 - 1.2</td>
<td>1.1 - 1.4 (as COD)</td>
<td>0.5</td>
<td>This paper</td>
</tr>
<tr>
<td>6.6 (as TOC)</td>
<td>2.4</td>
<td>6.3 (as TOC)</td>
<td>1.7 - 2.2</td>
<td>[4]</td>
</tr>
<tr>
<td>3.4 - 10.8 (as TOC)</td>
<td>1.7 - 6.5</td>
<td>1.9 - 4.4 (as TOC)</td>
<td>-0.01 - 0.03</td>
<td>[2]</td>
</tr>
<tr>
<td>1.24 - 1.75 (as COD)</td>
<td>0.21 - 0.3</td>
<td>0.82 - 1.50 (as COD)</td>
<td>No nitrification</td>
<td>[5]</td>
</tr>
</tbody>
</table>

*NO₃⁻ production rate.*

Table 3
Macroscale and microscale analyses of nitrification and denitrification in biofilms attached on membrane aerated biofilm reactors.

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Fig. 2
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