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Effect of Oxygen Concentration on Nitrification and Denitrification in Single Activated Sludge Flocs

A short running title : Effect of O₂ on Nitrification and Denitrification in Flocs

By

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Abstract

Simultaneous nitrification and denitrification (SND) was observed in the single aeration tank of a municipal wastewater treatment plant. Microelectrode measurements and batch experiments were performed to investigate the occurrence of SND. The microelectrodes recorded the occurrence of O_2 concentration gradients in individual activated sludge flocs. When the O_2 concentration in the bulk liquid was less than $45\ \mu\text{M}$, anoxic zones were detected within flocs with larger diameter (approximately $3,000\ \mu\text{m}$). The O_2 penetration depth in the floc was found to be dependent on the O_2 concentration in the bulk liquid. Nitrification was restricted to the oxic zones, whereas denitrification occurred mainly in the anoxic zones. The nitrification rate of the activated sludge increased with increasing O_2 concentration in the bulk liquid, up to $40\ \mu\text{M}$ and remained constant thereafter. SND was observed in the aerated activated sludge when O_2 concentration was in the range of $10\ \mu\text{M}$ to $35\ \mu\text{M}$.

Key words

Single activated sludge flocs, O_2 concentration, Nitrification, Denitrification, Microelectrodes

1 INTRODUCTION

2
3 The activated sludge process is the commonly used system for nitrogen removal
4 from both domestic and industrial wastewaters. Nitrogen is generally removed in two
5 steps; first microbial nitrification then denitrification. Since nitrification occurs under
6 aerobic conditions and denitrification occurs in an anoxic environment, nitrogen
7 removal is achieved by a sequence of aerobic and anoxic processes. The high density
8 of microorganisms in immobilized biomass results in the development of
9 microenvironments in the floc, which differ from that prevailing in the bulk liquid. The
10 existence of anoxic zones inside activated sludge flocs has not been suggested but
11 demonstrated by Schramm et al. (1999). The presence of the anoxic zones in the
12 aerated flocs facilitates the simultaneous occurrence of nitrification and denitrification
13 (SND) in the single aeration basin (Hao et al., 1997). Microelectrode measurements
14 have demonstrated the concentration gradient of O_2 and the occurrence of anoxic
15 zones in the aerated activated sludge flocs in which denitrification occurred (Schramm
16 et al., 1999; Lens et al., 1995). However, the effect of O_2 concentration on the
17 nitrification and denitrification processes taking place in the floc has not been
18 investigated.

19 The objective of this study was to investigate the effect of O_2 concentration on
20 nitrification and denitrification in single activated sludge flocs. We used O_2 , NH_4^+ ,
21 NO_3^- , and pH microelectrodes to determine O_2 penetration depth, nitrification, and
22 denitrification in the flocs at various O_2 concentrations. In addition, batch experiments
23 were performed in order to determine both nitrification and denitrification rates.

MATERIALS AND METHODS

Samples

Activated sludge samples were obtained from the primary aeration basin of a municipal wastewater treatment plant in Hachinohe, Japan. The volume of the aeration basin was 322 m³. The hydraulic retention time was approximately 10 h. The mixed liquor suspended solid (MLSS) was about 2.8 g liter⁻¹.

Batch Experiments

The nitrification and denitrification rates, represented as the NH₄⁺ and inorganic nitrogen (the sum of the concentrations of NH₄⁺, NO₂⁻, and NO₃⁻) consumption rates respectively were determined using a 1.0 liter batch reactor. The initial MLSS was determined before running the experiment. Air was supplied to the activated sludge sample at various flow rates. The O₂ electrode was inserted in the reactor for a continuous monitoring of O₂ concentration. After the O₂ concentration was adjusted, 2,000 μM of NH₄⁺ was added, resulting in a final concentration of approximately 3,000 μM of NH₄⁺. The NH₄⁺, NO₂⁻, and NO₃⁻ concentrations were measured as a function of time. During the initial 24 h incubation, the nitrification and denitrification rates [μmol g-MLSS⁻¹ h⁻¹] were calculated as the decrease in NH₄⁺ and inorganic nitrogen concentrations, respectively.

Microelectrode Measurements

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⁺, NO₃⁻, and pH (DeBeer et al., 1997) were constructed, calibrated, and used according to the protocol reported elsewhere (Okabe et al., 1999). All measurements were performed using a flow cell (4.0 Liter) that was filled at an average liquid velocity of 0.5 cm s⁻¹ with an artificial medium at 20°C. The artificial medium used to monitor the concentration profiles consisted of 280 μM C₆H₁₂O₆, 300 μM NH₄Cl, 100 μM NaNO₂, 300 μM NaNO₃, 570 μM Na₂HPO₄, 84 μM MgCl₂ · 6H₂O, 200 μM CaCl₂, and 270 μM EDTA. The activated sludge was analyzed by taking a grab sample of the mixed liquor from the aeration basin, of which a small portion was transferred to the analytical apparatus by glass capillary. Sample flocs were positioned in the flow cell reactor using five insect needles (Lens et al., 1995). The activated sludge was then acclimated in the medium for at least two hours before measurement to ensure that steady-state profiles were obtained. The concentration profiles in the floc were recorded using motor-driven micromanipulators (model MM-60V-H1 and MM-60XY-H1; Chuo Precision Industrial Co., Ltd., Tokyo, Japan) at intervals of 100 μm to 200 μm from the bulk liquid into the floc. Microelectrode measurements were performed at O₂ concentrations fixed in the range of 15 μM to 195 μM. At least three concentration profiles were measured at different positions in the activated sludge for each species and set of conditions. The number of concentration profile measurements

in a single floc was limited to three for technical reasons. The surface of the floc was determined using a dissecting microscope (model Stemi 2000; Carl Zeiss).

Calculations

Assuming that the flocs were absolutely spherical, the total NH_4^+ production rate ($J(\text{NH}_4^+)$) and the total NO_3^- production rate ($J(\text{NO}_3^-)$) were calculated using Fick's first law of diffusion : $J = D_s (dS/dz)$, where D_s is the molecular diffusion coefficient in water of compound S and dS/dz is the concentration gradient at the boundary layer near the surface of the floc (DeBeer et al., 1993). The molecular diffusion coefficients at 20°C for NH_4^+ and NO_3^- were $1.38 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and $1.23 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, respectively (Andrussow, 1969).

Analytical Methods

The dissolved organic carbon (DOC) was analyzed using a Shimadzu TOC analyzer (TOC 5000). The NH_4^+ concentration was determined colorimetrically. The NO_2^- and NO_3^- concentrations were determined using an ion chromatography (HIC-6A; Shimadzu) equipped with Shim-pack IC-A1 column. The samples were filtrated with 0.45 μm membrane filters before analysis. The MLSS was determined according to standard methods (Clesceri et al., 1998). The O_2 concentration and pH were determined using an O_2 electrode and a pH electrode, respectively.

RESULTS AND DISCUSSION

Performance of the Activated Sludge Reactor

The O_2 concentration ($15 \pm 10 \mu M$) in the aeration basin was relatively low (average $\pm$ standard deviation for 47 different samples). The concentrations of NH_4^+ , NO_2^- , and NO_3^- in the aeration basin were 780 ± 370 , 20 ± 20 , and $440 \pm 300 \mu M$, respectively, whereas the concentrations of the same substances in the influent were $2,080 \pm 330$, 10 ± 10 , and $10 \pm 10 \mu M$, respectively. Therefore, we can conclude that 60% of NH_4^+ and 40% of inorganic nitrogen (the sum of NH_4^+ , NO_2^- , and NO_3^-) have been eliminated and that SND occurred in the aeration basin. The DOC concentrations in the influent and the aeration basin were $4,930 \pm 2,160$ and $1,250 \pm 430 \mu M$, respectively. The temperature and pH in the aeration basin were $14 \pm 1 ^\circ C$ and 6.6 ± 0.2 , respectively.

Batch Experiments

Batch experiments were performed in order to investigate the effect of O_2 concentration on the rates of nitrification and denitrification of the activated sludge. Production of inorganic nitrogen at O_2 concentrations near $0 \mu M$ could be explained by biomass degradation and liberation of NH_4^+ adsorbed on biomass (Fig. 1). Nitrification occurred at O_2 concentrations greater than $10 \mu M$. However, when the O_2 concentration was lower than $40 \mu M$, the nitrification rates were low probably due to

insufficient O_2 . The nitrification rate reached its highest value ($24 \mu\text{mol g-MLSS}^{-1} \text{ h}^{-1}$) when the O_2 concentration was $70 \mu\text{M}$ and remained constant thereafter. Denitrification, observed at O_2 concentrations less than $35 \mu\text{M}$ peaked with a maximum rate of $6 \mu\text{mol g-MLSS}^{-1} \text{ h}^{-1}$ when the O_2 concentration was $25 \mu\text{M}$. It was likely that denitrification occurred in anoxic zones of the flocs and in stagnant zones in the batch reactors. Consequently, SND occurred at O_2 concentrations ranging between $10 \mu\text{M}$ and $35 \mu\text{M}$ by simply lowering the O_2 concentration in the activated sludge reactor although nitrification was incomplete. The absence of denitrification at O_2 concentrations near $0 \mu\text{M}$ or greater than $35 \mu\text{M}$ might be explained by the absence of NO_3^- , which was produced by nitrification and the inhibition of denitrification by O_2 , respectively. Since the average O_2 concentration ($15 \mu\text{M}$) in the aeration basin from which the activated sludge samples were obtained was in this range, this could explain the occurrence of SND in that basin. Other researchers have reported a denitrification enhancement in the aerated activated sludge when the O_2 concentration in the bulk liquid was less than $60 \mu\text{M}$ (Hao et al., 1997; Wistrom et al., 1996).

Microelectrode Measurements and Rate Calculation

Microelectrode measurements were performed in order to investigate the effect of the O_2 concentration on nitrification and denitrification in single activated sludge flocs. The concentration profiles were different between samples (see Fig. 3 for heterogeneity of the activities of the flocs). Typical concentration profiles of O_2 , NH_4^+ , NO_3^- , and pH in flocs are displayed in Fig. 2. O_2 penetrated the entire floc with a

diameter of approximately 800 μm at an O_2 concentration of 270 μM (Fig. 2A). The NH_4^+ and NO_3^- concentration profiles showed that nitrification occurred throughout the floc, whereas denitrification did not occur due to the absence of anoxic zones. NH_4^+ consumption did not match NO_3^- production because NO_2^- concentration was not measured. At an O_2 concentration of 195 μM nitrification occurred although denitrification was not detected in flocs with diameters about 3,000 μm (Fig. 2B). This was because O_2 present in the floc was not completely depleted. In contrast, when the floc was incubated at an O_2 concentration of 45 μM , O_2 was depleted at a depth of 1,200 μm (Fig. 2C). Nitrification was restricted to the outer oxic zone of the floc. The steeper concentration gradient of NO_3^- in the inner zones as compared to the outer zones indicated that denitrification occurred mainly in the inner anoxic zones. At an O_2 concentration of 15 μM , O_2 was depleted at the depth of 200 μm (Fig. 2D). No nitrification occurred, but NH_4^+ production did take place. Denitrification was detected just below the surface of the floc. Consequently, microelectrode measurements indicated that nitrification was restricted to the outer oxic zone, whereas denitrification occurred mainly in the inner anoxic zones and SND occurred at an O_2 concentration of 45 μM .

$J(\text{NH}_4^+)$ and $J(\text{NO}_3^-)$ at various O_2 concentrations in the bulk liquid are shown in Fig. 3. NO_3^- was added to the bulk liquid. The diameter of each floc was approximately 3,000 μm . $J(\text{NH}_4^+)$ decreased from 0.006 $\mu\text{mol cm}^{-2} \text{h}^{-1}$ to -0.027 $\mu\text{mol cm}^{-2} \text{h}^{-1}$ when the O_2 concentration was raised from 15 μM to 90 μM . However, it remained unchanged at O_2 concentrations greater than 90 μM . $J(\text{NO}_3^-)$ gradually increased from -0.065 $\mu\text{mol cm}^{-2} \text{h}^{-1}$ to 0.011 $\mu\text{mol cm}^{-2} \text{h}^{-1}$ when the O_2 concentration was increased

1 from 15 μM to 195 μM . These results suggested the possibility of SND in a single
2 activated sludge floc at O_2 concentrations ranging between 45 μM and 100 μM .
3 However, the activities of flocs were different in floc size and between samples, which
4 were obtained from different reactors (Schramm et al., 1999). Therefore, further
5 investigations are needed to clarify the difference of SND between samples. Although
6 our results were limited, we are convinced that the data on microelectrode
7 measurements can explain the occurrence of SND in the aeration basin.

8 Microelectrode measurements could hardly detect the anoxic zones in the flocs
9 with diameters of less than 2,300 μm (Schramm et al., 1999; Lens et al., 1995). In
10 contrast, the anoxic zones developed and denitrification occurred in the flocs analyzed
11 in this study since relatively larger flocs were used to fasten the flocs in the
12 microelectrode setup. The difference in the O_2 concentrations (45 μM to 100 μM for
13 microelectrode measurements and 10 μM to 35 μM for the batch experiments) for
14 SND might be explained by the difference in flow regimes and floc sizes. The batch
15 experiments were done under vigorous stirring whereas the microelectrode
16 measurements were done under a very quiet flow regime. The flow regime is important
17 for the processes inside flocs. Vigorous stirring leads to continuous aggregation and
18 disaggregation of the flocs and facilitates advection in the flocs. These dynamics have
19 a strong impact on the floc diameter, mass transport resistance, microenvironments,
20 and local activities inside the flocs. For instance, the average floc diameter was $210 \pm$
21 $70 \mu\text{m}$ in the aeration basin and about 3000 μm during the microelectrode
22 measurements. Therefore, the microprofiles presented in this paper are not profiles of
23 the flocs in the aeration basin. These problems with the microelectrode measurements

could be partly solved by using another flow system (Schramm et al., 1999). Furthermore, other conditions (e.g. medium composition) of the microelectrode measurements were different from those used in the batch experiments. Consequently, our results present the mechanisms of SND in activated sludge flocs rather than in situ conditions of the aeration basin.

CONCLUSIONS

Microelectrode measurements recorded the occurrence of anoxic zones in the inner zones of the single activated sludge floc. Nitrification was restricted to the outer oxic zone, whereas denitrification occurred mainly in the inner anoxic zones. O₂ penetration depth and the zones of nitrification and denitrification in the floc were dependent on the O₂ concentration in the bulk liquid. Batch experiment results showed that SND could be achieved at O₂ concentrations between 10 µM and 35 µM although nitrification was incomplete. These results could explain the occurrence of SND in the aeration basin.

REFERENCES

- Andrussow L. 1969. Diffusion. In: Landolt-Bornstein Zahlenw, Funct. vol. 2/5a. Springer, Berlin, Germany.
- Clesceri LS, Greenberg AE, Eaton AD. 1998. Standard Methods for the Examination of Water and Wastewater, 20th edn. American Public Health Association,

1 Washington, DC.

2 DeBeer D, Schramm A, Santegoeds CM, Kuhl M. 1997. A nitrite microsensor for
3 profiling environmental biofilms. *Appl Environ Microbiol* 63 : 973-977.

4 DeBeer D, van den Heuvel JC, Ottengraf SPP. 1993. Microelectrode measurements of
5 the activity distribution in nitrifying bacterial aggregate. *Appl Environ Microbiol*
6 59 : 573-579.

7 Hao X, Doddema HJ, van Groenstijn JW. 1997. Conditions and mechanisms affecting
8 simultaneous nitrification and denitrification in a Pasveer oxidation ditch. *Bioresour*
9 *Technol* 59 : 207-215.

10 Lens PN, De Poorter MP, Cronenberg CC, Verstraete WH. 1995. Sulfate reducing and
11 methane producing bacteria in aerobic wastewater treatment systems. *Wat Res* 29 :
12 871-880.

13 Okabe S, Satoh H, Watanabe Y. 1999. In situ analysis of nitrifying biofilms as
14 determined by in situ hybridization and the use of microelectrodes. *Appl Environ*
15 *Microbiol* 65 : 3182-3191.

16 Revsbech NP. 1989. An oxygen microelectrode with a guard cathode. *Limnol*
17 *Oceanogr* 34 : 474-478.

18 Schramm A, Santegoeds CM, Nielsen HK, Ploug H, Wagner M, Pribyl M, Wanner J,
19 Amann R, DeBeer D. 1999. On the occurrence of anoxic microniches,
20 denitrification, and sulfate reduction in aerated activated sludge. *Appl Environ*
21 *Microbiol* 65 : 4189-4196.

22 Wistrom AO, Schroeder ED. 1996. Enhanced nutrient removal by limiting dissolved
23 oxygen concentration in a continuously fed, intermittently decanted, activated

1 sludge plant. Environ Technol 17 : 371-380.

2

3

1

Fig. 1

1

Fig.2

Fig.3

List of Figures

Fig. 1. The consumption rates of NH_4^+ (○) and inorganic nitrogen (the sum of NH_4^+ , NO_2^- , and NO_3^-) (▲) as determined by the batch experiments at various O_2 concentrations in the bulk liquid. Rates are the mean values and error bars show standard deviations.

Fig. 2. Typical concentration profiles of O_2 (●), NH_4^+ (△), NO_3^- (□), and pH (solid lines) in activated sludge flocs incubated at O_2 concentrations of 270 μM (A), 195 μM (B), 45 μM (C), and 15 μM (D). The diameters of the flocs were 800 μm (A), 2,800 μm (B), 3,200 μm (C), and 3,200 μm (D), respectively. The shaded area indicates the floc. The surface of the floc is at a depth of 0 μm. Note the expanded

scales in panel A.

Fig. 3. $J(\text{NH}_4^+)$ (○) and $J(\text{NO}_3^-)$ (▲) in the flocs as a function of O_2 concentration in the bulk liquid. Rates are the mean values of triplicate measurements and error bars show standard deviations. Positive values indicate the release of the solutes and negative values indicate the uptake.