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ENDOGENOUS DENITRIFICATION IN BIOFILM

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ABSTRACT

The model on endogenous denitrification in a biofilm, previously presented by the authors, was verified well with bench scale experiments. Production of the secondary substrate due to lysis of microorganisms, which is necessary for the progress of endogenous denitrification, was experimentally proved. In order to investigate metabolic characteristics of the substrate, molecular weight distributions of the substrate on COD_{cr} or carbohydrate bases were measured. The percentage of the secondary substrate with molecular weight less than 10,000, was more than 50% on both bases. The experimental results demonstrated that the production rate of the substrate can be expressed by the first order type expression with respect to the concentration of biomass. The specific constants for microorganisms lysis and maximum endogenous denitrification rate were determined (0.45 day^{-1} and0.141 day^{-1}, respectively).

KEYWORDS

Biofilm; endogenous denitrification; secondary substrate, kinetic model.

INTRODUCTION

Aerobic biofilm reactor has high potential in removal of organic substances and nitrogen compounds. This is because the various bacteria species contributing to oxidation, nitrification, and denitrification coexist throughout the biofilm (Masuda et al., 1987, Chen et al., 1988), and the aerobic zone and/or anaerobic zone are available in biofilm with suitable conditions. Additionally, denitrification was observed even when organic substances were insufficient in a submerged biofilm reactor (Omori, 1978). The authors (1987) also found that at very low DO level (below 1 mgO_2/l), oxidized nitrogen was removed in a submerged biofilm reactor in which the concentration of organic substances was extremely low. Basically, the amount of organic substances stored as a source of electron acceptor was too small to be supplied to the denitrification, since artifical sewage was not added to the reactor for three days before the observation. It could, therefore, be inferred that the organic substances available to this denitrification (i.e., endogenous denitrification) were produced from microorganisms in the biofilm.In this research, this type of organic substance is named as secondary substrate. As the biomass density in biofilm is much bigger than that of activated sludge, the amount of the substrate produced in the biofilm is much higher. Therefore, if the endogeneous denitrification is effectively used, the nitrogen removal in biofilm is expected in wastewater of organic substances deficiency, such as effluent of advanced processes, in which the ratio of C/N is low. It is considered that the

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utilization of the endogenous denitrification will improve not only efficiency but also cost performance of nitrogen removal in biofilm reactor in treating general wastewater. Therefore, it is necessary to investigate the mechanism of the endogenous denitrification in biofilm. The specific object of this research is to investigate the source of the secondary substrate in the biofilm. The authors inferred that the substrate was produced from lysis of microorganisms (Terashima et al., 1987). Also the authors considered auto-decomposition of biomass resulted from combination of the lysis of microorganisms and the regeneration of microorganisms due to the catabolic reaction of the secondary substrate. In this research, first the metabolic characteristics of the substrate was investigated experimentally, and then mathematical model which describes the production of the substrate and the endogenous denitrification is proposed.

PRODUCTION OF THE SECONDARY SUBSTRATE AND MOLECULAR WEIGHT DISTRIBUTION OF THE SUBSTRATE

EXPERIMENTAL METHOD

Mechanism of the secondary substrate production in biofilm is considered to be the same as that in the activated sludge process. Since the activated sludge process is easy to operate, firstly an activated sludge cultivated with the artifical sewage (mainly glucose and acetate) was used for experiments to study the production of the secondary substrate and measure the molecular weight distributions of the substrate on COD_{cr} or carbohydrate bases.

If the secondary substrate is really produced from microorganisms, it should be observed easily in the biological reactor. However, in fact it is difficult to verify the existence of the substrate. This could be assumed to be continuous substrate consumption by catabolic reaction of microorganisms. Accordingly, if the activity of microorganisms is kept low. the substrate should be accumulated in the reactor. Therefore, first the validity of the assumption is evaluated.

According to Nakanishi (1966), when pH was controlled at 4 or 9-10, the oxygen consumption of activated sludge decreased 10% of that at pH7. It was proved that low pH and high pH conditions will inhibit the microorganism activity. The high pH, however, was reported to cause decomposition of microorganims (Yanagida, 1981). In contrast, it was found in the previous experiment with activated sludge that the activity is easily recovered when pH is readjusted to neutral from pH4. This inferred that damage of the microorganisms was negligible, although they were exposed to pH4. Based upon this consideration, the following experiments were carried out.

The activated sludge was sampled and was aerated for about one day without addition of the artificial sewage, and then was washed three times with tap water. The sample was equally separated and added into two 1L reactors with pH and DO controllers. Then, these two reactors were aerated without addition of the artificial sewage at the identical DO level controlled to 5 (mgO_2/l). The pH was controlled either to 4 or 7. Samples were withdrawn from each reactor at certain intervals during the aeration. MLSS and soluble COD_{cr} of the same samples were measured. COD_{cr} was measured with an autoanalyzer (Technicon Ltd.). At the end of the experiment, the liquor was separated from the sludge and was filtered with 0.45μm membrane filter. Ultra filtration was used to measure the molecular weight distributions of the substrate on CDO_{cr} and carbohydrate bases. The filtration was performed by ultra filtration equipment (TOYO, UHP-43, ultra filter UH, UK) with the filters covering from 1,000 to over 200,000 molecular weight. The COD_{cr} and carbohydrate in each range were measured. Carbohydrate was analyzed by "anthron method" (Tai, 1964).

RESULTS AND DISCUSSION

Figure 1 shows the change of COD_{cr} with time in these two reactors. In this figure, the concentration increased nearly linearly in the reactor of pH4 at which metabolic reaction was kept low, and the increase was very small in the reactor of pH7. The measured molecular weight distributions of the substrate on COD_{cr} and carbohydrate bases are indicated in Figures 2 and 3, respectively. Figure 2 illustrates that the percentage of the substrate on COD_{cr} basis with molecular weight less than 10,000 was about 60 at pH4, and that at pH7 was

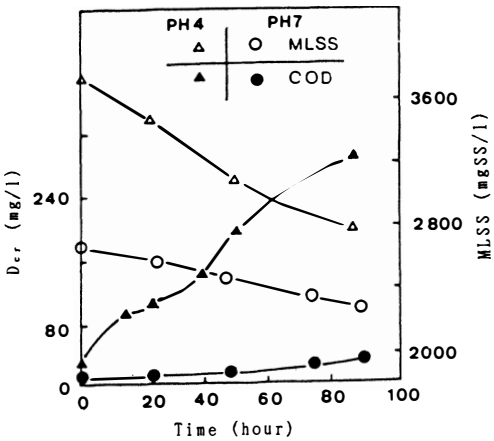

Figure 1 Changes of COD_{cr}, MLSS in an aeration tank during auto-decomposition of activated sludge

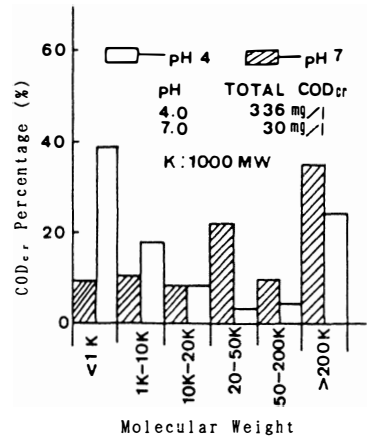

Figure 2 Molecular weight distribution of organic substances

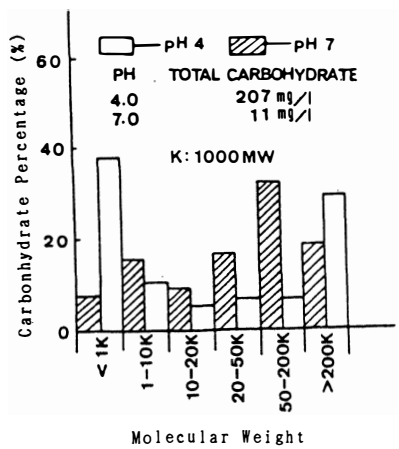

Figure 3 Molecular weight distribution of carbohydrate

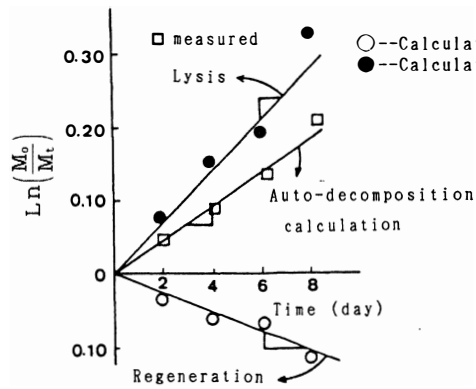

Figure 4 Analysis of auto-decomposition of activated sludge

not greater than 19%. Figure 3 shows that the percentage of the substrate on carbohydrate basis with molecular weight less than 100,000 was near 50 at pH4, and that at pH7 was below 25%. These experimental results proved the assumption on the production of the substrate is correct. Therefore, it could be concluded that organic substances (i. e., the secondary substrate) available to microorganisms, were always produced by lysis of microorganisms themselves, and more than half of the substrate at pH4 has lower molecular weight than that of pH7. Obviously, the secondary substrate produced from microorganisms can be consumed by catabolic reaction of microorganisms.

MODELLING OF THE PRODUCTION OF THE SUBSTRATE

MATHEMATICAL MODEL

Auto-decomposition of microorganisms could be expressed as (Park et al., , 1987):

$$\Delta M_{ob} = \Delta M_{ly} + \Delta M_{rg} \tag{1}$$

In Equation (1), ΔM_{ob} = observed biomass decrease by auto-decomposition (mgSS).
ΔM_{ly} = biomass decrease by lysis (mgSS).
ΔM_{rg} = biomass regeneration by catabolism of the secondary substrate (mgSS).

The regeneration of biomass could be described as:

$$\Delta M_{rg} = (-\Delta M_{ly} \cdot P - \Delta COD_{cr}) \cdot Y_s \tag{2}$$

In Equation (2), P = conversion coefficient from dried weight of biomass to COD_{cr} ($mgCOD_{cr}/mgSS$).
Y_s = yield coefficient of the secondary substrate ($mgSS/mgCOD_{cr}$).
ΔCOD_{cr} = change of COD_{cr} in reactor ($mgCOD_{cr}$).

The expressions of the ΔM_{ly} and ΔM_{rg} were obtained with combination of these two equations [(1) and (2)].

$$\Delta M_{ly} = \frac{\Delta M_{ob} + \Delta COD_{cr} \cdot Y_s}{1 - P \cdot Y_s} \tag{3}$$

$$\Delta M_{rg} = -\frac{(\Delta M_{ob} \cdot P + \Delta COD_{cr}) \cdot Y_s}{1 - P \cdot Y_s} \tag{4}$$

ΔM_{ob}, ΔCOD_{cr}, and P can be directly measured but Y_s can not. In order to determine Y_s, the authors assumed that the secondary substrate could be obtained by dispersing a sample of the activated sludge using an ultra panasonic disperser. The sample was sterilized with high pressure steam before the dispersal, and then filtered with 0.45μm membrane filter. Y_s was measured in an experiment with the sample. Therefore, ΔM_{ly} and ΔM_{rg} were determined from the equations (3) and (4) , respectively.

Figure 4 shows the change of MLSS during the aeration without addition of any artificial sewage, and the calculated values of ΔM_{ly} (filled circle) and ΔM_{rg} (circle) from the change by equations (3) and (4). In this figure, M_o was the initial MLSS and M_t was the MLSS at time t. This figure indicated that ΔM_{ly} could be expressed by first order equation with respect to the MLSS, and production rate of the secondary substrate could be described as:

$$\left(\frac{ds}{dt}\right)_{ly} = P \cdot (1-f) \cdot \left(-\frac{dM}{dt}\right)_{ly}$$

$$= P \cdot (1-f) \cdot b \cdot M_t \quad (5)$$

In Equation (5), $\left(\frac{ds}{dt}\right)_{ly}$ = production rate of the secondary substrate ($mgCOD_{cr}/L \cdot day$).

M_t could be considered to be a constant (M_c) at steady state, and Equation (5) was rewritten in this case.

$$\left(\frac{ds}{dt}\right)_{ly} = P \cdot (1-f) \cdot b \cdot M_c \quad (6)$$

From this Equation (6), the lysis coefficient of the activated sludge was measured, and it was 0.035 day^{-1} at steady state(Park et al., 1987).

DETERMINATION OF THE LYSIS COEFFICIENT IN A BIOFILM

METHOD

In general, microorganisms in biofilm are different from those in activated sludge, and lysis coefficient of the microorganisms in biofilm is also not identical to that in activated sludge. Since it is impossible to determine the coefficient in biofilm by using the method described for the activated sludge, an expression for biofilm was proposed in this research.

Mass balances on organic substrate and oxygen within biofilm at steady state could be respectively expressed with equations (7) and (8), provided that nitrification is negligible and secondary substrate was assumed to be similar to the organic substrate in the influent.

$$D_{fs} \cdot \frac{d^2S_f}{dZ^2} = \frac{K_{ms} \cdot S_f}{K_s+S_f} \cdot \frac{C_f}{K_o+C_f} \cdot X_f - b \cdot P \cdot (1-f) \cdot X_f \quad (7)$$

$$D_{fc} \cdot \frac{d^2C_f}{dZ^2} = r_2 \cdot \frac{K_{ms} \cdot S_f}{K_s+S_f} \cdot \frac{C_f}{K_o+C_f} \cdot X_f \quad (8)$$

In Equations [(7) and (8)],

D_{fs}, D_{fc} = diffusion coefficients of substrate and oxygen within biofilm (cm^2/day).
S_f, C_f = concentrations of substrate and oxygen within biofilm (mg/cm^3).
Kms = maximum specific rate of substrate removal (day^{-1}).
K_s, K_o = saturated constants of substrate and oxygen (mg/cm^3).
r_2 = oxidation ratio of substrate (-).
X_f = biofilm density ($mgSS/cm^3$).
Z = biofilm depth perpendicular to supported media(cm).

Both sides of Equation (7) were multiplied by r_2, and Equation (7) was linked with Equation (8). Then,

$$D_{fc} \cdot \frac{d^2C_f}{dZ^2} = r_2 \cdot D_{fs} \cdot \frac{d^2S_f}{dZ^2} + r2 \cdot b \cdot P \cdot (1-f) \cdot X_f \quad (9)$$

By integrating Equation (9) from surface of biofilm (Z=0) to supported media ($Z=L_f$).

$$\int_{0}^{\left(\frac{dC_f}{dZ}\right)_{Z=0}} D_{fc} \cdot \frac{d^2C_f}{dZ^2} \cdot dZ = \int_{0}^{\left(\frac{dS_f}{dZ}\right)_{Z=0}} r_2 \cdot D_{fs} \cdot \frac{d^2S_f}{dZ^2} \cdot dZ - \int_{L_f}^{0} r_2 \cdot b \cdot p \cdot (1-f) \cdot X_f \cdot dZ \qquad (10)$$

In Equation (10), L_f = biofilm thickness (cm).

It could be assumed to be $\frac{dC_f}{dZ}=0$ and $\frac{dS_f}{dZ}=0$ at the supported media. As the result of the integration of Equation (10), Equation (11) was obtained.

$$D_{fc} \cdot \left(-\frac{dC_f}{dZ}\right)_{Z=0} = r_2 \cdot D_{fs} \cdot \left(-\frac{dS_f}{dZ}\right)_{Z=0} + r_2 \cdot b \cdot P \cdot (1-f) \cdot X_f \cdot L_f \qquad (11)$$

$D_{fc} \cdot \left(-\frac{dC_f}{dZ}\right)_{Z=0}$ and $D_{fs} \cdot \left(-\frac{dS_f}{dZ}\right)_{Z=0}$ are the transport fluxes of oxygen (J_c) and organic substrate (J_s) on biofilm surface, respectively. Therefore, Equation (11) was arranged as the following Equation (12).

$$J_c = r_2 \cdot J_s + r_2 \cdot b \cdot P \cdot (1-f) \cdot X_f \cdot L_f \qquad (12)$$

In Equation (12), J_s includes two transport mechanisms: (1) mass transport of external substrate into biofilm; (2) liberation of the secondary substrate to bulk liquid phase. Under aeration without addition of any substrate, most of the secondary substrate could be completely oxidized within biofilm when DO level was high in bulk liquid phase. In this case, the liberation flux of the secondary substrate was so small that it could be negligible (Terashima et al., 1987), and J_c became the maximum (J_{mac}). Thus, the lysis coefficient (b) could be determined by Equation (13).

$$b = \frac{J_{mac}}{r_2 \cdot P \cdot (1-f) \cdot X_f \cdot L_f} \qquad (13)$$

J_{mac} MEASUREMENT

EXPERIMENTAL METHOD Biofilm was cultivated with the artificial sewage described above for two months before transferring into experimental reactor (250 ml) with pH and DO controllers as shown in Fig. 5. The reactor was filled with distilled water with nutrients, and was sealed at the beginning of the experiment. The DO level was measured for 24 hours in the reactor at pH7. After the measurement, biofilm density and thickness were measured following the methods described in (Terashima et al., 1987). The experiment was repeated under the same conditions except for the initial DO level in reactor.

EXPERIMENTAL RESULTS Figure 6 shows the relationship betwen initial DO level and rate of DO consumption, which became maximum, 4.8 $mgO_2/L \cdot day$ with the initial DO level of 8 mgO_2/l. The maximum flux of DO consumption (J_{mac}), 0.22 $mgO_2/cm^2 \cdot day$ was obtained by multiplying the maximum rate with volume of the reactor (250 ml) and dividing the rate with the area of biofilm surface (5.4 cm^2). The averages of the biofilm density and thickness were 26 $mgSS/cm^3$ and 1.25 mm, respectively. After those measurements, the sample of the biofilm was dispersed, and used to measure P and r_2. The measurements of these two parameters were 1.26 $mgCOD_{cr}/mgSS$ and 0.13 (-), respectively. All procedures were carried out at constant temperature (20 °C). The lysis coefficient (b) in biofilm was determined (0.45day^{-1}) from

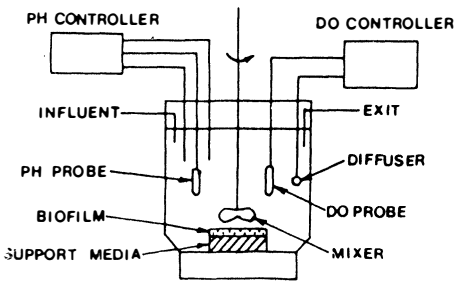

Figure 5 Experimental apparatus

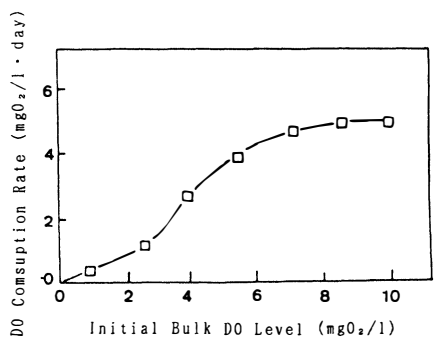

Figure 6 Relationship between initial DO Level in bulk liquid phase and DO consumption rate in a biofilm during auto-oxidation

Table 1 Mathematical Model of Nitrification in a Biofilm

① Bulk liquid phase

$$V\cdot\frac{dS_b}{dt}=\int_0^{L_o} A\cdot D_{ws}\frac{\partial^2 S}{\partial Z^2}\cdot dS \qquad (16)$$

$$V\cdot\frac{dC_b}{dt}=\int_0^{L_o} A\cdot D_{wc}\cdot\frac{\partial^2 C}{\partial Z^2}\cdot dC \qquad (17)$$

$$V\cdot\frac{dN_b}{dt}=\int_0^{L_o} A\cdot D_{wn}\cdot\frac{\partial^2 N}{\partial Z^2}\cdot dN \qquad (18)$$

② Diffusion layer

$$\frac{\partial S}{\partial t}=D_{ws}\cdot\frac{\partial^2 S}{\partial Z^2} \qquad (19)$$

$$\frac{\partial C}{\partial t}=D_{wc}\cdot\frac{\partial^2 C}{\partial Z^2} \qquad (20)$$

$$\frac{\partial N}{\partial t}=D_{wn}\cdot\frac{\partial^2 N}{\partial Z^2} \qquad (21)$$

③ Within biofilm

$$\frac{\partial S_f}{\partial t}=D_{fs}\cdot\frac{\partial^2 S}{\partial Z^2}-\frac{K_{ms}\cdot S}{K_s+S_f}\cdot\frac{C_f}{K_o+C_f}\cdot X_f-\beta\cdot\frac{K_{mn}\cdot N_f}{K_n+N_f}\cdot\frac{S_f}{K_s+S_f}\cdot\frac{K_{or}}{K_{or}+C_f}\cdot X_f+b\cdot P\cdot(1-f)\cdot X_f \qquad (22)$$

$$\frac{\partial C_f}{\partial t}=D_{fc}\cdot\frac{\partial^2 C}{\partial Z^2}-r_2\cdot\frac{K_{ms}\cdot S_f}{K_s+S_f}\cdot\frac{C_f}{K_o+C_f}\cdot X_f \qquad (23)$$

$$\frac{\partial N_f}{\partial t}=D_{fn}\cdot\frac{\partial^2 N}{\partial Z^2}-\frac{K_{mn}\cdot N_f}{K_n+N_f}\cdot\frac{S_f}{K_s+S_f}\cdot\frac{K_{or}}{K_{or}+C_f}\cdot X_f \qquad (24)$$

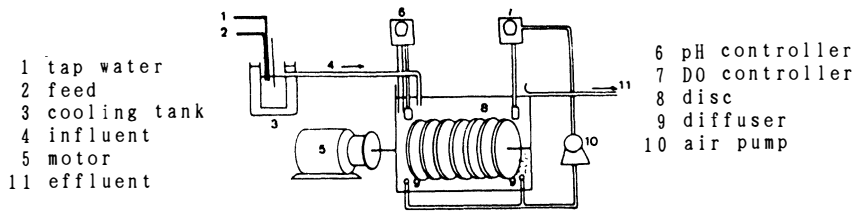

Figure 7 Schematic of a submerged biofilm reactor

Equation (13). The f value, biodegradable portion of cell body on CODcr basis, was 0.08 (Dold et al., 1980).

On the other hand, the previous expression, describing flux of oxygen consumption by auto-decomposition within biofilm (J_c'), was as follows:

$$J_c' = b' \cdot X_f \cdot L_f \tag{14}$$

In Equation (14), b' = auto-decomposition coefficient for oxygen consumption ($mgO_2/mgSS \cdot day$).

Relation between b and b' could be expressed with Equation (15) by linking Equation (13) and Equation (14).

$$b' = r_2 \cdot P \cdot (1-f) \cdot X_f \cdot L_f \tag{15}$$

From this Equation (15), b' was calculated to be 0.068 day^{-1} in this research, which is similar to that reported by Kim et al. (1985). Therefore, it could be considered that the value of b, 0.45 day^{-1}, obtained here was appropriate.

MODEL OF ENDOGENOUS DENITRIFICATION IN BIOFILM

EQUATIONS

Substrate and oxygen are transported through diffusion layer on biofilm surface into biofilm. Under the condition that nitrification is ignored, the equations expressing the transport of organic substrate, oxygen, and NO_x-N(NO_2-N+NO_3-N) in the diffusion layer were respectively shown as Equation (19) through Equation (21) in Table 1. The mass balance equations on these substrates within biofilm were respectively shown as Equation (22) through Equation (24) in the table, it was proposed that the kinetics of oxidation and denitrification were described by complex Monod type expression in which organic substrate, DO and NO_x-N are taken into account as influencing factors. Equation (16) through Equation (18) describes concentration changes of the substrates. In these equations, the organic substrate is the sum of the secondary substrate and external substrate from influent, and endogenous denitrification resulting from the production of the secondary substrate is considered in these equations.

The symbols, S_f, C_f, D_{fc}, D_{fs}, K_{ms}, K_o, X_f, t, Z, and r_2, were the same as those mentioned above and others are explained as follows.

- A = biofilm surface area (cm^2).
- S_b, C_b, N_b = concentrations of organic substrate, oxygen, and NO_x-N in bulk liquid phase ($mgCOD_{cr}/cm^3$, mgO_2/cm^3, mgN/cm^3).
- S, C, N = concentrations of organic substrate, oxygen, and NO_x-N in diffusion layer ($mgCOD_{cr}/cm^3$, mgO_2/cm^3, mgN/cm^3).
- D_{ws}, D_{wc}, D_{wn} = diffusion coefficients of organic substrate, oxygen, and NO_x-N in bulk liquid phase (cm^2/day).
- D_{fn} = diffusion coefficient of NO_x-N within biofilm (cm^2/day).
- L_o = diffusion layer thickness (cm).
- K_{mn} = maximum specific rate (day^{-1}).
- K_n = saturated concentration of NO_x-N (mgN/cm^3).
- K_{or} = DO inhibition coefficient of denitrification (mgO_2/cm^3).
- V = volume of reactor (cm^3).
- β = COD_{cr}-use coefficient for denitrification ($mgCOD_{cr}/mgN$).

It was assumed that the diffusion coefficient of NO_x-N was the average of those of NO_2-N and NO_3-N and that all of the diffusion coefficients within biofilm were equal to 80% of those in bulk liquid phase (Dolores et al., 1976, Chen et al., 1989).

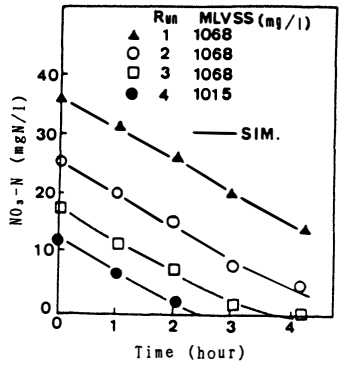

Figure 8 Change of NO_3-N concentration in a reactor

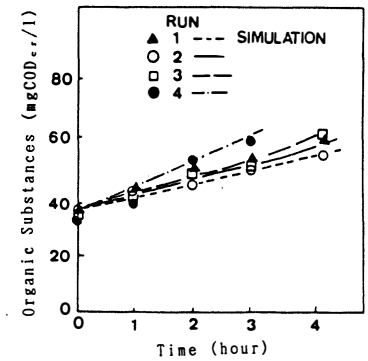

Figure 9 Change of concentration of organic substances in reactor

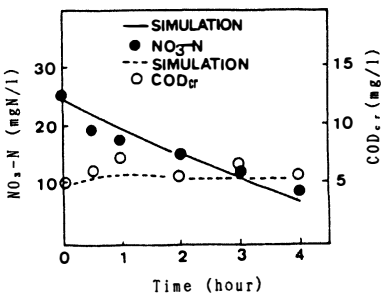

Figure 10 Comparison of model simulations with experimental results for endogenous denitrification in a submerged biofilm reactor

Table 3 Values of Kinetic and Stoichiometric Parameters

sym.	value	dimension	sym.	value	dimension
Dws	0.6	cm^2/day	Kn	0.8	mgN/ℓ
Dwc	1.6	cm^2/day	Xf	30.2	mgVSS /cm^3
Dwn	1.4	cm^2/day	Kms	3.0	1/day
Dfs	0.44	cm^2/day	Ko	0.5	mgO_2 /ℓ
Dfc	1.44	cm^2/day	b	0.45	1/day
Dfn	1.2	cm^2/day	P	1.26	mgCOD/mgVSS
Lf	0.145	cm	f	0.08	(−)
Lo	0.01	cm	r_2	0.13	mgO_2/mgCOD
Ks	10.41	mgCOD/ℓ	β	2.86	mgCOD/mgN
Kor	0.1	mgO /ℓ	Kmn	0.141	1/day
A	1562	cm^2	V	8.0	ℓ

Table 2 Values of Parameters of Endogenous Denitrification

Parameters	Run 1	Run 2	Run 3	Run 4	Average
K_{mn} (day^{-1})	0.134	0.143	0.144	0.144	0.141
K_n (mg/ℓ)	0.79	0.90	0.69	0.54	0.80
K_s (mg/ℓ)	11.6	9.9	9.6	15.6	10.4

DETERMINATION OF PARAMETERS

Most parameters in those equations shown in Table 1 have been experimentally estimated (Owen et al., 1976, Chen, 1990). However, those associated with endogenous denitrification (K_{mn}, K_n, and K_s) have not been investigated. In this research, these parameters were determined from batch experiments with the dispersed biofilm.

EXPERIMENTAL METHOD AND RESULTS Biofilm was cultivated in a submerged rotating disc reactor as shown in Fig. 7 (volume: 8 L, number of discs: 9, and disc diameter: 25 cm) with the artificial sewage composed of glucose, ammonium, and acetate, at hydraulic retention time 4.4 hours, rotation speed 38 rpm. Biofilm was sampled and dispersed by a high speed homogenizer and washed with tap water. The sample was equally separated into four reactors(1 L flask). The initial concentration of NO_3-N at the reactors was prepared at different levels with only N_aNO_3 and tap water. Four runs of the experiments were carried out with these reactors which were sealed up, and nitrogen gas was introduced and pH was controlled at 7. Concentrations of soluble COD_{cr} and NO_3-N were continuously measured in each of the runs.

The changes of COD_{cr} and NO_3-N were plotted in Fig. 8 and Fig. 9, respectively. Figure 8 shows that although the concentration of COD_{cr} was very low, the concentration of NO_3-N decreased with time. This demonstrated that endogenous denitrification occurred in these reactors. Figure 9 indicated the concentration of COD_{cr} increased with time in each run.

DETERMINATION METHOD AND RESULTS Because the biofilm was dispersed and DO level was zero in the reactors, the changes of COD_{cr} and NO_3-N can be described in Equations (25) and (26), which are simplified from Equations (22) and (24) in Table 1.

$$\frac{dS_b}{dt} = -\beta \cdot \frac{K_{mn} \cdot N_b}{K_n + N_b} \cdot \frac{S_b}{K_s + S_b} \cdot M_c + b \cdot P \cdot (1-f) \cdot M_c \tag{25}$$

$$\frac{dN_b}{dt} = -\frac{K_{mn} \cdot N_b}{K_n + N_b} \cdot \frac{S_b}{K_s + S_b} \cdot M_c \tag{26}$$

In Equations (25) and (25), K_{mn} = maximum specific rate of endogenous denitrification (day^{-1}). The MLSS was assumed constant in these experiments. The parameters of K_{mn}, K_s, and K_n were determined by Equations (25) and (26) based upon the experimental results, using a parameter determination method (i. e., the simplex method (Dixon, 1974)). The values of the parameters were shown in Table 3. The calculated changes of COD_{cr} and NO_3-N in each run, using Equations (22) and (23) with the parameters in Table 2, were indicated in Fig. 8 and Fig. 9, respectively.

VERIFICATION OF THE MODEL ON ENDOGENOUS DENITRIFICATION

EXPERIMENTAL METHOD AND RESULTS

Distilled water was introduced into the submerged rotating disc reactor for 24 hours with only aeration. After this, liquor in the reactor was renewed and then N_aNO_3 and nutrients were added, followed by pH adjustment. Changes of COD_{cr} and NO_3-N were investigated for 4 hours in this reactor in which nitrogen gas was introduced during the investigation. Biofilm density and thickness were measured after the investigation.

The experimental results were plotted in Fig. 10. This indicated that NO_3-N decreased in the reactor even when organic substrate was not added, and endogenous nitrification did occur in this case. Biofilm density and biofilm thickness were 30.2 $mgSS/cm^3$ and 1.45 mm, respectively.

MODEL SIMULATION

The numerical analysis of the proposed model [Equations (16) to (24)] was carried out by differentiating these equations, with the boundary conditions, such as : (a) each external mass flux correspondingly equals the internal mass flux at biofilm surface, (b) every

internal mass flux on the surface of supported media is zero. The parameters applied in the simulations were shown in Table 3. The simulations on changes of CODcr and NO_3-N were expressed in Fig. 10 by the solid and dashed lines, respectively. It was proved from this figure that the proposed model can appropriately evaluate the endogenous denitrification in a biofilm.

CONCLUSIONS

The production of the secondary substrate from lysis of microorganisms was studied in this research. A mathematical model describing endogenous denitrification using the secondary substrate in biofilm was proposed and evaluated by experimental results. The main conclusions obtained in this research were as follows.

1. Secondary substrate was produced from the lysis of microorganisms and the percentage of the substrate with molecular weight less than 10,000 on COD_{cr} or carbohydrate bases was over 50.

2. Production rate of the secondary substrate could be described by first type of expression with respect to biomass concentration.

3. An expression incorporating the coefficient of lysis in a biofilm was proposed, by which the coefficient was determined, $0.45day^{-1}$.

4. Endogenous denitrification was proved to occur in a submerged biofilm reactor.

5. The kinetic of endogenous denitrification in biofilm could be expressed by a complex Monod type equation, and the parameters governing the denitrification were measured.

6. The comparison of model simulations with experimental results showed that the proposed model can appropriately describe endogenous denitrification in a biofilm.

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