MATHEMATICAL MODEL OF SULFUR UTILIZING AUTOTROPHIC DENITRIFICATION IN AN UP-FLOW PACKED-BED REACTOR BASED ON BIOMASS DISTRIBUTION

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Abstract : A novel technology for the removal of nitrogen from wastewater, an autotrophic denitrification process with sulfur particles, has been developed. A respirometer was employed to monitor the nitrogen gas produced in the reactor, while 4',6-diamidino-2-phenylindole staining was employed to investigate the biomass distribution in terms of cell number according to the reactor height. From the respirometric monitoring, the denitrification reaction was defined as a first order reaction. The reactor was divided into 7 sections and biomass was analyzed in each section where cell number was ranged from 4.8×10^6 to 8.7×10^7 cells/g dry weight of sulfur. Cells placed mostly in the lower layer (≤ 10 cm of height). A function for biomass distribution was obtained with non-linear regression. Then a mathematical model has been developed by combining a plug-flow model with the biomass distribution function. The model could make a vertical profile of the up-flow packed-bed reactor resulting in a reasonable comparison with measured nitrate concentration with 5% of error range.

Key Words : Sulfur; denitrification; packed-bed; up-flow; biomass distribution; mathematical model

INTRODUCTION

Nitrate is one of the serious pollutants in water environment. Some industrial wastewaters, for example, from steel manufacturing and chemical synthesizing factories contain nitrate in high concentrations more than 500 mg N/L. However, most processes for nitrogen removal were based on heterotrophic denitrification, which require organic sources for nitrate

reduction but industrial wastewaters include little organics in common. Therefore, some external carbon source is required to denitrify those kinds of wastewaters. The external carbon source causes the increase of treatment price and other organic treatment processes should be followed in order to remove residual organics. Although lots of researches have been carried out to remove nitrogen from water and the removal efficiencies are more than 80% in most cases, the organic demand of the biological denitrification is still a stumbling block.[1,2] There has been an unique approach for nitrogen removal

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based on sequential heterotrophic and autotrophic reactions to reduce the organic demand in treating wastewaters with high nitrate concentrations.[3]

A novel technology for the removal of nitrogen from wastewater, the sulfur particle autotrophic denitrification (SPAD) process, has been developed in an up-flow packed bed type. This process is dominated by sulfur utilizing autotrophic denitrifiers, which utilize elemental sulfur to reduce nitrate. These autotrophs may take part in general water and/or wastewater treatment processes for the removal of nitrate as long as an exogenous sulfur source and anoxic environment are provided.[4-10] Many researchers have studied the autotrophic denitrification in the presence of elemental sulfur or thiosulfate as electron donors, using pure cultures of *Thiobacillus denitrificans, Thiomicrospira denitrificans, Thiobacillus versutus, Thiosphaera pantotropha and Paracoccus denitrificans.*[5,6,8,9-11] However, all the researches were carried out in suspended conditions and kinetic models derived were basically explaining the relationship between biomass and substrate. These researches could be useful to estimate the ability of autotrophic denifriers but may not be available for making a vertical profile of the packed bed reactor.

It is very important to determine at which height denitrification is finalized in the packed bed reactor because it could be used to determine the effectiveness and the capacity of the reactor.[9] However, there has been no report introducing the method to determine the height showing the vertical profile of the reactor. Of cause, the break-through curve has been used to make vertical profiles of packed bed reactors such as sand filter and granular activated carbon. It is a simple and effective method but not for biological reactors. Therefore, a mathematical model was developed in this research, which could be applied for biological up-flow packed bed reactor. In the up-flow packed bed reactor, it should be pointed out that the amount of biomass changed according to the reactor height

resulting in heterogeneity in denitrification capacity. Thus the model was established based on the change of the amount of biomass with the reactor height. Basically, two kinds of methods, respirometry and biomass analysis according to reactor height with 4,6-diamidino-2-phenylindole (DAPI) staining, were involved for the model development.

MATERIALS AND METHODS

Reactor Configuration and Monitoring System

Figure 1 shows the schematic diagram of the on-line gas monitoring system. The volume of the packed-bed was 2,356 mL. The system consisted of 8 parts. No. 3, 5, 6, 7 and 8 were important parts in the monitoring of nitrogen gas produced. The pressure balancing line, No. 3, was set to remove the pressure difference between the inner headspace and the headspace of the effluent part. The water level continuously went up and down due to the pressure difference when the reactor was closed. Therefore, the pressure balancing line played an
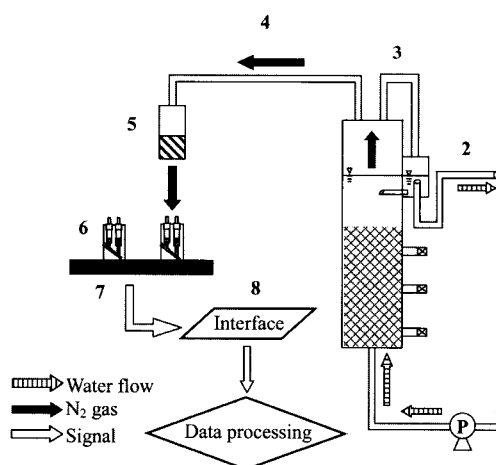

Figure 1. Schematic diagram of gas monitoring system for the sulfur utilizing autotrophic denitrification process (1. Influent, 2. Effluent, 3. Pressure balancing line, 4. Gas outlet, 5. Adsorption bottle for removal of undesirable gas, 6. Cell, 7. Cell base, 8. Interface).

important role in maintaining the water level for stable gas detection. The adsorption bottle, No. 5, was used to remove undesirable gases, such as CO_2, water vapor and H_2S, in order to detect nitrogen gas only. The bottle was packed with pellet-type KOH (95%, Dongyang Chemical Co., Ltd., Seoul, Republic of Korea) and silica gel blue (5-8 mesh, Dongyang Chemical Co., Ltd., Seoul, Republic of Korea) to remove CO_2 and water vapor. No. 6, 7 and 8 were the mechanical parts of the respirometer.

Feed and Seed

The wastewater used in the experiment was artificially made with tap water. The tap water was left open to the air for 24 hrs and then used for the artificial wastewater. The feed contained 1,000 mg $NaHCO_3$/L, 2,000 mg KH_2PO_4/L, 500 mg NH_4Cl/L, 10 mg $FeSO_4 \cdot 7H_2O$/L, and 500 mg $MgCl_2 \cdot 6H_2O$/L. Then, nitrate was added 120, 240, and 360 mg N/L with KNO_3, respectively to adjust the loading rate and verify the model obtained.

In the case of the seed, the autotrophic denitrifiers were cultured in a master culture reactor (MCR), using thiosulfate as the electron donor. The biomass concentration was approximately 200-400 mg /L, as mixed liquor volatile suspended solid (MLVSS). The MCR breeding autotrophic denitrifiers had been operated for about 10 months and it took about 2 days for the autotrophs to remove 100 mg/L of nitrate nitrogen under steady operation. Then, 1 L of the culture was transferred into the testing reactor and the reactor operated for 1 month before this research to make the microorganisms take their positions on the surface of sulfur particles.

Kinetic Model for SPAD Process

A simple kinetic equation for plug-flow reactor was modified in terms of biomass distribution and the effective bed (equation (1)). The porosity of the bed, packed with 2 to 4 mm sulfur particles, was measured as approximately 45% (see the section 2.4), and thus A_P,

could be assumed as 45% of the reactor surface area. The reaction had been determined as a first order in a previous research[12,13] and thus n in the equation (1) was employed as 1. $M_{avg.}$, M_0 and $k_{N,0}$ were employed to compensate for the change in biomass concentration according to height. $M_{avg.}$ was a function of reactor height. The reaction rate constant, $k_{N,0}$ was determined in the bottom layer ($\leq$ 4 cm of height in this study, Figure 2) of the packed bed, where there was no gradient in biomass concentration. If the reaction constant was determined in a higher layer, it would be smaller than $k_{N,0}$ because the amount of biomass should be smaller than that in the bottom layer. The denitrifying capacity would decrease with the reactor height because of the decrease of biomass. The loss of capacity was compensated by the combination of the three terms, $k_{N,0}$, $M_{avg.}$ and M_0.
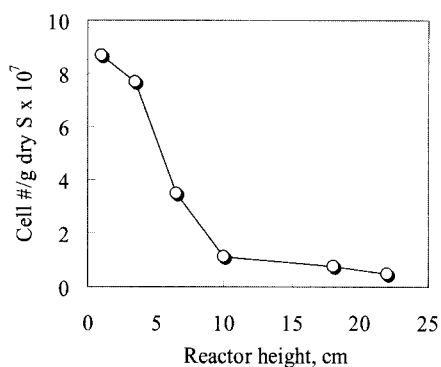

Figure 2. Biomass distributions according to reactor height.

$$\frac{dC}{dy} = -\frac{A_P}{Q} k_{N,0} \frac{M_{avg.}}{M_0} C^n = -\frac{\phi A_S}{Q} k_{N,0} \frac{\int f(y)/y}{M_0} C \qquad (1)$$

Determination of Porosity of Packed Bed

The porosity of the packed bed was measured based on the method introduced by Klute and Dirksen (1986).[14] A 10 L column was packed with sulfur and filled with water. The column was then left for 30 min and filled with water again, as a part of the water had been absorbed by the sulfur particles. After that, the water in the pores was drained and its volume was measured. This procedure was repeated three

times. The total volume of the bed (V_t) was assumed to be 10 L and that of the removed water was 4.52 L in average ($V_w = V_v$). Thereby, the porosity was simply calculated as 0.45 using equation (2).

$$\phi = \frac{(V_t - V_s)}{V_t} = \frac{V_v}{V_t}$$ (2)

Calculation of Reaction Rate Constant, $k_{N,0}$

The reaction rate constant, $k_{N,0}$ was calculated with an integrated model of the equation (1) based on the volume of the bottom layer where biomass distributed homogeneously. The layer was determined basically with respirometric calculation (equation (3)) and verified by biomass analysis with DAPI (Sigma Chemical Co., St. Louis, M.O., U.S.A) staining and nitrate measurement at each height. Nitrate loading rate was set low in order to make the denitrification reaction finished in the lower layer. In determining $k_{N,0}$, packed-bed contact time (PBCT) and the influent nitrate concentration were 11.8 hr and 120 mg NO_3^--N/L, respectively. The determination of the height at which nitrate is depleted has been explained in the former research.[13]

$$y_h = v \cdot (S / R_s)$$ (3)

Sulfur Particle Sampling for Measuring Cell Concentration

The reactor bed (24 cm height), packed with sulfur particles, was divided into 7 sections from the bottom to the top: one 2 cm, two 3 cm and four 4 cm sections. Sulfur particle samples were removed from each section at the end of the reactor operation. Each section was gently mixed and used for DAPI staining and moisture content analysis.

DAPI Staining and Epi-fluorescence Microscopy

Five grams of sulfur particles were taken from each section and used for DAPI staining to quantify the total microorganisms in each section, while another 5 g from each section were heated at 104°C for 1 h to determine the moisture content. For DAPI staining, the sulfur particles were mixed with 10 ml of phosphate buffered saline (PBS, 0.13 M NaCl and 10 mM sodium phosphate buffer, pH 7.2) in sterile 50 ml centrifuge tubes, and cells were released from the sulfur particles by vortexing vigorously for 1 min. The released cells were rinsed with PBS and fixed with 4% paraformaldehyde in PBS overnight at 4°C. The fixed cells were then rinsed and suspended in PBS. The suspended cells were mixed with an equal volume of 96% ethanol before storing at -20°C, which were then used within a week. The stored PBS-ethanol samples were serially diluted and 8 μl of each dilution immobilized on precleaned, gelatin-coated glass slides (Cel-Line Associate, New-filed, N.J., U.S.A.) by air-drying. The immobilized cells were stained with DAPI solution (1 μg/ml) for 5 min. The DAPI stained cells were briefly washed with distilled water and air-dried at room temperature.[15,16] The dried cells were subjected to epi-fluorescent microscopy.

The DAPI-stained cells were visualized using a Zeiss Axiolab, with a 50W-mercury lamp, and the images obtained with a digital camera (Model Coolpix995, Nikon, Japan) mounted on the microscope. The cell counting was achieved with at least 10 random microscopic fields in each well. MS Excel was used for the statistical analysis, in which gave the cell number per g (dry) of sulfur particle.

Analysis

The individual items analyzed were the NO_3^--N, the volume of N_2 gas and the composition of the gas in the headspace. NO_3^--N was analyzed with ion chromatography (DX-120, Dionex Inc.), the gas production with a respirometer (AER-200, CES Inc. U.S.A.) and the gas composition with gas chromatography (GC). The GC was equipped with a TCD detector and HAYESEP D 100/120 column, with carrier and reference gas (both He) flow rates of

30 and 20 mL/min, respectively. The oven and detector temperatures were 75±0.5 and 200± 1.0°C, respectively.

RESULTS AND DISCUSSION

Biomass Distribution with Reactor Height

The biomass distribution was determined with cell counting accompanied with DAPI staining. The concentration of biomass was expressed based on dry weight of sulfur[17] and ranged from 4.8×10^6 to 8.7×10^7 cells/g dry weigh of sulfur (Figure 3). The highest number occurred at the lowest layer and vice versa. Most of biomass placed in the lower layer below 10 cm of height. Thus it could be simply expected that denitrification mostly occurred in the lower layer. From the analysis, it could be noticed that there was little gradient in biomass concentration below 4 cm of height. The result was plotted and an equation for the distribution (equation (4)) was obtained through non-linear regression with Sigma Plot v. 6.0.

$$f(y) = M_{min} + \frac{M_{max} - M_{min}}{1 + \exp[(y-a)/b]} = 2.21 + \frac{22.61}{1 + \exp[(y-5.95)/1.08]}$$

(4)

Biomass placed mostly in the lower layer ($\leq$ 10 cm of height) in this process within the range of nitrate loading rate applied in this research. Thus it could be assumed that less than 50% of the packed-bed was utilized for denitrification. It could be suggested from the result that the growth of bacteria in the upper layer (>10 cm of height) would be limited because of the loss of nitrate source. Nitrate was mostly depleted in the lower layer (Figure 3). If the nitrate loading rate was increased and thus nitrate supplied to the upper layer, the distribution of biomass would be approaching to the homogenous condition since the growth of bacteria could be stimulated also in the layer. The homogenous distribution of biomass could contribute to the increase of process capacity.
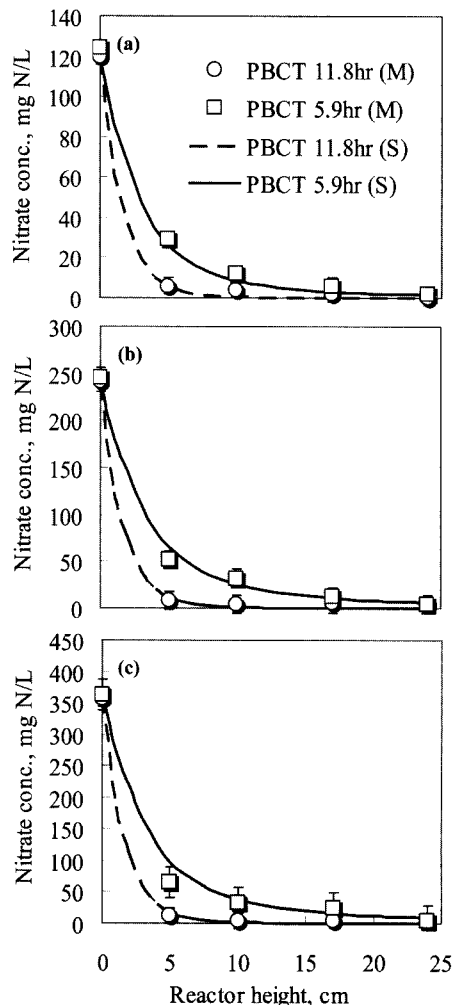

Figure 3. Simulated results and measured nitrate concentration according to reactor height. ((M) and (S) mean measured and simulated values, respectively. (a) Influent nitrate concentration = 120 mg N/L. (b) Influent nitrate concentration = 240 mg N/L, (c) Influent nitrate concentration = 360 mg N/L).

Model Evaluation

It was assumed that the surface of sulfur particles was fully occupied by bacteria in the bottom layer and the assumption could be supported by SEM (scanning electron microscope) image of sulfur particle (Figure 4). Then the mathematical model was evaluated using the parameters measured and/or calculated on the layer. M_0 was the biomass concentration in the

1st section from the bottom to 2 cm in height, which was 8.7×10^7 cells/g dry weight of sulfur. The reaction was determined as the first order in the previous researches in the same experimental conditions.[11,12] Therefore, equation (5) could be derived by combining with the equation (4) and integrating the equation (1).

$$C = C_0 \cdot \exp[-\frac{A_P}{Q} k_{N,0} \cdot \{1.04 + 0.13 \ln y + 1.51 (\ln y)^2 - 0.09 (\ln y)^3\}]$$

(5)

In the model, biomass distribution was expressed as a function of reactor height so that the model could make a vertical profile of the process. The concentration of nitrate at a certain height could be simply expected. Also it is possible to determine where nitrate will be exhausted with the model. It could be widely used for expecting the performance of packed-bed type bio-reactors by determining $k_{N,0}$. The value of $k_{N,0}$ could be varied according to bacterial species or the sort of reaction.
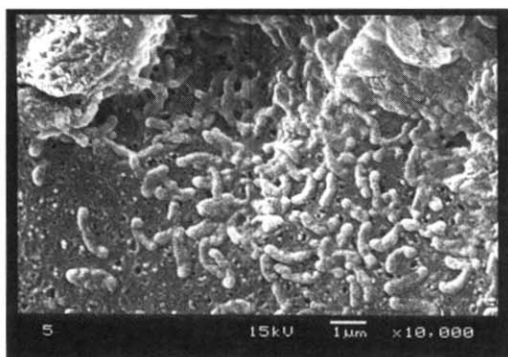

Figure 4. SEM (scanning electron microscope) image of the surface of sulfur particle occupied by bacteria.

Calculation of Reaction Rate Constant

The KOH removed the CO_2 and packed silica gel absorbed the water vapor. A little amount of H_2S could be produced by sulfate-reducing bacteria, but most of this gas would be used as electron donor for the sulfur-utilizing autotrophic denitrifiers. Therefore, most gas produced could be assumed as nitrogen gas. Even if a small

amount of the gas left the reactor, it would be dissolved in water and absorbed by the silica gel. The details on the gas analysis and data acquisition were mentioned in a previous research.[12]

The height of nitrate depletion was calculated with the equation (3). As mentioned above, the PBCT and the influent nitrate concentration were set at 11.9 hr and 120 mg NO_3^-/L. The rate of gas production, R_g was 18 mL N_2/hr where more than 99 % of the gas was nitrogen. R_N, thus, was calculated to 22.5 mg N/L. Then the height was determined as 5.5 cm by the respirometric calculation as previously described.[12] It was verified by measuring nitrate at each height (Figure 3(a)) where nitrate was nearly depleted at 5-cm of height. And it could be supported by the biomass analysis (Figure 2). According to the analysis, it could be concluded that biomass was distributed homogeneously within the height. Therefore, 5.5 cm could be utilized for the calculation of the reaction rate constant. The effluent concentration of nitrate was 6 mg N/L. Combining all the values, the constant could be calculated with the equation (5) and it was 3.62 hr^{-1}.

Model Verification

In order to verify the model, nitrate concentration was measured at each height, for each influent concentration of nitrate and at each PBCT. Then the measured values were compared to those simulated with the model. All the measured values were the average of 10 samples. As shown in Figure 3, the model showed the vertical profile of the packed-bed rector with respect to nitrate concentration and the measured values were well matched with the simulated lines within 5 % of error except those at 5 cm of height in Figure 3(b) and (c). Consequently, it could be concluded that the mathematical model could simply show the vertical profile of the paced-bed denitrification reactor. It could have a possibility as the basis of monitoring and control of packed-bed type bio-reactors.

CONCLUSIONS

The application of the respirometric method and biomass analysis with DAPI staining has been proved appropriate for the mathematical model development. The following are the intensive results of the research.

1. A function of biomass distribution could be developed on the basis of cell number analysis according to reactor height. Most of cells placed in the lower layer and the number was ranged from 4.8×10^6 to 8.7×10^7 cells/g dry weight of sulfur. The highest number stands for the lowest layer and vice versa.

2. From the result of biomass distribution analysis, it could be suggested that most reaction occurred in the lower layer since most bacteria placed there. This suggestion could be supported by the results of nitrate analysis at each height.

3. A mathematical model could be developed by combing a simple plug-flow model with the function of biomass distribution.

$$C = C_0 \cdot \exp[-\frac{A_p}{Q} k_{v,0} \cdot \{1.04 + 0.13\ln y + 1.51(\ln y)^2 - 0.09(\ln y)^3\}]$$

4. The model showed the vertical profile of the packed-bed rector with respect to nitrate concentration and the measured values were well matched with the simulated lines within 5% of error. Consequently, it could be concluded that the mathematical model could simply show the vertical profile of the paced-bed denitrification reactor. It could have a possibility as the basis of monitoring and controls of not only the SPAD process but also, probably, other packed-bed type bioreactors.

ACKNOWLEDGEMENT

Nomenclature

AP : specific cross-sectional area occupied by pores, cm^2

AS : specific cross-sectional area of reactor, cm^2

a : experimental coefficient

b : experimental coefficient

C : concentration of nitrate nitrogen, mg NO_3^--N/L

f(y) : function for biomass distribution according to reactor height

$k_{N,0}$: reaction rate constant, hr^{-1}

M_0 : average biomass concentration in the layer with no gradient of biomass concentration, cell#/g dry weight of sulfur

$M_{avg.}$: average biomass concentration up to a certain height, cell #/g dry weight of sulfur $= \int_0^y f(y)/y$

M_{min} : minimum biomass concentration, cell# / g dry weight of sulfur

M_{max} : maximum biomass concentration, cell# / g dry weight of sulfur

n : reaction order, dimensionless

Q : volumetric flow rate, mL/hr

R_N : nitrate removal rate based on nitrogen gas production, mg NO_3^--N/hr $= R_g/0.8$

R_g : rate of nitrogen gas production

S : the amount of nitrate nitrogen removed for 1 hour, mg

V_t : total volume of bed, L

V_s : volume occupied by sulfur particles, L

V_v : void volume, L

v : vertical linear velocity, cm/hr $= Q / A_P = Q / 0.45A_S$

y_h : height at which nitrate is depleted, cm $= v(S/R_N)$

y : reactor height, cm

$\emptyset$: porosity of sulfur packed bed, 0.45

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