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Effect of nitrifiers enrichment and diffusion on their oxygen half-saturation value measurements

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ABSTRACT

It has been recognized that the size of activated sludge floc affected the measurement of substrate half-saturation value. The impact of bacteria enrichment on the half-saturation value measurement has not been studied. This study highlighted the importance of bacteria enrichment on the evaluation of the half-saturation values. Due to the critical importance of oxygen half-saturation value in achieving partial nitrification, the impact of floc diameter and nitrifier enrichment on the measurement of oxygen half-saturation value for AOB ($K_{O,AOB}$) and NOB ($K_{O,NOB}$) was evaluated both in experiment and in mathematical simulation. The experiment results showed low measured $K_{O,AOB}$ and $K_{O,NOB}$ values under small floc diameter and low nitrifiers concentration condition. The increase in floc diameter and nitrifiers enrichment caused a significant increase in the measured $K_{O,AOB}$, while only slight increase in $K_{O,NOB}$ was noticed. The simulation results were consistent with the experiment results and indicated that the nitrifiers concentration and the floc diameter had higher impact on the $K_{O,AOB}$ measurement than on the $K_{O,NOB}$ measurement. The results could explain the conflicting reports on the measured $K_{O,AOB}$ and $K_{O,NOB}$ values.

1. Introduction

Activated sludge models (ASMs) based on the Monod-type equations offers a valuable tool for modeling and control of biological wastewater treatment process [1]. For effective application of the ASMs, the model parameters need to be calibrated and validated [2]. In the ASMs, the diffusion resistances within the activated sludge flocs were not explicitly considered and represented implicitly by the substrate half-saturation values.

It has been recognized that the diffusion resistance within flocs affected mass transfer, and therefore, affected the substrate half-saturation value measurement [3]. The measured substrate half-saturation value reflected both the external diffusion resistance and intrinsic substrate transport resistance due to enzymatic substrate binding at bacteria level [3,4]. It is generally accepted that high substrate half-saturation value is derived under large floc size (high diffusion resistance) conditions [5]. Therefore, the interpretation of substrate half-saturation value should consider the characteristics of activated sludge flocs.

Although the effect of floc size on the substrate half-saturation value measurement was widely known, the effect of bacteria concentration on the substrate half-saturation value measurement has not been investigated. The substrate concentration profile within the activated sludge flocs could be affected by the bacteria concentration. At high bacteria concentration, the substrate consumption by bacteria in the outside layer of flocs reduces the substrate available to the bacteria inside the flocs. On the other hand, the substrate could penetrate into the inner part of flocs under low bacteria concentration. Therefore, the bacteria concentration affects the substrate half-saturation measurement by changing the substrate availability to the bacteria inside the inner part of flocs. The bacteria concentration level should be considered in evaluating the measured substrate half-saturation value.

The recent development of autotrophic nitrogen removal by the ANAMMOX (anaerobic ammonium oxidation) [6,7] provided an opportunity for transforming the present energy-consuming into an energy-yielding wastewater treatment [8]. One of the key steps to achieve autotrophic nitrogen removal is the washout or suppression of NOB (nitrite oxidizing bacteria), in which, the ammonium is converted into nitrite by AOB (ammonium oxidizing bacteria) while the nitrite oxidation into nitrate is stopped or inhibited (called partial nitrification or nitritation).

The DO (dissolved oxygen) limitation technique was usually applied to achieve nitritation [9]. It is believed that the NOB has higher oxygen half-saturation value than AOB ($K_{O,NOB} > K_{O,AOB}$) [10,11]. The NOB was selectively suppressed under the low DO con-

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dition. However, a number of researches have shown that $K_{O,AOB}$ was larger than $K_{O,NOB}$ [3,12–14]. The lack of consensus in the AOB and NOB oxygen half-saturation values led to confusion in understanding the mechanism for NOB suppression at low DO condition.

It should be noted the oxygen half-saturation values of AOB and NOB in the recent reports were measured on sludge samples collected from nitrification reactor for ammonium removal alone [12,13]. These samples had high nitrifier (AOB and NOB) concentration than the sludge samples from reactors for combined organic material and ammonium removal. The effect of nitrifier concentration was not considered in evaluating the measured oxygen half-saturation value. It is not known how the nitrifier concentration affects their oxygen half-saturation value measurement.

Therefore, in this study, we tried to elucidate the effect of nitrifier concentration and diffusion on their oxygen half-saturation values measurements by: (1) cultivating sludge samples of different nitrifier concentration and flocs characteristics; (2) measuring their oxygen half-saturation values; (3) characterizing nitrifier community by high throughput sequencing technology; (4) evaluating the impact of nitrifier concentration and flocs size on the oxygen half-saturation value by the mathematical biofilm model.

2. Material and methods

2.1. The two sludge samples

The first sample (A) was collected from the aeration tank of the local sewage treatment plant for organic material and nitrogen removal. The plant operated at SBR (sequencing batch reactor) mode, with non-aerated influent filling period for de-nitrification and aeration period for nitrification. The nitrifier concentration in the sample A was low because the majority of bacteria were heterotrophic bacteria for organic material removal. The sample A was characterized by small pin floc and had an average floc diameter less than 50 μm.

The other sample (B) was cultivated in the 6 L lab-scale SBR for ammonium nitrification without de-nitrification. It was expected high nitrifier concentration could be achieved in the lab-scale SBR. The DO concentration during the aeration phase was maintained at above 4.0 mg O_2 L^{-1}. The artificial wastewater feed mainly contained NH_4^+ at 100 mg N L^{-1}. The $NaHCO_3$ was provided as alkalinity and other trace gradients were added according to the recipe shown in [15]. The sludge sample B was collected from the lab SBR after 105 days of operation. Compared to the heterotrophic biomass, the autotrophic nitrifying biomass could form large dense flocs more easily [16]. The average floc diameter of the sludge sample B was measured at 280 μm.

2.2. Respirometric characterization of sludge samples

The respirometric tests were used to indicate the biomass activity (heterotrophic and autotrophic) and measure the oxygen half-saturation value for AOB and NOB. The respirometric tests were carried out in a 1 L batch reactor at temperature of 25 °C. More detailed information on the respirometer for OUR (oxygen uptake rate) measurement could be found in Wu et al. [17].

To assess the biomass activity, SOURs (specific oxygen uptake rate, mg O_2 L^{-1} h^{-1} g^{-1} VSS) were measured at low F/M (food to mass) ratio. To test heterotrophic activity, SOUR was measured after pulse addition of acetate at 100 mg COD L^{-1} into both sludge samples. The AOB activity was assessed by pulse addition of 10 and 40 mg NH_4^+-N L^{-1} for sample A and B respectively during the SOUR measurement. For NOB activity, 10 mg NO_2^--N L^{-1} was added to the both samples during the SOUR measurement.

The oxygen half-saturation values ($K_{O,AOB}$, $K_{O,NOB}$ (mg O_2 L^{-1})) were assessed by measuring the OUR (oxygen uptake rate, mg O_2 L^{-1} h^{-1}) for AOB and NOB at different DO concentrations [3,10,18]. At NH_4^+ or NO_2^- non-limiting condition, the exogenous OURs for AOB and NOB (OUR_{AOB} and OUR_{NOB}) could be expressed by the following equation:

$$OUR_{AOB} = OUR_{AOB}^{max}\frac{S_O}{S_O + K_{O,AOB}},\ OUR_{NOB} = OUR_{NOB}^{max}\frac{S_O}{S_O + K_{O,NOB}} \quad (1)$$

where, OUR_{AOB}^{max}, OUR_{NOB}^{max} (mg O_2 L^{-1} h^{-1}) were the maximum OUR_{AOB} and OUR_{NOB} and were assumed to be constant during the OUR test with stable VSS concentration; S_O (mg O_2 L^{-1}) was the DO concentration.

2.3. Mathematical simulation

A biofilm model was used to investigate the effect of nitrifier concentration and floc size on the measurement of $K_{O,AOB}$ and $K_{O,NOB}$. The floc was assumed to be rigid sphere and have a fixed diameter. The solid concentration of the activated sludge sample was fixed at 2000 mg VSS L^{-1}. The weight percentage of AOB and NOB (P) within the flocs was varied to represent the nitrifier concentration. The AOB and NOB was assumed to be evenly distributed within the spherical flocs. The weight ratio of AOB and NOB was assumed to be the ratio of their yield coefficient (Y_{AOB}:Y_{NOB}, the yield coefficients were taken from Wiesmann [11]). The biomass density within floc was set to be 30 kg VSS m^{-3}, corresponding to 42.6 kg COD m^{-3} (assuming a conversion factor of 1.42 g COD g^{-1} VSS). The floc porosity was 0.8. Only the solutes diffusion and biodegradation were considered in the model. The growth and of AOB and NOB was ignored because the simulation was carried out in a short time scale of 1 h to represent the actual experiment condition for oxygen half-saturation value measurement.

The diffusion and biodegradation of soluble components (S) within the spherical flocs could be expressed by the following equation:

$$\frac{\partial S}{\partial t} = \frac{1}{\varepsilon}r^{-2}\frac{\partial}{\partial r}\left(r^2 D\frac{\partial S}{\partial r}\right) + \frac{R_S}{\varepsilon} \quad (2)$$

where, ε is the porosity (0.8); r is the distance to the floc centre (m); D is the diffusion coefficient (m^2 d^{-1}); R_S is the biodegradation rate of soluble components ($mg\,L^{-1}$ d^{-1}).

The R_S for the various soluble components was shown in the Table 1.

The NH_4^+ removal rate (dS_{NH4}/dt, mg N L^{-1} d^{-1}) in the bulk liquid could be calculated by the following equation:

$$\frac{dS_{NH4}}{dt} = J_{NH4}A,\ J_{NH4} = D_{NH4}\frac{\partial S_{NH4}}{\partial r}\Big|_{r=R},\ A = 4\pi R2N \quad (3)$$

where, J_{NH4} was the flux of NH_4^+ into the flocs (mg N L^{-1} d^{-1} m^{-2}); A was the total flocs surface area (m^2); D_{NH4} was the NH_4^+ diffusion coefficient within flocs (m^2 d^{-1}); $\frac{\partial S_{NH4}}{\partial r}|_{r=R}$ was the NH_4^+ concentration gradient at the floc surface (r = R); R was the floc radius and N was the number of floc.

Similarly, the NO_2^- removal rate (dS_{NO2}/dt mg N L^{-1} d^{-1}) in the bulk liquid could be expressed by the following equation:

$$\frac{dS_{NO2}}{dt} = J_{NO2}A,\ J_{NO2} = D_{NO2}\frac{\partial S_{NO2}}{\partial r}\Big|_{r=R} \quad (4)$$

where, J_{NO2} was the flux of NO_2^- into the flocs (mg N L^{-1} d^{-1} m^{-2}); D_{NO2} was the NO_2^- diffusion rate within floc(m^2 d^{-1}); $\frac{\partial S_{NO2}}{\partial r}|_{r=R}$ was the NO_2^- concentration gradient at the floc surface.

By calculating the dS_{NH4}/dt and dS_{NO2}/dt at different DO concentrations and NH_4^+ or NO_2^- non-limiting condition respectively, the