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Modeling of Simultaneous Partial Nitrification, Anammox and Denitrification Process In a Single Reactor

Yang Xiao[1], Qiang Xiao[1,2]* and Shi Xiang[2]

[1]*State Key Laboratory of Urban and Regional Ecology, Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*
[2]*Chongqing University of Arts and Sciences, Chongqing 402160, China*

Abstract

One sequencing batch reactor (SBR) seeded with anaerobic granular sludge was started-up to enrich simultaneous partial nitrification and anaerobic ammonia oxidation (Anammox) bacteria. The SBR could be operated successfully through first enriching Anammox bacteria under strictly anaerobic condition during the first stage and then controlling the dissolved oxygen (DO) in the reactor between 0.3 and 0.6 mg/L during the second and third stage, and eventually the maximum total nitrogen removal rate could reach 63.7%. Besides, a dynamic mathematical model including nitrification, Anammox, COD oxidation and denitrification was developed for the simulation of the performance of biological processes. The dynamic mathematical model was extended with two parts: model correction factors of DO, and Haldane kinetics for Anammox. Subsequently, a set of experiments and simulations were carried out to analyze the effects of substrate nitrite and DO on SBR system, and to validate the model. Research results indicated that biological processes could be inhibited by a certain nitrite concentration 20~30mg/L, and high or low DO concentration was not in favor of operation of SBR system. In addition, the simulation results were consistent with the experimental results, which illuminated that the mathematical model was appropriate for the modeling of simultaneous partial nitrification, Anammox and denitrification process in a single reactor, thus it could estimate the impact of specific parameters and predict the efficiency of system.

Keywords: Partial nitrification; Anammox; SBR; DO; Model

Nomenclature: S_{O2} dissolved oxygen, S_S readily biodegradable substrate, S_{NH4} ammonium nitrogen, NH_4+-N, S_{NO2} nitrite nitrogen, NO_2−-N, S_{NO3} nitrate, NO_3−-N, S_{N2} dinitrogen, N_2, X_{NH} aerobic ammonium oxidizers, X_{NO} aerobic nitrite oxidizers, X_{AN} Anammox bacteria, X_H hetertrophic organisms, X inert particulate organic material, Y_{NH} yield coefficient of X_{NH}, Y_{NO} yield coefficient of X_{NO}, Y_{AN} yield coefficient of X_{AN}, Y_H yield coefficient of X_H, fx Fraction of inert COD generated in biomass lysis, η anoxic reduction factor, μ^m_{NH} maximum growth rate of X_{NH}, K_{O2NH} affinity constant for oxygen , K_{NH4NH} affinity constant for ammonium, K_{NO3} affinity constant for nitrate, b_{NH} aerobic endogenous respiration rate, n anoxic reduction factor, μ^m_{NO} maximum growth rate of X_{NO}, K_{O2NO} affinity constant for oxygen, K_{NO2NO} affinity constant for nitrite, b_{NO} aerobic endogenous respiration rate, μ^m_{AN} maximum growth rate of X_{AN}, K_{O2AN} inhibiting constant for oxygen, K_{NO2AN} affinity constant for nitrite, Ki inhibiting coefficient for the nitrite, K_{NH4AN} affinity constant for ammonium, b_{AN} aerobic endogenous respiration rate, μ^m_H maximum growth rate of X_H, K_{O2H} affinity constant for oxygen, K_S affinity constant for substrate, K_{NO2} affinity constant for nitrite, b_H aerobic endogenous respiration rate.

Introduction

Nitrogen removal is one of the crucial steps in wastewater treatment. Nowadays, the most common way to remove nitrogen from wastewater is the combination of two sequential biological processes: autotrophic nitrification and heterotrophic denitrification. But in many wastewaters, the low level of organic carbon is hardly sufficient for complete heterotrophic denitrification, and addition of an external organic matter source, such as methanol, is often necessary to achieve complete denitrification [1].

In the mid-1990s, a new microbial process for nitrogen removal, anaerobic ammonium oxidation (Anammox), was discovered in which ammonium was directly converted to nitrogen gas under anaerobic conditions with nitrite as the electron accepter [2] and offered new opportunities for wastewater engineers and microbiologists [1]. The Anammox process was first observed in an anaerobic denitrifying fluidized bed reactor in 1986 [3]. Recently the process has also been shown to occur in nature in marine sediments and anoxic water columns [4,5].

Later on, several microbial processes which are combination of partial nitrification and Anammox have been made based on this new discovery, such as: Sharon/Anammox (a combined process with two separate reactors in series of Sharon and Anammox; [6]), CANON (completely autotrophic nitrogen removal over nitrite; [7]), OLAND (oxygen limited autotrophic nitrification denitrification; [8]) and aerobic deammonification [9]) etc. Compared with conventional biological nitrogen removal processes, these biological processes has many advantages, e.g. low oxygen demand, no requirement for external carbon sources, minimized surplus sludge and reduced CO_2 emissions [10]).

However, the application of these simultaneous partial nitrification and Anammox processes can be limited by its inhibition by certain compounds. One of the most important inhibitors is dissolved oxygen (DO), which reversibly inhibits Anammox bacteria [1,11]. The other one is substrate nitrite which negatively impact Anammox bacteria when its concentrations reach a certain level [12]. This nitrite inhibition could be overcome by addition of trace amounts of either of the Anammox intermediates including hydrazine and hydroxylamine [12] and full recovery is observed only several weeks after nitrite concentrations have returned below the detection limit [11]. Apart from the two major external compounds inhibition, the extremely low growth rate

***Corresponding author:** Qiang Xiao, Chongqing University of Arts and Sciences, Chongqing 402160, China, Tel: +86-10-64889011; E-mail: xiaoqiang7598@126.com

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(0.0027·h^{-1}, doubling time 11 days) of these Anammox bacteria [13] as an inner inhibitory factor also limit these microbial processes. These inhibitory effects make the start-up of nitrogen removal process difficult and complex, so a model-based approach would be very useful. Especially, using modeling in the development of Anammox process is more crucial. Even if a model is not fully correct, the trends provided by model simulations greatly help the development of processes based on slow growing bacteria [14].

For the evaluation and the optimization of this nitrogen removal process, various models have been developed and edited, such as the CANON model [15]. However, most previous studies of mathematic model concerning the combination of partial nitrification and Anammox have not deeply considered the influence of concentration gradient of DO in granular sludge and inhibitory effect of nitrite. Therefore, the extension of such models to these neglected parts which could significantly affect these biological processes appears particularly interesting but requires specific experiments and research.

The aim of this work was to enrich simultaneous partial nitrification and Anammox bacteria and to validate a dynamic mathematical model for the simulation of biological processes. First, Anammox process was started-up in one single SBR seeded with anaerobic granular sludge, and the performance of the SBR system was analyzed based on the experimental results. Then, an extended mathematical model was proposed and validated by comparing the simulated output with the experimental data.

Materials and Methods

Reactors and operation

The experiment was carried out in a Plexiglas cylindrical reactor with a working volume of 7.0L (height 74 cm, diameter 11 cm). The temperature was maintained at (30 ± 2)°C through a water jacket of reactor. The pH of the reactor was controlled between 7.5 and 8.3 with HCl (1 M) and NaOH (1 M) stock solution. A black-vinyl sheet enclosure was used to keep the bacteria away from the light. The synthetic wastewater was fed to the reactor after deoxygenating by stripping with nitrogen gas in a water tank. Before feeding, the sludge was allowed to settle for 1 h. About 5 L of the supernatant was removed, and 5 L of the freshly prepared synthetic wastewater was fed. Nitrogen gas was sparged from the bottom of the reactor at a maximum gas flow of 40 mL/min controlled by a mass-flow controller for fluidization of the biomass and the maintenance of anaerobic condition in the reactor.

Origin of biomass

The anaerobic methanogenic granular sludge used for inoculation originated from an internal circulation reactor in a wastewater treatment plant. The main characteristics were mixed liquor suspended solids (MLSS) 50.43 g/L and mixed liquor volatile suspended solids (MLVSS) 21.30 g/L.

Media

Synthetic wastewater was composed as described in Table 1 [2]. Synthetic wastewater A contained mainly nitrite and ammonium to support Anammox activity and this wastewater was used for experiments under anaerobic conditions. Synthetic wastewater B contained no nitrite but did contain ammonium to establish aerobic ammonium oxidation and, due to oxygen limitation, only part of the ammonium was converted to nitrite. The resulting mixture of ammonium and nitrite supports subsequently Anammox. Synthetic wastewater C contained lower concentration of calcium salts and phosphate to increase organic ingredients of granular sludge. The composition of trace element solution (g/L) as follows (Table 1).

Substance	Synthetic waste A	Synthetic waste B	Synthetic waste C
$(NH_4)_2SO_4$	0.31	0.31	0.31
$NaNO_2$	0.345	0	0
$KHCO_3$	1.258	1.258	1.258
NaH_2PO_4	0.05	0.05	0.005
$MgSO_4$•$7H_2O$	0.20	0.20	0.20
$CaCl_2$•$2H_2O$	0.30	0.30	0.005
Trace element solution	1.0 mL/L	1.0 mL/L	1.0 mL/L

Table 1: Composition of the synthetic wastewater used in this study. Values are in g/L.

EDTA 15, $ZnSO_4·7H_2O$ 0.43, $CuSO_4·5H_2O$ 0.25, $CoCl_2·6H_2O$ 0.24, $FeSO_4·7H_2O$ 6.25, $MnCl_2·4H_2O$ 0.99, $NaMoO_4·2H_2O$ 0.22, $NiCl_2·6H_2O$ 0.19, H_3BO_4 0.014, $NaWO_4·2H_2O$ 0.054

Analytical methods

For monitoring the performance of the reactor, samples were retrieved from the reactor at regular intervals. NH_4+-N, NO_2–-N and NO_3–-N were determined spectrophotometrically according to standard methods [16]. Total inorganic nitrogen (TIN) was calculated as: TIN = [NH_4+-N] + [NO_2–-N] + [NO_3–-N]. COD was measured in microwave digestion method. A volatile suspended solid (VSS) was determined by weight method. The pH was determined with a digital, portable pH meter. The DO level was measured with a digital, portable DO meter.

Mathematical model

A mathematical model describing the kinetics of aerobic ammonium oxidizers, aerobic nitrite oxidizers, Anammox bacteria and heterotrophic organisms was proposed, similar to the CANON model. The description of aerobic and denitrifying conversions by heterotrophic organisms was taken directly from ASM3 [17]. Anoxic growth processes of heterotrophic organisms were described for both nitrite and nitrate reduction. Similar to the ASM3, the anoxic endogenous respiration processes were expressed by consumption of nitrate only. Hydrolysis of particulate COD, the storage of COD, and the endogenous respiration processes of XSTO were omitted in the model. Such simplification had little influence on simulation. In addition to Monod equations in the model, Haldane equations were added to describe the inhibitory effect of substrate. The Anammox process was not inhibited by ammonium or by the by-product nitrate up to concentrations of at least 1 g of nitrogen per liter. However, in the presence of more than 0.1 g of nitrite nitrogen per liter, the process was completely inhibited [12]. Therefore, the inhibitory effect of substrate was expressed by nitrite only. Model correction factors of DO and Anammox restraint rate were introduced as well. The stoichiometric and kinetic matrix is shown in Appendix Table I and Table II. The values for stoichiometric and kinetic parameters referred to ASM3 and some literatures [13,18-20], with summarizing in Appendix Table III. For the modeling, the mathematical equations were implemented in the Matlab software using 4-order Runge-Kutta method.

Results and Discussion

Enrichment of granular sludge in SBR

Anammox process was started-up in a SBR, which was inoculated with anaerobic granular sludge, and experienced three phases. Figure 1 shows the performance of SBR system during start-up period. In the first phase (0~55 days), the Anammox population was enriched under strictly anaerobic condition kept by sparging nitrogen gas, and synthetic waste A was supplied to the reactor. For the very low growth rate of Anammox bacteria, the initial hydraulic retention time (HRT) was controlled at 22 days to enrich Anammox bacteria, and then gradually shorten the HRT in order to increase the loading rate. As seen in Figure 1, no reduction of ammonium was observed in the reactor, and only nitrite decreased rapidly in the initial 10 days. The nitrite removal rate reached 21.7 g nitrite/(m^3·day). At the same time, COD decreased from 916 mg/L to 520 mg/L. However, the synthetic wastewater did not contain organic carbon source, and the presence of high COD load could be produced by the lysis of some biomass due to the changes of their living environment. These results indicated that nitrite removal was caused mainly by heterotrophic denitrification. Heterotrophic denitrification as the dominant process competed nitrite with Anammox during the initial 30 days. To compensate the inhibiting effect caused by heterotrophic denitrifies, nitrite was added to reactor on time after the nitrite in the reactor was completely depleted. Ammonium and nitrite simultaneously remove from day 46, which indicated that Anammox biomass had been accumulated and the Anammox activity had enhanced in the reactor. In the second phase (56~155 days), synthetic waste B was supplied to the reactor with a HRT of 5 days, and DO in the reactor was controlled at about 0.5~0.8 mg/L by sparging mixture gas (air and nitrogen gas). The sludge transformed to autotrophic granular sluge (combined partial nitrification and Anammox granular biomass) in this phase. Ammonium over converted into nitrite from day 56 to 65 which indicate the impact of oxygen on the reaction system. After 5 days of synthetic waste B and mixture gas supply, the nitrite concentration increased to 30.1 mg/L, and ammonium concentration only decreased 32.5 mg/L, which indicated that Anammox activity was completely inhibited in the reactor. Two weeks after the impact of oxygen on the reaction system, Anammox activity was recovered and the concentrations of ammonium and nitrite showed no further change and the reactor was therefore considered to be at "steady state". Then, a total nitrogen removal efficiency of 72.3% was obtained. From day 105 on, the substrate load was increased by further reducing the HRT to 3 days. In the third phase (156~200 days), DO in the reactor was slightly decreased to 0.3~0.5 mg/L to increase Anammox activity, and synthetic waste C was supplied. After diminishing adequately the Ca and P concentrations of the feeding medium both the activity and the nitrogen uptake of the SBR system increased quickly. It was similar to the result of some research papers that salt precipitation might interfere with microbial activity and caused a decrease of the nitrogen removal rate of reactor [21]. From day 180 on, the reactor was considered to be in a new "steady state" mode, because from this point onward, the ammonia conversion rate in the reactor was constant. The phenomena illuminated that the interspecific competition of aerobic ammonium oxidizers and Anammox bacteria reached a new balance that could remove ammonium efficiently and stably. During the next 10 days' cultivation, the activity of reactor was high and stable and a total nitrogen removal rate reached 19.2 g N/(m^3·day) (Figure 1).

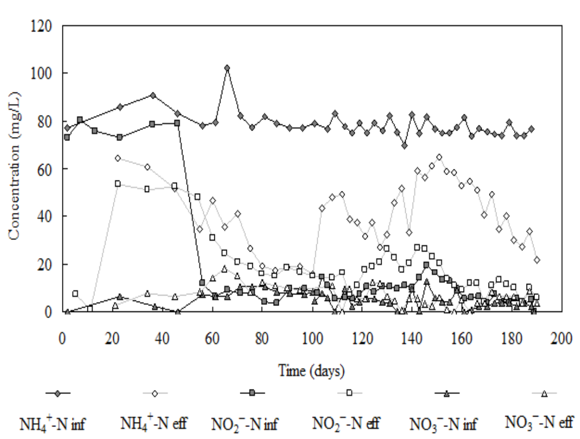

Figure 1: Performance of SBR during start-up period.

Sludge evolution

The experiment had been operated for over 6 months up to this point. Some characteristics of granular sludge, such as concentration, shape and color, were changed extremely. With the improvement of activity, the sludge concentration decreased from 7.21 g/L to 5.224 g/L (at day 52), and then to 3.67 g/L (at day 190) due to the decay of the biomass. The color of the granular sludge in the reactor changed from dust black to brown and then to brownish red, at the same time, the shape of granules changed from granules to fragmentized granules, and then to compact granules. The change of biomass color and shape could indicate the start-up course of the bioreactor.

The morphology and inner structure of the granules observed in more detail with photographs and SEM (scanning electron microscopy) are shown in Figure 2. As seen in Figure 2, the granules developed in SBR had compact structure and a clear spherical outer shape. Compact spherical granules mainly composed of spherical and filamentous bacteria, and lots of cavities were present. These cavities can enhance substrate transfer from the bulk to granules and intermediate or by-product transfer from inside granules to the bulk (Figure 2).

The distribution of DO in granular sludge

Under oxygen limiting conditions, partial nitrification took place dominantly in the aerobic region of granular sludge. The aerobic ammonium oxidizers oxidize ammonium to nitrite, consume DO and so create an anaerobic microenvironment for the inner granular sludge. Therefore, aerobic/anaerobic microenvironments formed in granular sludge mainly depend on mass transfer of oxygen and microorganisms metabolism. It is significant to understand the mass transfer and reaction of oxygen in granular sludge for enhancing removal efficiency of granular sludge system. From ordinary experiments and operations, only the actual DO in main solution is generally known while DO in granular sludge is difficult to assess. According to substrate reaction-diffusion equations in biofilm, the simulations concerning the distribution of DO in granular sludge were implemented in the Matlab software.

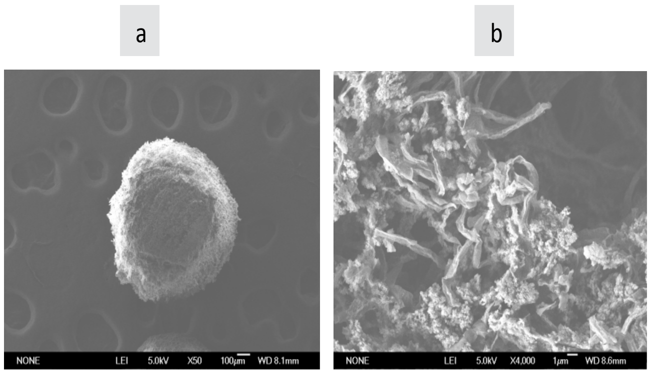

Figure 2: Scanning electron micrographs of autotrophic granular sludge (a: at 50× magnification; b: at 4000× magnification).

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Monod equation is used to describe the oxygen consumption (DC) rate of biological degradation process at any point in biofilm:

$r_{ut} = -q \times X_f \times S_f/(K+S_f)$ (1)

Where r_{ut} is the DO consumption rate, q is the DO max consumption rate, X_f is the biofilm density, S_f is the DO concentration, K is the affinity constant.

Molecular diffusion of oxygen in biofilm observed Fick's second law:

$r_{diff} = D_f \times d^2S_f/dr^2$ (2)

Where r_{diff} is the DO diffusion rate, D_f is the O_2 diffusion coefficient in biofilm ($D_f = 0.8 \times D_{O2}$), DO_2 is the O2 diffusion coefficient in water, r is the distance from inner wall of granular sludge.

Assumptions: 1.Biological reaction do not involve the main solution and only exist in granular sludge; 2. Mass transfer effect in the interface between granular sludge surface and mixed liquid is so small that it can be neglected; 3.The distribution of substrate, biomass aggregates and porosity in granular sludge is homogenous; 4.The distribution of microorganisms is only influenced by DO. According to the assumptions above, the diffusion and reaction of DO is simultaneous under the "steady-state". Balance equation (dimensionless unit) is given:

$D_f \times d^2S_f/dr^2 - q \times X_f \times S_f/(K+S_f) = 0$ (3)

Boundary conditions: $dS_f/dr(r=0) = 0$ (There are no mass transfer effect in inner wall of granules), Sf(r=1) = 1.

Aerobic ammonium oxidation was considered to be the primary course of oxygen consumption. Higher-order differential equation (3) was changed into a first-order differential equation by reducing order. According to the boundary conditions, the simulation of oxygen distribution in granular sludge was performed in the Matlab software using ODE-BVP solution. The simulation results are shown in Figure 3.

This simulation indicated a distinct distribution of DO in granular sludge, from where we could predict the existent range of aerobic bacteria and anaerobic bacteria within the aggregate. While aerobic bacteria were located at the surface layer of the granule with dimensionless radius range from 0.8~1, anaerobic bacteria occupied most of the interior parts with dimensionless radius range from 0~0.8.

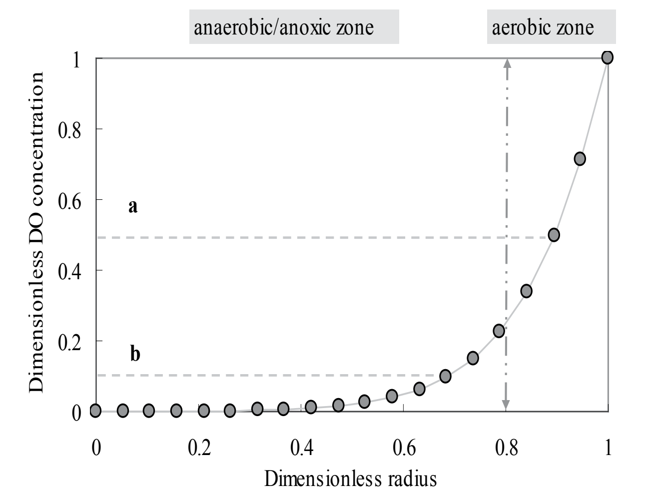

Figure 3: Oxygen distribution in granular sludge.

Model correction factors of DO (a and b) were introduced. a: correction factor of DO for aerobic bacteria, value equal to 0.5; b: correction factor of DO for anaerobic bacteria, value equal to 0.1. Larger type aggregates (>500μm) accounted for 68% of the Anammox potential whereas 65% of the nitrification potential was found in the smaller aggregates (<500μm) [22]. The sludge granule size differed from one another in reality [23], and its shape was not the regular sphere, so not all the granular sludge could be suitable for the formation of living environment of Anammox bacteria. Anammox restraint rate (c) was introduced for auxiliary adjustment (Figure 3).

Appliance of the model

Influence of nitrite on granular sludge: Related literatures reported that nitrite can inhibit the activity of granular sludge in a certain concentration and some researchers had measured the value of nitrite inhibitory coefficient for Anammox Ki=5.401~11.995 mmol/L [24]. During this test, an initial 20~30 mg/L nitrite was set for reactor and DO was controlled at 0.3~0.5 mg/L, to determine whether or not the inhibition happened. In addition, the model was implemented to validate its availability. The simulative conditions including the heterotrophic and autotrophic biomass were estimated based on final "steady state" of the reactor. Results showed in Figure 4, document that process inhibition caused by nitrite seemed to appear. Unsaturated fatty acids of Anammox bacteria would be subject to certain destruction due to excessive nitrite accumulation which was toxic to Anammox bacteria in a certain concentration, thus the activity of Anammox bacteria was limited. When inhibitory effect caused by nitrite appeared in the reactor, both high nitrite concentration like 70-150 mg/L and long duration like 12 hours that could result in complete Anammox activity lost were excepted [12]. By always keeping the Anammox potential higher than the nitrification potential, the occurrence of fatal nitrite build-up and following irreversible inhibitory effect of nitrite on Anammox biomass could be reduced. Consequently, the efficiency of total nitrogen removal recovered through reducing aeration just in time or keeping anaerobic microenvironment (Figure 4c). With much lower DO (0.2-0.3mg/L),

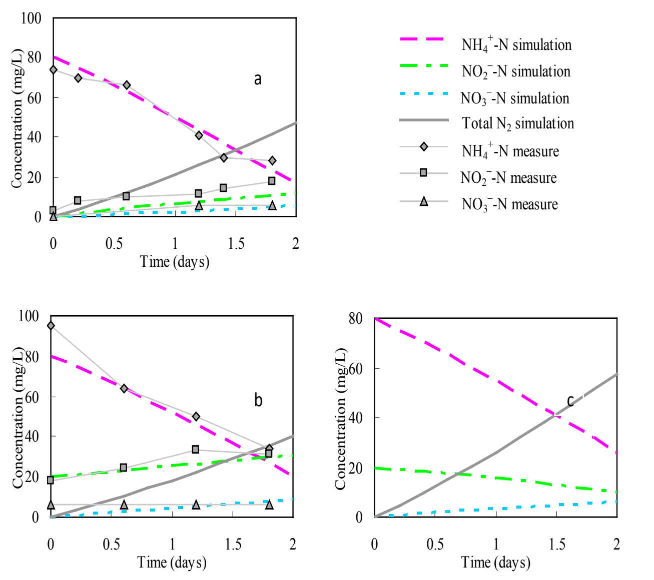

Figure 4: Influence of nitrite concentration on nitrogen conversions, a: NO_2^--N=0 mg/L, b: NO_2^--N=20 mg/L, c: NO_2^--N=20 mg/L, reduce aeration.

the consumption rate of nitrite consumed by Anammox bacteria was higher then the production rate of nitrite produced by ammonium oxidizers, so the inhibition caused by accumulative nitrite gradually reduced in the reactor. Other literature also reported that when inhibition occurred, 100 µM hydrazine addition seems to recover the activity of SBR system from inhibitory effect of nitrite (Figure 4) [25].

Influence of DO on granular sludge: The oxygen concentration in granular sludge was identified as the main variable influencing the nitrite accumulation and Anammox activity during the whole star-up period. To further explore the influence of DO on biological processes, some tests with DO in the range of 0.1~0.2 mg/L (t=190 d), 0.3~0.6 mg/L (t=185 d), 0.7~1.0 mg/L (t=128 d) were carried out in the reactor. Moreover, the simulation was performed to validate its availability. Except biomass and the amount of aeration all other parameters were identical for the simulation. The test results are shown in Figure 5. When DO was controlled at 0.1~0.2 mg/L, the activity of aerobic ammonium oxidation was inhibited, and the reactor had a lower nitrite production rate. At the same time, the absence of nitrite indirectly restricted the activity of Anammox bacteria, consequently a total nitrogen removal efficiency of the reactor reduced as well. However the efficiency of the reactor could still keep high if reaction time extended. When DO was maintained at 0.3~0.6 mg/L, the accumulation of a small amount of nitrite met the growing necessary of Anammox bacteria and a higher removal efficiency was obtained in the reactor. When DO was kept at DO 0.7~1.0 mg/L, the elevated concentrations of toxic nitrite negatively impacted Anammox. Apart from it, higher DO concentration in reactor also reversibly inhibited Anammox bacteria, thus the activity of the reactor drop quickly in the reactor. The results of Figure 5, reflect that a total nitrogen removal can simultaneously reach higher levels by controlling appropriate DO related ammonium concentration of inflow. In an ammonium concentration of 80 mg/L, optimal DO level for the maximal nitrogen gas production is 0.3~0.6mg/L. From Figure 4 and 5 we can see that the simulation results are found to be in good agreement with the experimental ones, consequently no modification of the parameters is necessary for the simulation (Figure 5).

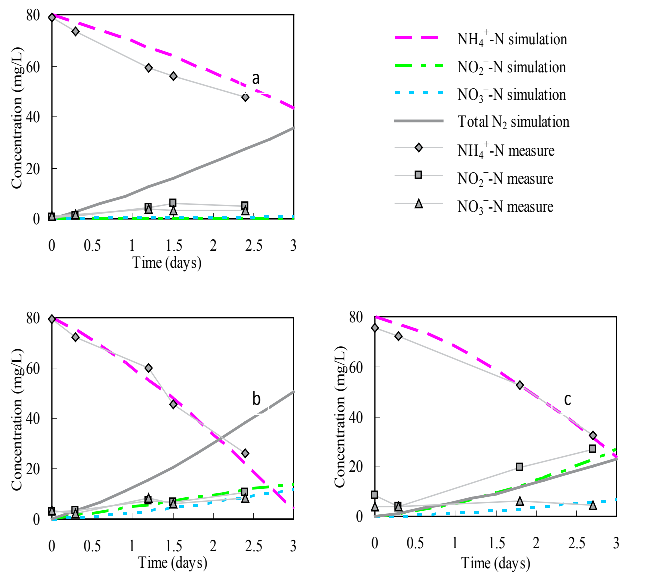

Figure 5: Influence of DO concentration on nitrogen conversions, a: DO 0.1~0.2 mg/L, b: DO 0.3~0.6 mg/L, c: DO 0.7~1.0 mg/L.

Conclusions

1. Simultaneous partial nitrification, Anammox and denitrification process could be cultivated successfully in single SBR after several months acclimation. Initially, anaerobic granular sludge as seeding sludge was utilized to cultivate Anammox granular biomass. Synthetic wastewater (80 mg/L ammonium, 80mg/L nitrite) was supplied to the SBR with a HRT of 22 days. Anammox activity developed quickly in the reactor, which indicated that the anaerobic granular sludge was a suitable inoculum and the longer HRT was in favor of the growing of Anammox bacteria. Then in the medium-term of the experiment, granular sludge changed to autotrophic granules by sparging mixture gas (air and nitrogen gas) and by further reducing HRT. Finally, the SBR system was operated successfully with DO in the reactor kept at 0.3-0.6 mg/L and the Ca and P concentrations of the feeding medium diminished adequately. The activity of the system was high and stable and a total nitrogen removal rate reached 19.2 g N/(m^3·day).

2. A dynamic mathematical model was developed for the simulation of biological processes in SBR. This model based on ASM3 and CANON model, allowed to simulate nitrification, Anammox, COD oxidation and also of denitrification. The mathematical model was introduced model correction factors of DO and extended to Haldane kinetics for Anammox. A set of experiments and simulations were carried out to analyze the effect of two major inhibitors (substrate nitrite and DO) on SBR system, and to validate the model. The biological processes in the reactor could be inhibited by a certain nitrite concentration 20~30mg/L. The inhibition by nitrite, however, could be recovered by reducing the amount of aeration, which was in favor of Anammox. In an ammonium concentration of 80 mg/L, the optimal DO level for the maximal nitrogen gas production is at 0.3~0.6mg/L. In addition, the simulation results shows good agreement with the experiment ones, thus the model can estimate the impact of specific parameters and predict the efficiency of system.

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