IV Symposium on Agricultural and Agroindustrial Waste Management
May 5-7, 2015 - Rio de Janeiro, Brazil

KINETIC MODELS FOR NITROGEN INHIBITION IN ANAMMOX PROCESS ON DEAMMONIFICATION SYSTEM

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SUMMARY: The performance of the deammonification process depends on the microbial activity of ammonia oxidizing bacteria (AOB) and ANAMMOX bacteria, and the autotrophic organisms involved in this process have different preferences for substrate, that may cause inhibition or imbalance of the system. The aim of this work was to adjust the best kinetic model for nitrogen inhibition by ammonium and nitrite in an ANAMMOX system (AMX) and in a deammonification system (DMX), both with the same operational conditions and suspended biomass. Four kinetic inhibition models were processed through non-linear regression to represent the inhibitions by substrate of the AMX and DMX processes: Edwards I, Monod, Teissier and Andrews models. The statistical criteria proved that the model proposed by Edwards I, was the best model to describe ammonium and nitrite inhibition of the AMX system, and show that the inhibitory effect of substrate concentrations were more evident and rapid for NO_2^--N than NH_3-N in the ANAMMOX process. While in the DMX system, the Monod model was the best model to describe the performance of the deammonification process, show that increasing in the substrate concentration is not limiting for the ammonia consumption rate.
Keywords: ANAMMOX, kinetic study, mathematical models.

INTRODUCTION

Since the discovery of anaerobic ammonium oxidation (ANAMMOX) (Mulder et al., 1995) several processes using ANAMMOX activity bacteria have been implemented to improve the autotrophic nitrogen removal in wastewater. Among these processes, the deammonification was recently proposed seeking more effectiveness for the treatment of concentrated effluents with low carbon/nitrogen ratio, as digestate swine wastewater.

Deammonification process combines partial nitritation with ANAMMOX process, both working together in two (Okabe et al., 2011) or in a single reactor (Chang et al., 2013). The reaction consists in the partial oxidation of ammonia to nitrite (by the ammonia oxidizing bacteria - AOB - activity) under limited oxygen conditions and subsequently in conversion of the nitrite produced and ammonium remaining to nitrogen gas (by the ANAMMOX bacteria activity), producing a small amount of nitrate. The overall nitrogen removal reaction is described by Equation 1.

$$NH_4^+ + 0.85\ O_2 \rightarrow 0.44\ N_2 + 0.11\ NO_3^- + 1.43\ H_2O + 1.14\ H^+ \qquad \textbf{Equation 1}$$

The main advantage of this process is it being completely autotrophic, without the need for carbon source addition, as well as having reduced energy requirements and lower biomass production compared to other processes. The deammonification in a single-step could be very economical compared with two-step nitrogen removal process since it requires less operational control and reach nitrogen removal in a single reactor.

The performance of the deammonification process depends on the microbial activity of AOB and ANAMMOX bacteria, and the autotrophic organisms involved in this process have different preferences for substrate, operating conditions and some external factors that may cause inhibition or imbalance of the system. Knowing this, the study of the kinetics and

modeling of deammonification process can be valuable tools to better understand and use this technology more efficiently.

The aim of this work was to adjust the best kinetic model for nitrogen inhibition by ammonium and nitrite in an ANAMMOX system (AMX) containing only ANAMMOX bacteria and in a deammonification system (DMX) containing AOB and ANAMMOX bacteria, both with the same operational conditions and suspended biomass.

MATERIAL AND METHODS

Systems for kinetics of substrate consumption were developed and chosen such methodology to quantify the rates at different ammonium and nitrite concentrations.

ANAMMOX system

One glass conical flask (1000 mL) with usable volume of 600 mL was used as a reactor for batch tests, coupled with a system for sample collection. The experiments were conducted in an Incubator Shaker (Lucadema, Luca-222) with temperature control at 25 ^{o}C and a rotation speed of 60 rpm, making it possible to maintain the constant temperature and homogenized system. To evaluate the effect of substrate in the bacteria consumption rate, tests were performed using concentrations of approximately 20, 50, 100, 150, 200, 250, 300, 400, 600 and 800 mg L^{-1} NT, these being 50% in the NH_3-N form and 50% in the NO_2^--N form, as required by the stoichiometry of the ANAMMOX process. Samples were collected every 30 minutes for 8 hours or until substrate concentrations below 10 mg L^{-1}. The cellular concentration of ANAMMOX biomass used for the tests was 1.7 gVSS L^{-1} and derived from the batch reactor with stable activity. The system monitoring was performed by analysis of pH, temperature, ammonia, nitrite and nitrate, all according to the methodology established by APHA, 2012.

Deammonification system

The batch tests were performed in a reactor with stable deammonification activity (Pra, 2013) and usable volume of 1.1 L. The temperature was controlled at 25 ^{o}C and the air flow rate was set at 20 mL min^{-1} in all batch tests. Intermittent aeration was used and controlled by Programmable Logical Controller (Dexter, model µDX series 100), with cycles of 30 minutes (22 minutes for oxic cycle and 8 minutes for anoxic cicle). The total cellular concentration of AOB:ANAMMOX used for the tests was 2.4 gVSS L^{-1}, in proportion to AOB:ANAMMOX 58:200 mL v/v. To evaluate the effect of substrate in the microorganisms consumption rate, tests were performed using concentrations of 50, 100, 150, 200, 250, 300, 400, 550, 650, 800 and 1000 mgNH_3-N L^{-1}. Samples were collected at the end of each cycle (30 minutes) for 8 hours. All samples were filtered and system monitoring was performed by analysis of pH, temperature, dissolved oxygen, total alkalinity, ammonia, nitrite and nitrate (APHA, 2012).

Fitting of mathematical models to experimental data

The substrate consumption rates was determined from the linear regression of substrate concentrations versus time for AMX and DMX systems. Starting from these and the respective substrate concentrations used, four kinetic models were processed through non-linear regression (SAS 9.2 software, 2008) to represent the inhibitions by substrate of the AMX and DMX processes, as shown in Table 1. The Akaike Information Criterion (AIC) was used to choose the best model.

RESULTS AND DISCUSSION

Figure 1 shows, in graphical form, the response of kinetic models fitting for experimental data AMX system. Due to the ANAMMOX bacteria consume ammonia and nitrite as a substrate simultaneously, the statistical analysis was performed separately, thus enabling to obtain the kinetic coefficients and the specific kinetic model for each substrate. One realizes that the kinetic model of Edwards I was the best fit to the experimental data for both NH_3-N and NO_2^--N, obtaining a correlation coefficient of R^2 = 0.962 and R^2 = 0.919, respectively. This model may have the best fit because it has been proposed considering the inhibition constant K_I, which in

this case represents the inhibitory effect of substrate concentration on biomass when exposed to high concentrations.

For AMX system, the Edwards I model found a maximum specific consumption rate of 9.971 $mgNH_3$-N $gVSS^{-1}$ h^{-1} for ammonia and 9.573 $mgNO_2^-$-N $gVSS^{-1}$ h^{-1} for nitrite. The K_S values were 96.435 mg L^{-1} for ammonia and 56.402 mg L^{-1} for nitrite. Already the substrate inhibition constant K_I was 603.53 $mgNH_3$-N L^{-1} for ammonia and 525.34 $mgNO_2^-$-N L^{-1} for nitrite. The values for these kinetic coefficients presented, associated with Figure 1, shows that the inhibitory effect of substrate concentrations were more evident and rapid for NO_2^--N than NH_3-N, i.e. while the NO_2^- activity began to decrease around 100 $mgNO_2^-$-N L^{-1} (Figure 1B), the NH_3 activity decreased only close to 800 $mgNH_3$-N L^{-1} (Figura1A).

Figure 2 shows the curves of kinetic models fitting to the experimental data for DMX system. Despite the similar behavior, you can see that the kinetic model of Monod was the best fit to the experimental data for NH_3-N, obtaining a correlation coefficient of $R^2 = 0.978$. The data intersection after non-linear regression allows estimating the values of the kinetic coefficients of DMX biomass and were of 7.513 $mgNH_3$-N gVSS h^{-1} for the maximum specific consumption rate of NH_3-N and 175.48 $mgNH_3$-N L^{-1} for the constant substrate saturation K_S. The absence of KI variable associated with the values provided by the Monod model and Figure 2, show that increasing in the substrate concentration is not limiting for the ammonia consumption rate, displaying the large capacity of the DMX system to withstand high removal loads without inhibiting activity of the bacteria.

The interpretation of these results confirms that the AMX system is much more sensitive to substrate inhibition than the DMX system. Musabyimana (2008) assessing the impact of nitrite concentration on the ANAMMOX bacteria during deammonification process concluded that ANAMMOX bacteria are more resistant to nitrite concentrations when they are in the DMX process than found themselves in enriched mixed cultures. This is because deammonification reactors with suspended biomass tend to eliminate nitrogen by forming granules which on the outside are OAB bacteria and inside are ANAMMOX bacteria (Chang et al., 2013). Thus, the ANAMMOX bacteria would not be totally exposed substrate concentrations, existing a concentration gradient in the aggregate which makes them more tolerant to inhibition than if they were alone. Finally, from an operational standpoint, the results provided by the kinetic models allow better recognize and control the substrate inhibition in DMX and AMX systems, considering that these concentrations are very important for the stability of the process, since they can both favor or inhibit activity of the bacteria involved.

CONCLUSIONS

The statistical criteria proved that the model proposed by Edwards I, was the best model to describe ammonium and nitrite inhibition of the AMX system, and show that the inhibitory effect of substrate concentrations were more evident and rapid for NO_2^--N than NH_3-N in the ANAMMOX process. While in the DMX system, the Monod model was the best model to describe the performance of the deammonification process, show that increasing in the substrate concentration is not limiting for the ammonia consumption rate.

The comparison between the kinetic coefficients in both systems, especially K_I, suggest that the DMX coefficients were influenced by the internal mass transfer in the granule and arrangement of bacteria, making ones more resistant than when found themselves in enriched mixed cultures.

ACKNOWLEDGEMENT

Authors thanks financial support from CAPES.

REFERENCES

APHA – American Public Health Association. **Standard methods for the examination of water and wastewater.** 22 ed. Washington, DC: American Public Health Association, 2012.

CHANG, X. et al. **Performance of a completely autotrophic nitrogen removal over nitrite (CANON) process for treating wastewater with different substrates at ambient temperature.** Environmental Sciences, 25: 688-697, 2013.
MULDER, A. et al. **Anaerobic ammonium oxidation discovered in a denitrifying fluidized reactor.** FEMS Microbiology and Ecology, 16: 177-184, 1995.
MUSABYIMANA, M. **Deammonification process kinetics and inhibition evaluation**. Virginia Polytechnic Institute and State University, Blacksburg, 2008.
OKABE, S. et al. **Development of long-term stable partial nitrification and subsequent ANAMMOX process.** Bioresource Technology, 102: 6801-6807, 2011.
PRA, M. C. de. **Estabelecimento e Estudo Cinético do Processo de Desamonificação utilizando-se um reator único para remoção de nitrogênio à temperatura ambiente**. Master Thesis, Federal University of Santa Catarina, 2013.

Tabela 1. Kinetic models used for fitting data during the experiment.

Equations			
Monod	Andrews	Teissier	Edwards I
$\mu_X = \mu_m \cdot \frac{S}{K_S+S}$	$\mu_X = \mu_m \cdot \frac{S}{K_S + S + \frac{S^2}{K_I}}$	$\mu_X = \mu_m \cdot \left(1 - e^{\frac{S}{K_S}}\right)$	$\mu_X = \mu_m \left(\exp\left(-\frac{S}{K_I}\right) - \exp\left(-\frac{S}{K_S}\right)\right)$

μ_X = specific growth rate (d^{-1}); μ_m = maximum specific growth rate (d^{-1}); S= substrate concentration (mg L^{-1}); K_S = saturation substrate constant (mg L^{-1}); K_I = inhibition substrate constant (mg L^{-1});

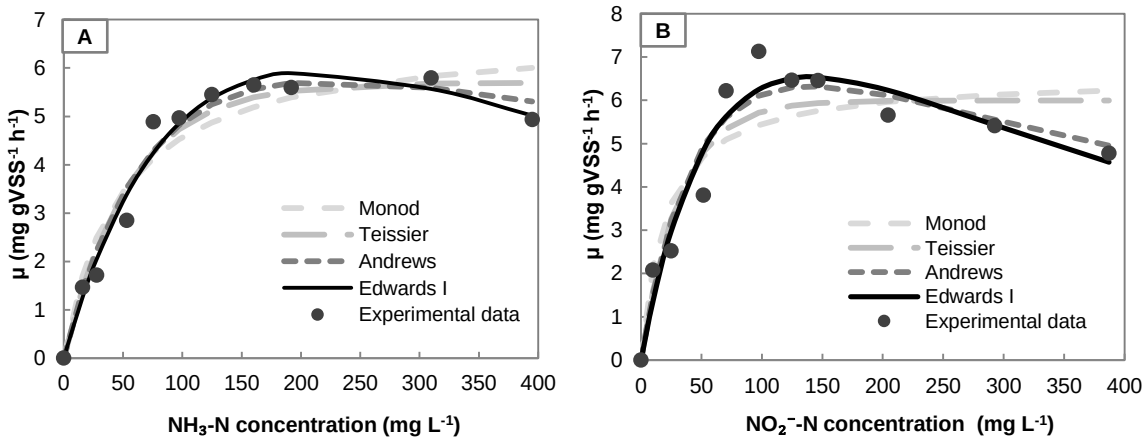

Figure 1. Models fitting to the experimental data for AMX system. A: data from the specific ammonia consumption rate versus substrate concentration, B: data from the specific nitrite consumption rate versus substrate concentration.

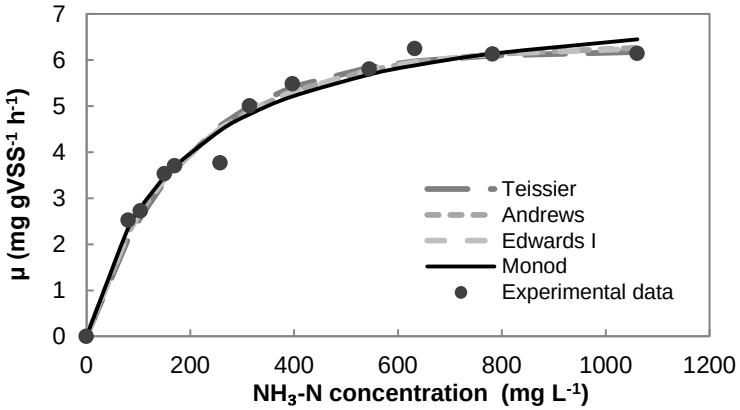

Figure 2. Models fitting to the experimental data for DMX system.