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Kinetic models for nitrogen inhibition in ANAMMOX and nitrification process on deammonification system at room temperature

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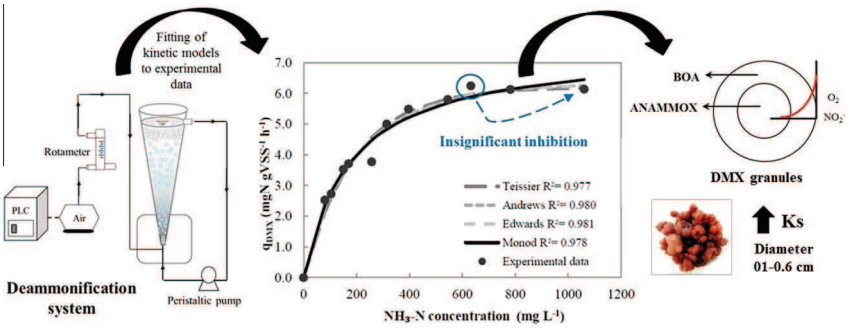
HIGHLIGHTS

- Andrews model describes NH_3 inhibition for nitrification process.
- Edwards model describes NH_3 and NO_2^- inhibition for ANAMMOX process.
- The inhibitory effect was more significant for NO_2^- than NH_3 in the ANAMMOX process.
- Monod model describes the absence inhibition for deammonification process.
- Deammonification process was stronger for N-inhibition than nitrification and ANAMMOX.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study were fitted the best kinetic model for nitrogen removal inhibition by ammonium and/or nitrite in three different nitrogen removal systems operated at 25 °C: a nitrifying system (NF) containing only ammonia oxidizing bacteria (AOB), an ANAMMOX system (AMX) containing only ANAMMOX bacteria, and a deammonification system (DMX) containing both AOB and ANAMMOX bacteria. NF system showed inhibition by ammonium and was best described by Andrews model. The AMX system showed a strong inhibition by nitrite and Edwards model presented a best system representation. For DMX system, the increased substrate concentration (until 1060 mg NH_3-N/L) tested was not limiting for the ammonia consumption rate and the Monod model was the best model to describe this process. The AOB and ANAMMOX sludges combined in the DMX system displayed a better activity, substrate affinity and excellent substrate tolerance than in nitrifying and ANAMMOX process.

1. 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,

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the deammonification was recently proposed seeking more effectiveness for the treatment of concentrated effluents with low carbon/nitrogen ratio, as sanitary and domestic sewage or digestate from agricultural wastewater.

Deammonification process combines partial nitritation process with ANAMMOX process, both working together in two (Okabe et al., 2011) or in a single reactor (Zhang et al., 2012). The combined reaction consists in the partial oxidation of ammonia to nitrite under limited oxygen conditions (partial nitritation by the aerobic ammonia oxidizing bacteria, AOB, activity – Eq. (1)), and a subsequent conversion of the nitrite produced and ammonium remaining to nitrogen gas and a small amount of nitrate under anaerobic conditions (by the ANAMMOX bacteria activity – Eq. (2))

$$NH_4^+ + 0.75O_2 \xrightarrow{AOB} 0.5NO_2^- + 0.5NH_4^+ + 2H^+ + H_2O \quad (1)$$

$$NH_4^+ + 1.31NO_2^- + 0.066HCO_3^- + 0.13H^+ \xrightarrow{ANAMMOX} 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (2)$$

The overall nitrogen removal reaction by deammonification process is described by Eq. (3)

$$NH_4^+ + 0.85O_2 \xrightarrow[ANAMMOX]{AOB} 0.44N_2 + 0.11NO_3^- + 1.43H_2O + 1.14H^+ \quad (3)$$

The main advantage of this process is that it develops in a complete autotrophic medium, without the need for carbon source addition, as well as having reduced energy requirements and lower biomass production compared to other classical processes such as nitrification/denitrification. The deammonification process performed in a single-step could be very economical compared to a two-step process since it requires less operational control and all the nitrogen removal is done in a single reactor (Chang et al., 2013; Cho et al., 2011; Jaroszynski and Oleszkiewicz, 2011; Zhang et al., 2014). On the other hand, the performance of the deammonification process depends on the microbial activity of AOB and ANAMMOX bacteria, and these autotrophic organisms have different preferences for substrate, operating conditions and some external factors that may cause inhibition or imbalance of the system.

In this way, the study of the kinetics and modeling can be a valuable tool to better understand and use deammonification technology more efficiently. The kinetic study of a given process allows obtaining a relationship between the reaction rate with the different specific conditions of the environment in which it occurs. To be able to represent the main processes involved and use them in engineering, mathematical models are commonly used.

Several mathematical models have been developed in recent decades to represent the variations in removal rate of certain substrates in biological systems under different operating conditions. For example, different kinetic models have been used to express the kinetic of growth of different microorganisms inhibited by phenol (Meriç et al., 2002), glucose (Davis et al., 2015), and BOD_5 (Tsuneda et al., 2005). Kinetics models have also been used for nitrification and ANAMMOX. For nitrification, Tanyolaç et al. (2001) found that the best model to express the kinetic behavior of nitrification inhibited by ammonium sulfate was the Monod model (1949), including a death factor. Nowadays, the Andrews model (1968) frequently has been used in modeling the inhibition of nitrification by substrate (Carrera et al., 2004). For ANAMMOX, the Monod and Andrews model were recently used to describe the response of a variety of systems: Expanded Granular Sludge Bed (EGSB) reactor response after feeding with strong ammonium-containing (Chen et al., 2011), Sequencing Batch Reactor (SBR) behavior under high organic and inorganic conditions (Tang et al., 2013), membrane bioreactor (MBR) of a suspended cell (Lotti et al., 2014) and still in marine ANAMMOX bacterial culture (Huang et al., 2014).

This knowledge with the reaction rate is very important to characterize the behavior of bacteria, which are quite sensitive to medium changes, and to be able to predict future adverse inhibitions. However, there are little or no references evaluating the best kinetic models for substrate inhibition in the deammonification system (DMX) that combines nitrification and ANAMMOX processes in a single tank. Furthermore, limited information is available on the process kinetics of substrate removal in these processes. In this study were fit the best kinetic model for nitrogen inhibition by ammonium and nitrite in three different systems: (1) a nitrifying system (NF) containing only AOB bacteria, (2) an ANAMMOX system (AMX) containing only ANAMMOX bacteria, and (3) a deammonification system (DMX) containing both AOB and ANAMMOX bacteria, both with suspended biomass and running at room temperature 25 °C.

2. Methods

Three specific systems were developed to quantify the reaction rates at different ammonium and nitrite concentrations: (1) nitrifying system (NF), (2) ANAMMOX system (AMX), and (3) single reactor deammonification system (DMX).

2.1. Source of nitrification and ANAMMOX sludges

The anaerobic ammonium oxidation (ANAMMOX) bacteria used was *Candidatus Jettenia asiatica* deposited under the provisions of the Collection of Microorganisms of Interest for Swine and Poultry (CMISEA) at Concórdia, Santa Catarina, Brazil (accession number: BRMSA 00323) (Viancelli et al., 2011). It was maintained at the Embrapa Swine and Poultry laboratory in a 3.3 L column up-flow continuous reactor. At the time of sludge harvesting, the parent reactor was being fed with synthetic wastewater containing 100 mg NH_3-N/L and 130 mg NO_2^--N/L and operated with a flow rate of 27 L/d, a nitrogen loading rate (NLR) of 1882 mg N/L/d, and a water temperature of 35 °C (De Prá, 2013).

The nitrification bacteria was maintained at the Embrapa Swine and Poultry laboratory in a 3.5 L Sequencing Batch Reactor (SBR). At the time of sludge harvesting, the parent reactor was being fed with synthetic wastewater containing 300 mg NH_4^+-N/L and the control of the whole system is automated through the use of a programmable logic controller (PLC). The SBR reaction cycle was 8 h (30 min for settling, 15 min to discard and 15 min for food). The HRT was 1.41 days and the aeration was intermittent at 15 min/h (De Prá, 2013).

2.2. Nitrifying system (NF)

Respirometry was the methodology chosen to quantify nitrification activity at different ammonium concentrations. The respirometer consisted of a glass conical flask of 500 mL internal volume with three exits at the top of the respirometer for a DO probe insertion (Hanna, HI 98186), a pH probe (Sincrontec, S2123-6606) and the injection of the test compounds. All NF system respirometric tests were carried out at the same biomass concentration (1.3 g VSS/L). A stirring plate and magnetic stirring bar set at a 750 rpm provided internal mixing of liquor and biomass particles. Aeration was provided through a porous stone diffuser at the bottom of the respirometer connected to an air compressor (Big Air A230, 2.5 W). A nutrient solution without ammonia was prepared to carry out the washing of biomass between tests through suspension, centrifugation and discarding the supernatant liquid. This washing procedure was repeated for three times, until the previous

substrate was annulled and it should not interfere in the new tests. The washed system was kept without ammonia substrate for 5 h before every respirometric experiment in order to establish endogenous respiration (ER), evaluated as a constant oxygen uptake rate (OUR). Temperature was kept at 25 ± 0.5 °C and pH at 7.5 ± 0.1 using NaOH 1 mol/L. Once the mixed liquor reached DO saturation, ammonia substrate (using NH_4Cl) was added to the test liquor at concentrations of 10, 25, 50, 100, 150, 200, 250, 350, 475, 600, 850 and 1200 mg NH_3-N/L. DO depletion was monitored until the concentration reached close to 15% of the DO saturation and the OUR was determined in duplicate for every substrate concentration (totaling 24 tests with substrate), considering as statistical error two times the standard deviation.

2.3. ANAMMOX system (AMX)

One glass conical flask with working volume of 600 mL was used as a reactor for AMX 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 °C and a rotation speed of 60 rpm. This set-up made 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 of total nitrogen (TN), 50% in the NH_3-N form and 50% in the NO_2^--N form, approaching the stoichiometry of the ANAMMOX process (Eq. (2)). Samples were collected every 30 min for 8 h or when substrate concentrations were below 10 mg N/L. The concentration of ANAMMOX biomass used for the tests was 1.7 g VSS/L collected from a batch reactor in our laboratory with stable ANAMMOX activity. The system monitoring was performed by analysis of pH, temperature, ammonia, nitrite and nitrate.

2.4. Deammonification system (DMX)

The batch tests were performed in a reactor with stable deammonification activity (De Prá, 2013) and working volume of 1.1 L. The temperature was controlled at 25 °C and the air flow rate was set at 20 mL/min in all batch tests. Intermittent aeration was used and controlled by Programmable Logical Controller (PLC Dexter, model μDX series 100), 30 min per cycle (22 min for oxic cycle and 8 min for anoxic cycle). The total cellular concentration of AOB + ANAMMOX used for the tests was 2.4 g VSS/L, obtained by mixing 58:200 mL AOB:ANAMMOX wet sludges in the 1.1 L volume reactor. To evaluate the effect of substrate in the microorganisms consumption rate, tests were performed using concentrations of 80, 100, 150, 200, 250, 300, 400, 550, 650, 800 and 1000 mg NH_3-N/L. Samples were collected during the anoxic cycle at the end of each complete cycle (30 min) for 8 h. All samples were filtered through a 0.4 μm pore-size disposable capsule filter and system monitoring was performed by process analysis: pH, temperature, dissolved oxygen, total alkalinity, ammonia, nitrite and nitrate.

2.5. Fitting of mathematical models to experimental data

The substrate consumption rate was determined from the linear regression of substrate concentrations versus time for NF, AMX and DMX systems. The results, with initial substrate concentrations, were processed by non-linear regression using four kinetic models (SAS 9.2 software, 2008). The best model was chosen using two statistical criteria. Firstly, the Akaike Information Criterion (AIC) was applied, with the lowest AIC values indicating a better model (Akaike, 1974). The AIC is founded on information theory: it offers a relative estimate of the information lost when a given model is used to represent the process that generates the data. In this way, it deals with the trade-off between the goodness of model fit and the complexity of the model, where the lowest value is the best model fit. Secondly, the adjusted R^2 was assessed, which relates the certainty between the experimental data and the proposed model (considering the number of independent variables).

2.5.1. Monod model

Monod model (Eq. (4)) is commonly used to describe the kinetics of substrate consumption. It provides a maximum substrate conversion rate (q_{max}) and a half saturation constant (K_S). The Monod model is represented below (Eq. (4)):

$$q = q_{max} \cdot \frac{S}{K_S + S} \tag{4}$$

where q is the specific substrate conversion rate (d^{-1}); q_{max} is the maximum specific substrate conversion rate (d^{-1}); S is the substrate (ammonium or nitrite) concentration (mg/L); K_S is the half saturation constant (mg N/L).

2.5.2. Andrews model

At low concentrations, the substrates serve as nutrients for microorganisms, but at high concentrations they behave as inhibitors. High substrate concentrations inhibit microbial growth and disturb their metabolism. Andrews model (Eq. (5)) is often used to describe the kinetics of substrate inhibition involving microorganisms (Andrews, 1968):

$$q = q_{max} \cdot \frac{S}{K_S + S + \frac{S^2}{K_I}} \tag{5}$$

where q is the specific substrate conversion rate (d^{-1}); q_{max} is the maximum specific substrate conversion rate (d^{-1}); S is the substrate (ammonium or nitrite) concentration (mg/L); K_S is the half saturation constant (mg N/L); K_I is the inhibition constant (mg N/L).

2.5.3. Edwards model

Other models can be considered to describe inhibitions by substrate. Edwards (1970) proposed the following kinetic model (Eq. (6)) for describing the inhibition by substrate:

$$q = q_{max}\left(\exp\left(-\frac{S}{K_I}\right) - \exp\left(-\frac{S}{K_S}\right)\right) \tag{6}$$

where q is the specific substrate conversion rate (d^{-1}); q_{max} is the maximum specific substrate conversion rate (d^{-1}); S is the substrate (ammonium or nitrite) concentration (mg/L); K_S is the half saturation constant (mg N/L); K_I is the inhibition constant (mg N/L).

2.5.4. Teissier model

Teissier (1942) proposed the application of substrate consumption to the microorganism growth with the following kinetic model (Eq. (7)):

$$q = q_{max} \cdot \left(1 - e^{-\frac{S}{K_S}}\right) \tag{7}$$

where q is the specific substrate conversion rate (d^{-1}); q_{max} is the maximum specific substrate conversion rate (d^{-1}); S is the substrate (ammonium or nitrite) concentration (mg/L); K_S is the half saturation constant (mg N/L).

2.6. Analytical methods

Ammonia (NH_3-N) was analyzed potentiometrically using a selective electrode method. Nitrite (NO_2^--N) and nitrate (NO_3^--N) were determined based on a colorimetric method using a flow injection analysis system (FIAlab – 2500). Alkalinity was determined using the titrimetric method (Titronic T-200 semi-automatic) and

Table 1
Substrate conversion rates (r) and specific substrate conversion rates (q) for each substrate concentration (S) used during the tests performed with the NF, AMX and DMX systems.

Tests	S_{NF} (mg NH_3-N/L)	S_{AMX} (mg/L)		S_{DMX} (mg NH_3-N/L)	OUR_{NF} (mg O_2/L/min)	$SOUR_{NF}$ (mg O_2 g/VSS/min)	r_{AMX} (mg/L/h)		q_{AMX} (mg g/VSS/h)		r_{DMX} (mg NH_3-N/L/h)	q_{DMX} (mg NH_3-N g VSS/h)
		NH_3-N	NO_2^--N				NH_3-N	NO_2^--N	NH_3-N	NO_2^--N		
ER	0	–	–	–	0.0575	0.0432	–	–	–	–	–	–
1	13.33	15.83	9.94	80.63	3.288	2.425	−2.52	−3.58	1.46	2.08	−6.403	2.524
2	25.71	27.74	25.06	104.36	4.965	3.685	−2.95	−4.33	1.72	2.52	−6.911	2.724
3	46.79	52.99	51.62	150.51	5.386	4.001	−4.89	−6.55	2.85	3.81	−8.953	3.529
4	99.85	75.61	70.38	170.07	5.711	4.245	−8.40	−10.70	4.89	6.22	−9.402	3.706
5	154.78	97.31	97.40	257.89	6.029	4.484	−8.54	−12.26	4.97	7.13	−9.559	3.768
6	211.19	124.80	124.80	314.29	5.942	4.419	−9.36	−11.12	5.45	6.47	−12.682	4.999
7	258.78	160.23	146.63	397.20	6.166	4.587	−9.71	−11.10	5.65	6.46	−13.911	5.483
8	354.52	191.94	204.40	545.19	6.099	4.537	−9.61	−9.73	5.59	5.66	−14.713	5.799
9	476.50	309.76	292.75	632.71	5.767	4.287	−9.96	−9.31	5.80	5.41	−15.842	6.244
10	605.60	395.00	387.20	814.00	5.597	4.160	−8.48	−8.22	4.93	4.78	−15.544	6.127
11	849.05	–	–	1060.68	5.663	4.209	–	–	–	–	−15.571	6.138
12	1244.50	–	–	–	5.078	3.770	–	–	–	–	–	–

expressed as mg $CaCO_3$/L. pH and DO were determined using a pH meter (S123-6606, Sincrontec) and a DO meter (55, YSI), respectively. Biomass concentration was determined as VSS. All methodologies were used according APHA (2012).

3. Results and discussion

3.1. Nitrification inhibition by ammonium in NF system

The concentration of dissolved oxygen during the respirometric test to determine the endogenous respiration (ER) of the nitrifying biomass showed a linear behavior, obtaining an oxygen uptake rate (OUR_{NF}) of 0.057 mg O_2/L/min, and a specific oxygen uptake rate ($SOUR_{NF}$) of 0.043 mg O_2/g SSV/min. Nitrification rates were obtained from the OUR measurements with ammonium sulfate as substrate and all taking into consideration the ER test. Twelve different initial concentrations of NH_3 were tested and the $SOUR_{NF}$ calculated from the respirometry test. The results for each test performed are available in Table 1 and subsequently fit to a model to obtain the kinetic parameters (Fig. 1 and Table 1).

Through $SOUR_{NF}$ data of Table 1, it is possible to see a tendency of inhibition of the nitrification process due to increased concentration of substrate S. During the initial tests at NH_3-N low concentrations (Tests 1–5, Table 1), the $SOUR_{NF}$ had ascent behavior, reaching the highest specific oxygen consumption rate in the test 7 ($SOUR_{NF}$ = 4.59 mg O_2/g VSS/min) under an initial NH_3-N concentration of 258.78 mg/L. After this concentration S in subsequent tests, the $SOUR_{NF}$ behavior became downward, reducing the oxygen uptake rate and consequently indicating substrate inhibition.

To find a mathematical model that best describe quantitatively the kinetic behavior of the nitrification process in NF system at different ammonium concentrations, the experimental data were fitted to each model and the most appropriate kinetic model was selected based on statistical criteria. These criteria included the Akaike Information Criterion (AIC) and the R^2 statistic (Fig. 1). Fig. 1A shows experimental data of the specific uptake rate SOUR as a function of substrate concentration in the NF system and the fitted curves of the four models studied with corresponding R^2, and Fig. 1B shows the corresponding AIC analysis results for the four models. These results show that among the four kinetic models, the best for describing nitrification inhibition by ammonium in the NF system was the Andrews model that incorporates nitrification inhibition by ammonium. This model had the lower value of AIC (−6.69, Fig. 1B) and the highest coefficient of determination R^2 (0.989, Fig. 1A).

3.1.1. Kinetic characteristics of NH_3 conversion in NF system

The data comparison after non-linear regression made possible to estimate the kinetic parameters of NF system determined by Andrews model ($SOUR_{max}$ = 4.89 mg O_2/g VSS/min). However, the parameter $SOUR_{max}$ for Andrews model is related to maximum substrate consumption in the absence of inhibition. The $SOUR_{max}$ parameter under inhibition is overestimated and needs an adjustment. Therefore, Andrews (1968) proposed Eq. (8), which is the result derived from Eq. (5) equal to zero:

$$SOUR^{\star}_{max} = \frac{\mu_m}{1 + 2\sqrt{\frac{K_S}{K_I}}} \tag{8}$$

where $SOUR^{\star}_{max}$ is the corrected maximum specific oxygen uptake rate (mg O_2/g VSS/min).

Thus, the theoretical $SOUR^{\star}_{max}$ was 4.39 mg O_2/g VSS/min for the substrate concentration of 300 mg NH_3-N/L. It agrees with the actual specific oxygen uptake rate of 4.587 mg O_2/g VSS/min with a ratio of actual value to theoretical value of 1.04. These SOUR

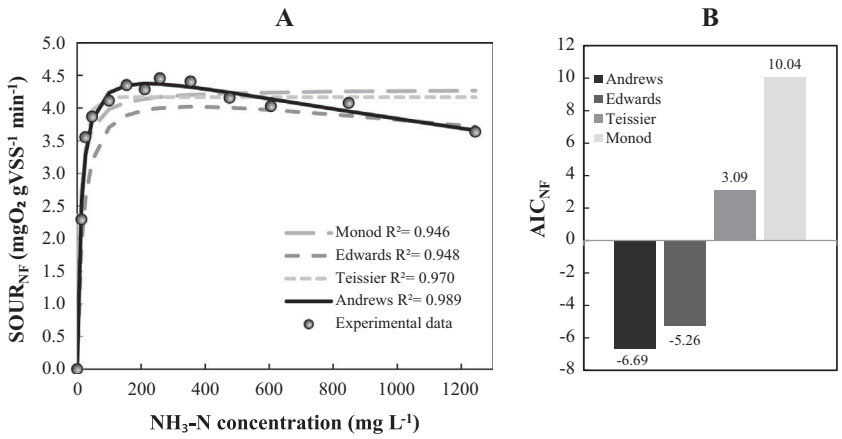

Fig. 1. Non-linear regression analysis for NF system. (A) Fitting of models to the experimental data with R^2; (B) analysis of Akaike Information Criterion (AIC).

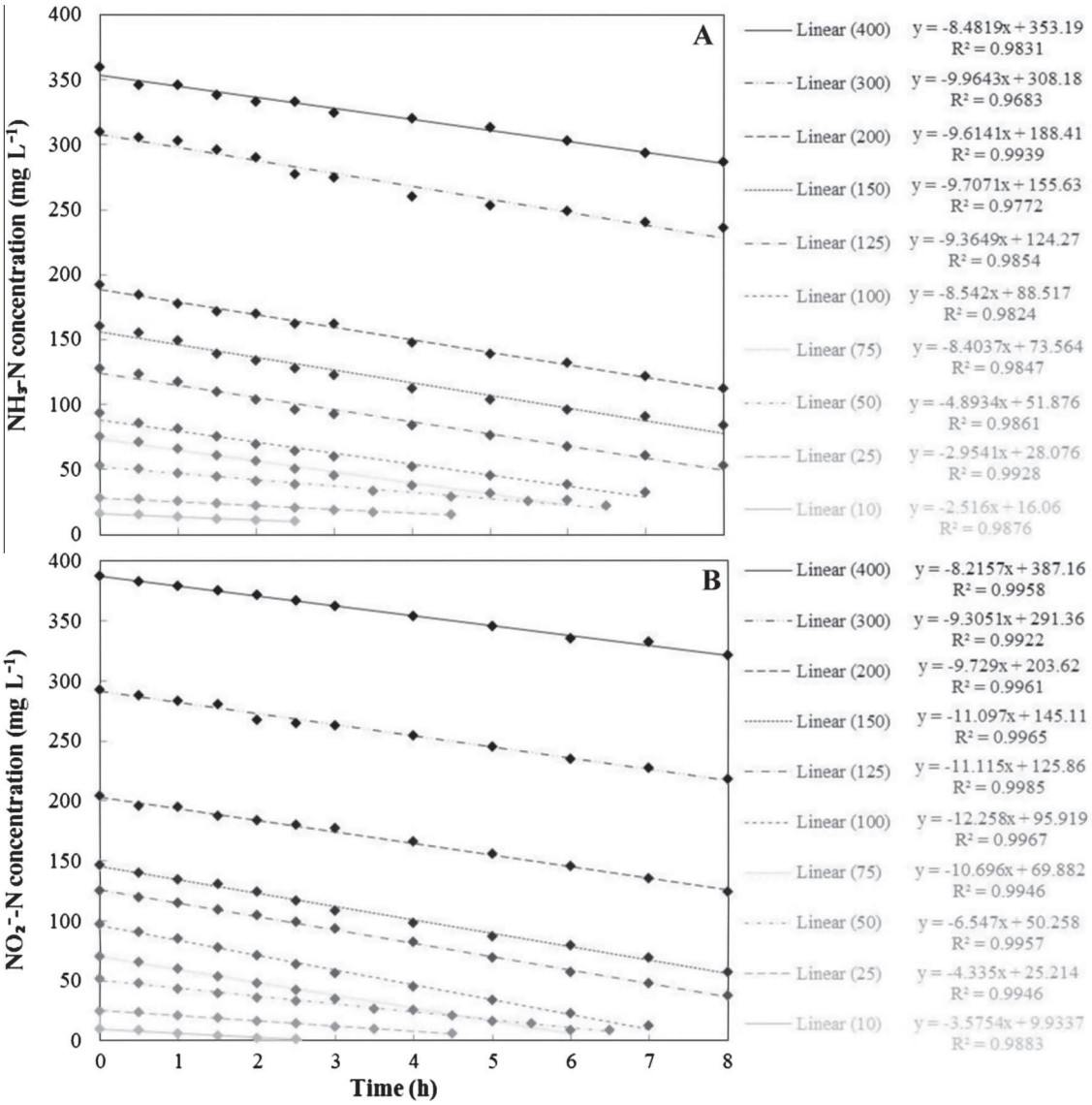

Fig. 2. Lines of substrate conversion rates for different concentrations of Table 1 during the tests in the AMX system. (A) Ammonia substrate; (B) nitrite substrate.

values are above those found in literature that are between 3.8 and 4.4 (Carrera et al., 2004; Jubany et al., 2005). This information suggest that the ammonia removal ability of the tested NF system was fully utilized. K_S and K_I values were respectively, 12.38 and 3867.12 mg N-NH_3/L. These results allow us to conclude that despite the slight drop in specific rate, the nitrifying biomass

studied presents high resistance to inhibition when subjected to high substrate concentrations. This enhances its ability to the treatment of concentrated nitrogen effluent such as animal wastewater biodigestor digestate, the fertilizer industry effluent and other effluents with low C/N ratio and high ammonia concentration. More importantly, we were able to establish a benchmark kinetics of our nitrifying biomass operating alone so as to better understand it behavior when combined with ANAMMOX in the DMX system.

3.2. ANAMMOX inhibition by ammonium and nitrite in AMX system

Since 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. Table 1 shows the values of substrate conversion rates (r_{AMX}) for ammonia and nitrite and their corresponding specific substrate conversion rates (q_{AMX}) obtained in the kinetic tests for AMX system. These r_{AMX} values were obtained from the slopes of the regression lines of substrate consumption obtained in each test performed as shown in Fig. 2.

The peak specific consumption rate occurred at higher substrate concentration for ammonia than for nitrite. For example, the highest specific consumption rate of ammonia found during the tests was 5.80 mg NH_3-N/g VSS/h (Test 9, Table 1) at an S_{AMX} concentration of 309.76 mg NH_3-N/L. On the other hand, the highest nitrite specific consumption rate was 7.13 mg NO_2^--N/g VSS/L (Test 5, Table 1) at a concentration of 97.31 mg NO_2^--N/L. Fig. 2, shows that the curves slopes generally increase with increasing substrate concentration, but this is less evident for substrate ammonia (Fig. 2A) than for substrate nitrite (Fig. 2B). To check a possible inhibition by substrate, the data were submitted to a nonlinear statistical analysis to adjust the best kinetic model to represent the behavior of the ANAMMOX biomass in the AMX system.

Fig. 3 shows the non-linear regression analysis for AMX system including the fitting of models to the experimental data with R^2 and analysis AIC for both substrates: ammonia (Fig. 3A and C) and nitrite (Fig. 3B and D). The Edwards kinetic model was the best fit to the experimental data for both NH_3-N and NO_2^--N, consistently obtaining the lower value of AIC and highest R^2 (0.962 and 0.919, respectively). The second model that best fitted both the ammonia and nitrite data was the Andrews model (Fig. 3). This is not surprising because both considered the inhibition constant K_I, which in this case represents the inhibitory effect of substrate concentration on biomass when exposed to high concentrations.

3.2.1. Kinetic characteristics of NH_3 and NO_2^- conversion in AMX system

For the AMX system, the Edwards model found a maximum specific theoretical conversion rate "q_{max}" of 9.971 mg NH_3-N/g VSS/h for ammonia removal and 9.573 mg NO_2^--N/g VSS/h for nitrite removal, both obtained at 25 °C ± 0.1. Despite extensive research on the microbiology and biochemistry of the ANAMMOX process, the information on the kinetics of this process is still highly variable in the literature. At 35 °C, the theoretical maximum specific theoretical ammonium conversion rate (q_{max}) reported was 12.38–15.88 mg N/g VSS/h and the maximum specific nitrite conversion rate in the range of 8.4–12,7 mg N/g VSS/h (Chen et al., 2011; Dapena-Mora et al., 2007). Malovanyy et al. (2012) reported that specific activity of ANAMMOX bacteria consumption increases on average from 8.7% to 12.5% for every 1 °C increase in process temperature. In this case, the estimated value using the minimum percentage (8.7%) would result in a much higher value of specific ammonium and nitrite conversion rate, above 20 mg N/g VSS/h for both substrates. Based on this, the AMX system showed a very positive q_{max} value for the ANAMMOX process maintained at 25 °C. The actual specific ammonium conversion rate q_{AMX} was 5.80 mg N/g VSS/h (Table 1, Test 9) and the ratio of actual value to theoretical

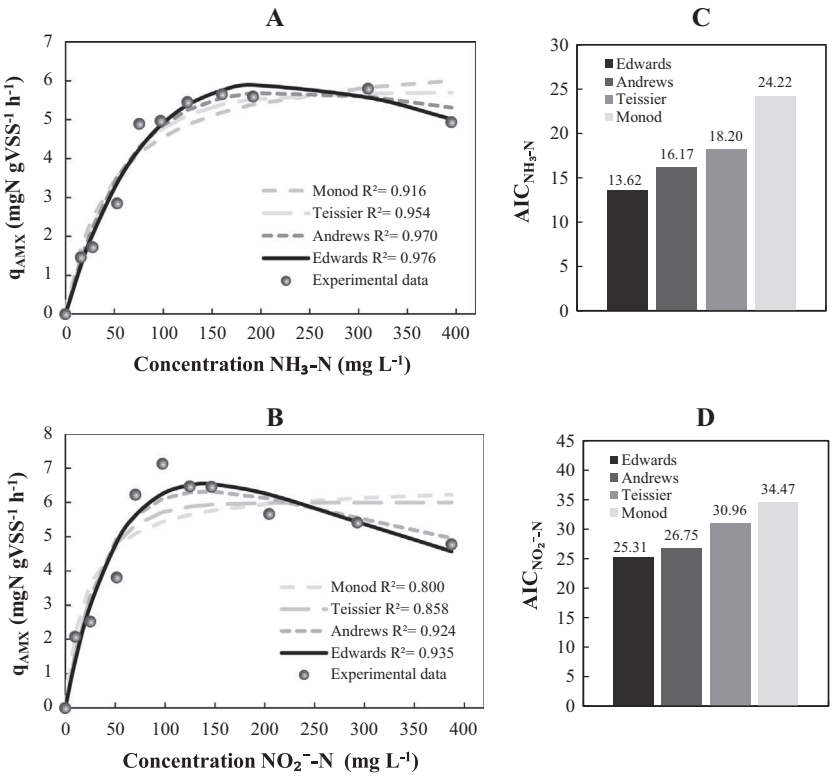

Fig. 3. Non-linear regression analysis for AMX system. (A) and (B) Fitting of models to the experimental data with R^2; (C) and (D) Analysis of Akaike Information Criterion (AIC).

value was 0.582, which indicates that the tested ANAMMOX bacteria sludge might have a higher conversion rate. Already the actual specific nitrite conversion rate q_{AMX} was 7.14 mg N/g VSS/h (Table 1, Test 5) with the ratio of actual value to theoretical value of 0.746. Such information suggests that the nitrite removal ability of the ANAMMOX bacteria was almost totally utilized.

For the substrate affinity K_S, the values obtained at 25 °C were 96.4 mg/L for ammonia and 56.4 mg/L for nitrite, while the corresponding literature values for 35 °C were 25–36 mg/L and 0.66–21 mg/L, respectively (Chen et al., 2011; Tang et al., 2013). Strous et al. (1999) reported that the half saturation constants of the ANAMMOX bacteria to ammonia and nitrite were equal to or less than 0.1 mg/L for aggregate diameters (80%) smaller than 50 μm. In this study, the diameter of the aggregates was bigger than 1 mm, 0.1–0.6 cm. As a result, the resistance to mass transfer in this study was much stronger and the substrate affinity constants were greater than those reported by Tang et al. (2013), Chen et al. (2011) and Strous et al. (1999). In addition to temperature and diameter, other factors can contribute to the difference between the kinetic characteristics among studies: the reactor operating conditions, the species of bacteria used in each experiment, and the possibility of inactivating a part of the microorganism population because of the limited mass transfer in the ANAMMOX granules.

The substrate inhibition constant K_I was 603.53 mg NH_3-N/L for ammonia and 525.34 mg NO_2^--N/L for nitrite (Edwards model, Fig. 3). This indicates that the inhibitory effect of substrate concentrations was more evident and rapid for NO_2^--N than NH_3-N, i.e. while the NO_2^- activity began to decrease around 100 mg NO_2^--N/L (Fig. 3B), the NH_3 activity decreased only nearby to 800 mg NH_3-N/L (Fig. 3A). The inhibitory effect of substrate in the ANAMMOX bacteria is not new and has been studied by other researchers. Similar to this study, ammonium has been found to cause low inhibition even at high concentrations (Dapena-Mora et al., 2007; Jin et al., 2012). On the other hand, different levels of ANAMMOX inhibition by nitrite have been reported in batch and continuous reactors, with concentrations around 100 mg N/L as in this study (Bettazzi et al., 2010; Jetten et al., 2005; Strous et al., 1999) or increased tolerance around 300 mg N/L (Carvajal-Arroyo et al., 2014; Lotti et al., 2014; Magrí et al., 2012).

3.3. Performance of deammonification inhibition by substrate

Fig. 4 shows the values of ammonia conversion rates (r_{DMX}) for each level of initial substrate concentration S_{DMX} used in the kinetic tests for DMX system. Eleven levels of initial concentrations of NH_3 were used in the range of 80–1060 mg N/L. As done previously for the AMX system, the specific substrate conversion rates (q_{DMX}, Table 1) for each batch test in the DMX system were subsequently fit to the various models to obtain the kinetic parameters.

Fig. 5A shows the experimental data in the DMX system and the curves of the specific conversion rate as a function of substrate concentration NH_3-N and R^2 fit of the models studied, and Fig. 5B shows the AIC analysis results. These results show that the best kinetic model for describing deammonification inhibition by ammonium in the DMX system was the Monod model, because it had the lowest value of AIC (9.40, Fig. 5B), and a high coefficient of determination of R^2 = 0.978 (Fig. 5A). The comparison of data after statistical analysis made possible to estimate the kinetic parameters of DMX system, determined by Monod model.

3.3.1. Kinetic characteristics of nitrogen conversion in DMX system

The theoretical maximum specific nitrogen conversion rate in the DMX system was 7.513 mg NH_3-N/g VSS/h. The actual nitrogen specific conversion rate was 6.24 mg NH_3-N/g VSS/h obtained at >600 mg NH_3-N/L substrate concentration (Test 9, Table 1) with actual value to theoretical value ratio of 0.83, which indicated that the tested DMX system still had potential deammonification activity. There is no doubt that q_{max} is the most critical parameter in modeling and nitrogen removal system projects, as it plays a dominant role in loading rate and treatment efficiency. The values obtained by DMX system were very good for reactor operation at 25 °C which favors the scale-up process by saving the coupled power consumption.

The constant of substrate affinity K_S in the DMX system was 175.48 mg N/L and the absence of inhibition constant K_I associated with the values provided by the Monod model (Fig. 5A and B), demonstrated that increased substrate concentration was not limiting for the ammonia conversion rate. This results indicate that, unlike the AMX system, the DMX system displays a large capacity

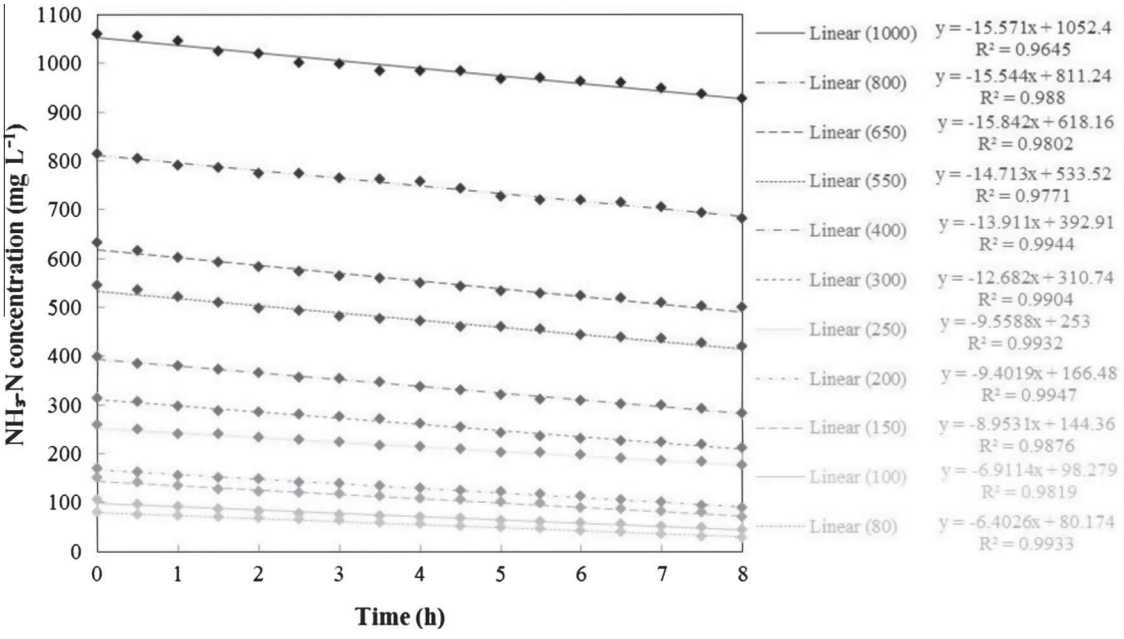

Fig. 4. Lines of substrate ammonia conversion rates for different concentrations of Table 1 during the tests in the DMX system.

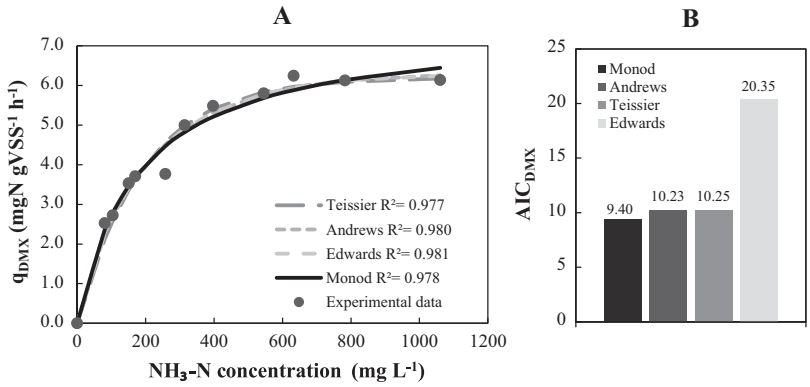

Fig. 5. Non-linear regression analysis for DMX system. (A) Fitting of models to the experimental data; (B) Analysis of Akaike Information Criterion.

to withstand high removal loads without inhibiting activity of the bacteria. This may seem strange since the deammonification process depends on the ANAMMOX activity to be effective. However, Zhu et al. (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 operate in an DMX system than found themselves in enriched mixed cultures. This is because deammonification reactors with suspended biomass tend to eliminate nitrogen by forming granules that on the outside are ammonia oxidizing bacteria (AOB) and on the inside are ANAMMOX bacteria (Chang et al., 2013). Thus, the ANAMMOX bacteria would not be totally exposed to substrate concentrations, existing a concentration gradient in the aggregate which makes them more tolerant to inhibition than if they were alone. Theoretically, substrate concentrations will first reach the surface of the aggregate where the AOB exists to carry out the partial nitritation and secondly, with a substrate concentration gradient, nitrite formed and residual ammonia will be converted to N_2 by the ANAMMOX activity.

The low specific ANAMMOX growth rate, as well as inhibition by NO_2^-, have historically been reported as major challenges of ANAMMOX process operation, however, the influence of each in system efficiency differs widely in the literature. The results obtained in this study by the kinetic characteristics and modeling of experimental data lead us to believe that the ANAMMOX bacteria can tolerate concentrations as high as 400 mg NO_2^--N/L in the short-term, but keeping concentrations above 100 mg NO_2^--N/L will probably cause a gradual decline in a long-term activity. Although the NO_2^- concentration proved to be a limiting factor for the specific nitrogen conversion rate in the AMX system, in the DMX system no accumulation of this substrate was observed, as well as little decrease in specific conversion nitrogen rate, indicating that the *S* concentrations were not limiting to the combined DMX process during the kinetic tests performed.

Finally, from an operational standpoint, the results provided by the kinetic models allow practitioners to better recognize and control the substrate inhibition in DMX and AMX systems, considering that these substrate concentrations are very important for the stability of the process, since they can both favor or inhibit activity of the bacteria involved. Knowing and controlling the conditions that favor the activity of the bacteria involved are a first step to development of economical nutrient removal systems based on DMX in the near future. In this regard, the results obtained in this study provide critical information toward achieving this goal.

4. Conclusions

The NF system showed inhibition by ammonium and the Andrews model was the best to describe this process. The AMX system also showed inhibition by ammonium but stronger by nitrite and the Edwards model was the best to describe it. Surprisingly for the DMX system, the increased substrate concentration (until 1060 mg NH_3-N/L) was not limiting for the ammonia consumption rate and the Monod model was the best model to describe this process. The AOB and ANAMMOX sludges combined in the DMX system displayed a better activity, substrate affinity and substrate tolerance compared to themselves in enriched mixed cultures.

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