

Kinetics and thermodynamics of anaerobic ammonium oxidation process using *Brocadia* spp. dominated mixed cultures

D. Puyol, J. M. Carvajal-Arroyo, B. Garcia, R. Sierra-Alvarez and J. A. Field

ABSTRACT

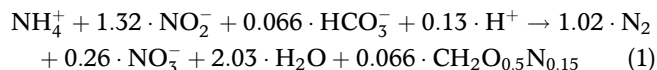
Anaerobic ammonium oxidation (anammox) is a recently discovered microbial process commonly applied to treat ammonium pollution in effluents with low organic carbon content. Modeling anammox processes is important for simulating and controlling full-scale plants. In this study, the anammox process was simulated using three models, and substrate and growth parameters obtained by different research groups. Two *Brocadia* spp.-dominated mixed cultures, one granular and the other flocculent, were used for this purpose. A very good correlation between experimental data using both sludges and model predictions was achieved by one of the models, obtaining correlation coefficients higher than 0.997. Other models and stoichiometric equations tested were unable to predict the anammox kinetics and stoichiometry. Furthermore, the thermodynamic behavior of the two mixed cultures was compared through the determination of the energy of activation of the anammox conversion at temperatures ranging from 9 to 40 °C. Optimum temperature for anammox activity was established at 30–35 °C in both cases. The energy of activation values calculated for granular sludge and flocculent sludge were 64 and 124 kJ mol⁻¹, respectively.

Key words | anammox, *Brocadia* spp., kinetics, modeling, simulation, thermodynamics

D. Puyol (corresponding author)
 J. M. Carvajal-Arroyo
 B. Garcia
 R. Sierra-Alvarez
 J. A. Field
 Department of Chemical and Environmental
 Engineering,
 The University of Arizona,
 Tucson, AZ 85721,
 USA
 E-mail: danielpuyol@email.arizona.edu;
 daniel.puyol@uam.es

INTRODUCTION

Anammox (anaerobic ammonium oxidation) is a recently discovered microbial process involved in the nitrogen biogeochemical cycle (Schmidt *et al.* 2003). Anammox has interesting engineering applications and allows efficient and economic removal of nutrient nitrogen from wastewaters with high NH₄⁺ concentration and relatively low chemical oxygen demand (COD)/ammonium ratio (e.g. landfill leachate and sewage sludge centrate) (Kuenen 2008). The anammox process involves the anaerobic oxidation of ammonium (NH₄⁺) using nitrite (NO₂⁻) as a terminal electron acceptor. This reaction results in the formation of dinitrogen gas. Some nitrate (NO₃⁻) is also produced from partial oxidation of nitrite for carbon fixation from inorganic carbon dioxide. The stoichiometry has been calculated as (Strous *et al.* 1998)



Anammox bacteria are slow-growing microorganisms with a complex cell biology comprising an intracellular organelle surrounded by ladderane lipids (anammoxosome) which have to deal with an inert substrate (ammonium) under highly competitive and anoxic conditions (Kartal *et al.* 2012). Anammox activity has been observed at temperatures ranging from 10 to 45 °C, with an optimum at 30–35 °C. The energy of activation (E_a) of the anammox reaction has been reported to be around 60 kJ mol⁻¹ (Strous *et al.* 1999; Dosta *et al.* 2008).

To improve the understanding and the modeling of the anammox process there is a need to have reliable estimates of kinetic parameters, which enable an increased predictability and better design of the biological process, facilitating its scale-up (Pavlostathis 2011). Modeling of the anammox process has received increasing attention in the last decade. Early approaches mainly consisted of simple Monod models that were used to estimate the maximum specific

doi: 10.2166/wst.2014.074

growth rate (μ_{\max}) and the half-saturation constants for the substrates of the anammox reaction ($K_{S_{\text{NH}_4^+}}$ and $K_{S_{\text{NO}_2^-}}$) (Strous et al. 1998; van der Star et al. 2008; Ni et al. 2010). However, multi-substrate biological processes are complex, so simulation of the anammox reaction has been previously described by double Monod equations, which are adaptations of the general IWA activated sludge models (Dapena-Mora et al. 2004; Volcke et al. 2006; Cema et al. 2012). Recently, an improved model considering all the nitrogen species in the parameters' estimation has been proposed (Puyol et al. 2013). This model was calibrated and validated with very strong statistical and stoichiometric support.

In this work, a multi-substrate solution approach to an anammox kinetic model was utilized to simulate the anammox process. The approach was tested on substrate consumption data obtained with two mixed cultures dominated by *Brocadia* spp. A thermodynamic study was also conducted to investigate the effect of temperature from 9 to 40 °C on the anammox activity of these cultures and calculate the E_a of the anammox reaction according to the Arrhenius equation.

MATERIALS AND METHODS

The anammox granular sludge (GS) and the flocculent sludge (FS) were collected from an expanded granular sludge bed reactor (EGSB) and a membrane bioreactor (MBR), respectively. The EGSB reactor was operated in continuous mode for 250 d treating a synthetic wastewater composed by a stoichiometric relationship between NH_4^+ and NO_2^- (1:1.32). The N-loading rate of the reactor was 3.36 g N L⁻¹ d⁻¹, and the sludge retention time (SRT) was around 145 d. The original inoculum was granular biomass from a full-scale anammox plant treating sludge centrate in The Netherlands, which was provided by Paques BV. The MBR was operated for 360 d treating the same synthetic wastewater as the EGSB reactor, but the N-loading rate was 0.35 g N L⁻¹ d⁻¹ and the SRT was 78 d. The original inoculum was an anammox enrichment culture derived from return activated sludge (Sun et al. 2011) originally obtained from Ina Road treatment plant in Tucson, AZ (USA). Both reactors were located in a temperature-controlled room set at 30 ± 2 °C and the influents were always prepared and maintained in anaerobic conditions.

The average measured particulate size was 2.4 ± 0.6 and 0.22 ± 0.18 mm for GS and FS, respectively. The microbial diversity in both GS and FS was characterized by generating a clone library (Sun et al. 2011). Two unique anammox species

were detected, the GS with *Candidatus B. fulgida* and the FS with *Candidatus B. carolinensis*.

Activity kinetics were studied in triplicate batch assays using 1.62 g volatile solids (VS) L⁻¹ and 0.36 g volatile suspended solids (VSS) L⁻¹ of the GS and FS, respectively. NO_2^- and NH_4^+ were spiked at 3.93 and 2.71 mM, respectively. Experimental values used in the thermodynamic study were obtained in triplicate batch assays inoculated with GS (0.36 g VS L⁻¹) and FS (0.77 g VSS L⁻¹), and then spiked with NO_2^- (3.57 mM) and NH_4^+ (2.71 mM). Batch assays were performed as previously described (Sun et al. 2011).

VS and VSS content were determined according to *Standard Methods* (APHA 2005). Biomass particle size was measured by means of the software ImageJ using five representative pictures of GS and FS (Puyol et al. 2013). The N₂ content in gas samples was measured by gas chromatography with thermal conductivity detection, NO_2^- and NO_3^- using ionic chromatography with suppressed conductivity detection, and NH_4^+ using an ammonium selective electrode (Sun et al. 2011).

Double Monod kinetics was used to describe the dependency of the growth rate of the anammox bacteria on NH_4^+ and NO_2^- concentrations as follows:

$$r = \mu_{\max} \frac{[\text{NH}_4^+]}{K_{S_{\text{NH}_4^+}} + [\text{NH}_4^+]} \frac{[\text{NO}_2^-]}{K_{S_{\text{NO}_2^-}} + [\text{NO}_2^-]} X \quad (2)$$

where μ_{\max} is the anammox specific growth rate (h⁻¹), $K_{S_{\text{NH}_4^+}}$ and $K_{S_{\text{NO}_2^-}}$ are the saturation constants for NH_4^+ and NO_2^- (mM) and X is the biomass concentration (mM C-biomass). Simulations were conducted using the model parameters and stoichiometry determined by Puyol et al. (2013), and the model parameters of Ni et al. (2009) and Oshiki et al. (2011) using the stoichiometry proposed by Strous et al. (1998). The stoichiometric and kinetic matrixes, as well as model parameters, are presented in Table 1. The experimental data were compared with the simulated data and the statistics were provided according to Puyol et al. (2012). The biomass stoichiometry used was $\text{CH}_2\text{O}_{0.5}\text{N}_{0.15}$ (Strous et al. 1998). Both simulation and statistical calculations were performed using MicroMath Scientist 3.0 (<http://www.micromath.com/>).

The E_a was calculated by estimating the maximum specific growth rate (μ_{\max}) of the anammox bacteria by using the empirical growth parameters determined by Puyol et al. (2013). The natural logarithm of μ_{\max} was compared with the inverse of the temperature (1,000/K), and the linear regression parameters were used for calculating

Table 1 | Stoichiometric and kinetic matrix of the models used for the simulation, parameter values and goodness of fitting of every simulation model to the experimental data. Kinetic equation for the simulation is described in Equation (2)

Process ^a	Components					Parameters					Reference
	S _{NH4+}	S _{NO2-}	S _{NO3-}	S _{N2}	X	K _S NH4+	K _S NO2-	Y _{X/S}	μ _{max}	R ²	
X-GS	-1/Y _{X/S}	-1.278/	0.353/	0.944/	1	0.64	0.35	0.105	0.0017	0.997	Puyol et al. (2013) ^b
X-FS	-i _{NBM}	Y _{X/S}	Y _{X/S}	Y _{X/S}		0.53	0.37		0.0034	0.999	
X-GS	-1/Y _{X/S}	-1/Y _{X/S}	1/	1.02/	1	0.05	0.04	0.06	0.0006	0.987	Ni et al. (2009) ^c
X-FS	-i _{NBM}	-1/0.258	0.258	Y _{X/S}					0.0013	0.992	
X-GS	-1/Y _{X/S}	-1.32/	0.256/	1.02/	1	0.03	0.09	0.034	0.0003	0.943	Oshiki et al. (2011) ^c
X-FS	-i _{NBM}	Y _{X/S}	Y _{X/S}	Y _{X/S}					0.0007	0.974	

i_{NBM} (N content of biomass) = 0.15 mmol N mmol⁻¹ C. S and K_S are in mM.

Y_{X/S} is in mmol C mmol⁻¹ NH₄⁺, μ_{max} is in h⁻¹.

^aX-GS = growth of X using GS. X-FS = growth of X using FS.

^bAccording to the stoichiometry by Puyol et al. (2013).

^cAccording to the stoichiometry by Strous et al. (1998).

the E_a values for both GS and FS as follows:

$$\frac{d[N_2]}{dt} = \frac{\mu_{\max}}{Y_{X/S}} X \frac{([N_{2\max}] - [N_2])}{K_{S_{N_2}} + ([N_{2\max}] - [N_2])} \quad (3)$$

$$\mu_{\max} = A \cdot e^{-E_a/R \cdot T} \Rightarrow \ln(\mu_{\max}) = \ln A - \frac{E_a}{R \cdot T} \quad (4)$$

where $Y_{X/S}$ is the biomass yield (mmol C-biomass mmol⁻¹ NH₄⁺ produced), $K_{S_{N_2}}$, $[N_2]$ and $N_{2\max}$ are the pseudo-Monod constant, the nitrogen produced and the maximum nitrogen production for an infinite time of reaction, respectively (mM N₂), E_a is the energy of activation (kJ mmol⁻¹ N₂ produced), A is the pre-exponential factor (h⁻¹), T is the temperature (K), and R is the universal gas constant (8.314 × 10⁻⁶ kJ mmol⁻¹ N₂ K⁻¹).

RESULTS AND DISCUSSION

Kinetics of the anammox reaction

Figure 1 shows the time evolution of the nitrogen species involved in the anammox process using both GS and FS. Ammonium and nitrite were consumed in both cases, with concomitant production of nitrogen and nitrate. Specific anammox activities (SAA) of 0.414 ± 0.004 and 0.848 ± 0.021 mmol N₂ g⁻¹ VSS h⁻¹ were calculated for GS and FS, respectively. The higher SAA of the FS can be related to the growth conditions of both sludges, since a higher specific loading rate was maintained in the MBR than in the EGSB (1.33 versus 0.60 mmol N₂ g⁻¹ VSS h⁻¹, respectively). The SAA values determined are comparable to those reported by other authors for sludge from high-rate

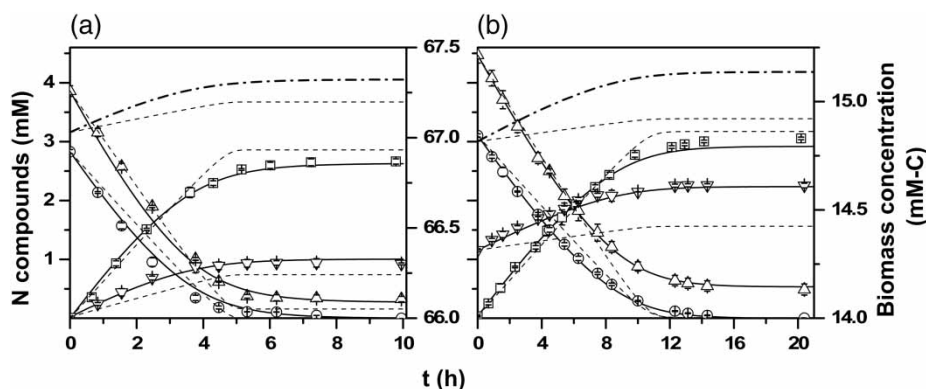


Figure 1 | Time course of N₂ (□), NH₄⁺ (○), NO₂ (Δ) and NO₃⁻ (▽) in the kinetic experiments using granular (a) and flocculent (b) sludge. Continuous lines and dash-dot lines are simulations of the N-compounds and biomass growth, respectively, obtained using the model described by Puyol et al. (2013). Dot lines are simulations obtained using the Oshiki et al. (2011) (a) and Ni et al. (2009) (b) models. Error bars represent standard deviations from triplicate experiments.

anammox reactors (from 0.42 to 0.94 mmol N₂ g⁻¹ VSS h⁻¹) (Chen et al. 2011; Ni et al. 2012).

The molar ratio between the NO₂⁻ and NH₄⁺ uptakes were very similar in the assays with GS and FS (1.25 ± 0.04 versus 1.28 ± 0.00), suggesting the same stoichiometry for the anammox reaction.

Simulation of the anammox reaction

Simulations of the anammox reaction and biomass growth were performed according to models described by Ni et al. (2009), Oshiki et al. (2011) and Puyol et al. (2013). Table 1 lists the substrate and growth parameters as well as the goodness of fitting through the correlation coefficient (R^2). Simulation curves for the consumption of nitrate and ammonium and for the production of N₂, nitrate and biomass by the GS and FS cultures are depicted in Figure 1. As can be seen, the model described by Puyol et al. (2013) accurately predicted the conversion of N species by both mixed cultures, GS and FS, and simulated the corresponding biomass growth. The two literature models were unable to describe properly the anammox reaction under the experimental conditions used in his study, as can be seen in Figure 1. Main differences are related to the stoichiometry of the nitrate production, since the Strous equation (Strous et al. 1998) predicts a nitrate stoichiometry significantly lower than that predicted by Puyol et al. (2013) (0.256 versus 0.353 mol NO₃⁻ mol⁻¹ NH₄⁺). Statistical analysis was used to further evaluate the accuracy of every model.

A high correlation between the simulation data from the Puyol et al. (2013) model and the experimental values was achieved with both biomass sources ($R^2 = 0.997$ and 0.999 for GS and FS simulation, respectively), whereas simulations of data using the models of Ni et al. (2009) and Oshiki et al. (2011) were somewhat less reliable, which can be deduced by lower values of the correlation coefficients (Table 1).

The main difference between the three simulation models was the values estimated for the substrate saturation constants (Table 1). Generally, model parameters used for calibrating the anammox reaction have been extracted from those calculated by Strous et al. (1999). In all the cases analyzed, calibration of the model parameters led to significantly increased values of the saturation constants of both nitrite and ammonium, compared with the initial parameters. The authors tried to explain those findings as kinetic limitations due to substrate diffusion through biogranules (Dapena-Mora et al. 2004; Volcke et al. 2006; Cema et al. 2012). However, in this work two anammox cultures with very different aggregation state (granular and

flocculent) were used, and the saturation constants determined in both cases are very similar (Table 1). This finding suggests that kinetic limitation by diffusion of substrates seems to be negligible in comparison with the anammox reaction under the present working conditions, as has been previously claimed (Puyol et al. 2013).

In order to further validate the model developed by Puyol et al. (2013), the stoichiometry of the anammox process was evaluated by comparing the measured with the literature values (Table 2). A nitrogen mass balance at the end of the experimentation was performed. N balances predicted by the Strous stoichiometry are also shown. The Strous stoichiometry was not able to predict the behavior of the anammox biomass under the experimental conditions used in this work. Differences in the strategy of growth (K-strategy versus r-strategy) of the anammox cultures used in the different works could explain these divergences (Puyol et al. 2013). In fact, values of μ_{\max} calibrated by using the Ni and Oshiki models, where the Strous stoichiometry was used, seem to be not reliable, since they are much lower than the experimentally calculated by Puyol et al. (2013) ($\mu_{\max} = 0.0041$ h⁻¹), whereas values of μ_{\max} calibrated by using the Puyol et al. model were very close to those experimentally calculated, overall for the FS (see Table 1). The low values of K_s and $Y_{X/S}$ of Ni and Oshiki models had a relevant effect on the low μ_{\max} determined.

Effect of temperature on the anammox reaction: thermodynamics

Analysis of the effect of temperature on anammox kinetics was performed in bioassays with GS and FS, thus mirroring the simulation experiments. The time course of N₂ production at temperatures ranging from 9 to 40 °C is depicted in Figure 2. The highest activities were achieved at 30 °C for both cultures, and a rapid activity decrease was observed at lower and higher temperatures. At the lowest temperature tested (9 °C), the GS was more active than the FS. This observation may be related to the different

Table 2 | Experimental and literature molar stoichiometries of the anammox reaction based on ammonium consumption

Element	Experimental data		Literature stoichiometry Strous et al. (1998)
	GS	FS	
NO ₂ ⁻	1.249 ± 0.038	1.283 ± 0.002	1.32
N ₂	0.942 ± 0.027	0.985 ± 0.006	1.02
NO ₃ ⁻	0.319 ± 0.012	0.354 ± 0.007	0.256

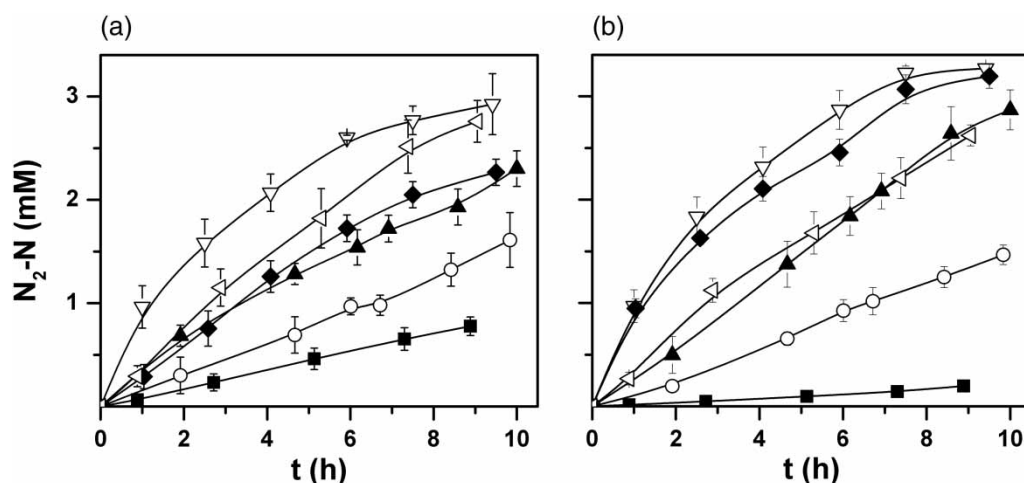


Figure 2 | Time course of the N_2 production in thermodynamics assays conducted at 9 (■), 18 (○), 25 (▲), 30 (▽), 35 (◆), and 40 °C (◄) using GS (a) and FS (b). Error bars are standard deviations from triplicates.

temperature sensitivity of the dominant anammox bacteria in the GS (*Candidatus B. fulgida*) and FS (*Candidatus B. caroliniensis*).

The experimental N_2 production data were fitted to Equation (3). Growth parameters were extracted from Puyol et al. (2013), the $Y_{X/S}$, $[N_{2max}]$ and saturation constant values were fixed in the model calibration, and maximum specific growth rates (μ_{max} , h^{-1}) were calculated. Figure 3(a) shows the effect of the temperature in the μ_{max} . As can be seen, μ_{max} values increased exponentially up to a temperature around 30–35 °C. At higher temperatures, the μ_{max} decreased, which can be explained by deactivation caused by temperature. Other authors have reported similar values for the optimum temperature of the anammox reaction (Strous et al. 1999; Dosta et al. 2008).

Table 3 shows the optimum temperature and the temperature range of the main biological N-removal processes (BNPs). As can be seen, optimal temperature levels range from 20 to 30 °C in all cases, with the exception of the nitrification and anammox processes. The temperature of operation is key for the operation of BNPs and, in particular for hybrid processes in which more than one BNP occurs simultaneously (Van Hulle et al. 2010). In this sense, selection of a suitable working temperature to favor growth of the desired microbial population can be critical to achieve complete N removal in systems such as the CANON process, where nitrification and anammox processes succeed in the same reactor, and the efficiency of the anammox process is strongly dependent on the stability of the nitrification process.

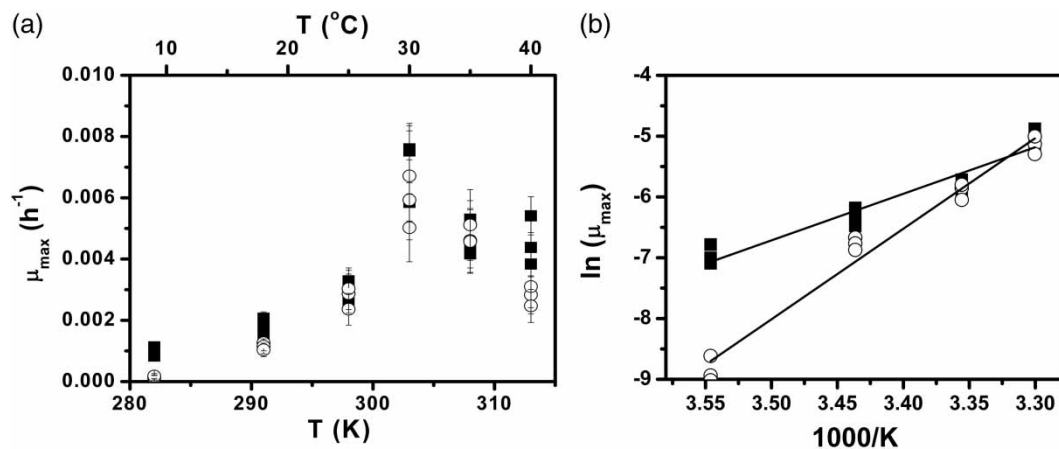


Figure 3 | Effect of temperature on the maximum SAA (a) and Arrhenius plots (b) for GS (■) and FS (○). Error bars represent standard deviations of the μ_{max} values resulted from fitting of every set of data to Equation (2).

Table 3 | Optimum and working ranges of temperatures of the main nitrogen-consuming biological processes

Process	Sub-process	T range ^a (°C)	Optimum T (°C)	Reference
Nitrification	Nitritation	4–50	22–30	Grunditz & Dalhammar (2001)
	Nitratation	4–52	25–35	
Denitrification	Autotrophic	< 15–50	20–25	Koenig & Liu (2001)
	Heterotrophic	(0)–10–35–(75)	20–25	Saleh-Lakha et al. (2009)
Anammox		10–45	30–35	This work; Strous et al. (1999); Dosta et al. (2008)

^aExtreme (psychrophilic and thermophilic) values in brackets.

Values of μ_{\max} in the exponential range (up to 30 °C) were used for estimating E_a values according to Equation (4). Plots of $\ln(\mu_{\max})$ versus $1,000/K$ are presented in Figure 3(b). Estimated values for the E_a by linear regression were different for GS (0.064 ± 0.006 kJ mmol⁻¹, $R^2 = 0.91$) and FS (0.124 ± 0.006 kJ mmol⁻¹, $R^2 = 0.97$). The E_a value determined for GS, unlike that for FS, is comparable with those previously reported by other authors for anammox cultures (Strous et al. 1999; Dosta et al. 2008). The observed differences between GS and FS thermodynamics may be related to the presence of different dominant anammox species in the various cultures.

CONCLUSIONS

Activity and growth kinetics of *Brocadia* spp. anammox cultures were successfully predicted by using a multi-substrate anammox model. Statistical analysis confirmed that the model has a high goodness of fit to the experimental data. Also, the experimentally measured N balances were very close to those predicted by the model, providing an independent validation. The optimum temperature of the two anammox cultures tested was very similar (30–35 °C); however, thermodynamic studies revealed a marked difference in their E_a values, which may be related to the dominance of different *Brocadia* spp. in these anammox cultures.

ACKNOWLEDGEMENTS

This work was partly funded by University of Arizona Water Sustainability Program, the NSF (Project #1234211) and the USDA EGYPT-USA (Project #1851). D. Puyol greatly acknowledges the Spanish Ministry of Education, the Spanish Fulbright Commission and the US Council for

International Exchange of Scholars (CIES) for receiving a post-doctoral Fulbright grant.

REFERENCES

- APHA 2005 *Standard Methods for the Examination of Water and Wastewater* 21st edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.
- Cema, G., Sochacki, A., Kubiawicz, J., Gutwiński, P. & Surmacz-Górska, J. 2012 Start-up, modelling and simulation of the Anammox process in a membrane bioreactor. *Chemical and Process Engineering* **33** (4), 639–650.
- Chen, T., Zheng, P., Tang, C., Wang, S. & Ding, S. 2011 Performance of ANAMMOX-EGSB reactor. *Desalination* **278** (1–3), 281–287.
- Dapena-Mora, A., Van Hulle, S. W. H., Luis Campos, J., Méndez, R., Vanrolleghem, P. A. & Jetten, M. 2004 Enrichment of anammox biomass from municipal activated sludge: experimental and modelling results. *Journal of Chemical Technology & Biotechnology* **79** (12), 1421–1428.
- Dosta, J., Fernández, I., Vázquez-Padín, J. R., Mosquera-Corral, A., Campos, J. L., Mata-Álvarez, J. & Méndez, R. 2008 Short- and long-term effects of temperature on the Anammox process. *Journal of Hazardous Materials* **154** (1–3), 688–693.
- Grunditz, C. & Dalhammar, G. 2001 Development of nitrification inhibition assays using pure cultures of *Nitrosomonas* and *Nitrobacter*. *Water Research* **35** (2), 433–440.
- Kartal, B., van Niftrik, L., Keltjens, J. T., Op den Camp, H. J. M. & Jetten, M. S. M. 2012 Anammox – growth physiology, cell biology, and metabolism. In: *Advances in Microbial Physiology* (K. P. Robert, ed.). Academic Press, London, pp. 211–262.
- Koenig, A. & Liu, L. H. 2001 Microbial aspects of autotrophic denitrification of wastewaters. In: *Advances in Water and Wastewater Treatment Technology* (M. Tomonori, H. Keisuke, T. Satoshi & H eds). Elsevier Science, B.V., Amsterdam, pp. 217–226.
- Kuenen, J. G. 2008 Anammox bacteria: from discovery to application. *Nature Reviews Microbiology* **6** (4), 320–326.
- Ni, B. J., Chen, Y. P., Liu, S. Y., Fang, F., Xie, W. M. & Yu, H. Q. 2009 Modeling a granule-based anaerobic ammonium

- oxidizing (Anammox) process. *Biotechnology and Bioengineering* **103** (3), 490–499.
- Ni, S.-Q., Lee, P.-H. & Sung, S. 2010 The kinetics of nitrogen removal and biogas production in an anammox non-woven membrane reactor. *Bioresource Technology* **101** (15), 5767–5773.
- Ni, S.-Q., Sung, S., Yue, Q.-Y. & Gao, B.-Y. 2012 Substrate removal evaluation of granular anammox process in a pilot-scale upflow anaerobic sludge blanket reactor. *Ecological Engineering* **38** (1), 30–36.
- Oshiki, M., Shimokawa, M., Fujii, N., Satoh, H. & Okabe, S. 2011 Physiological characteristics of the anaerobic ammonium-oxidizing bacterium '*Candidatus Brocadia sinica*'. *Microbiology-Sgm* **157**, 1706–1713.
- Pavlostathis, S. G. 2011 6.31 – Kinetics and modeling of anaerobic treatment and biotransformation processes. In: *Comprehensive Biotechnology*, 2nd edn (M.-Y. Murray, ed.). Academic Press, Burlington, pp. 385–397.
- Puyol, D., Sanz, J. L., Rodriguez, J. J. & Mohedano, A. F. 2012 Inhibition of methanogenesis by chlorophenols: a kinetic approach. *New Biotechnology* **30** (1), 51–61.
- Puyol, D., Carvajal-Arroyo, J. M., Garcia, B., Sierra-Alvarez, R. & Field, J. A. 2013 Kinetic characterization of *Brocadia* spp.-dominated anammox cultures. *Bioresource Technology* **139**, 94–100.
- Saleh-Lakha, S., Shannon, K. E., Henderson, S. L., Goyer, C., Trevors, J. T., Zebbarth, B. J. & Burton, D. L. 2009 Effect of pH and temperature on denitrification gene expression and activity in *Pseudomonas mandelii*. *Applied and Environmental Microbiology* **75** (12), 3903–3911.
- Schmidt, I., Sliemers, O., Schmid, M., Bock, E., Fuerst, J., Kuenen, J. G., Jetten, M. S. M. & Strous, M. 2003 New concepts of microbial treatment processes for the nitrogen removal in wastewater. *FEMS Microbiology Reviews* **27** (4), 481–492.
- Strous, M., Heijnen, J. J., Kuenen, J. G. & Jetten, M. S. M. 1998 The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology* **50** (5), 589–596.
- Strous, M., Kuenen, J. G. & Jetten, M. S. M. 1999 Key physiology of anaerobic ammonium oxidation. *Applied and Environmental Microbiology* **65** (7), 3248–3250.
- Sun, W., Banihani, Q., Sierra-Alvarez, R. & Field, J. A. 2011 Stoichiometric and molecular evidence for the enrichment of anaerobic ammonium oxidizing bacteria from wastewater treatment plant sludge samples. *Chemosphere* **84** (9), 1262–1269.
- van der Star, W. R. L., Miclea, A. I., van Dongen, U., Muyzer, G., Picioreanu, C. & van Loosdrecht, M. C. M. 2008 The membrane bioreactor: a novel tool to grow anammox bacteria as free cells. *Biotechnology and Bioengineering* **101** (2), 286–294.
- Van Hulle, S. W. H., Vandeweyer, H. J. P., Meesschaert, B. D., Vanrolleghem, P. A., Dejans, P. & Dumoulin, A. 2010 Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams. *Chemical Engineering Journal* **162** (1), 1–20.
- Volcke, E. I. P., van Loosdrecht, M. C. M. & Vanrolleghem, P. A. 2006 Continuity-based model interfacing for plant-wide simulation: a general approach. *Water Research* **40** (15), 2817–2828.

First received 20 November 2013; accepted in revised form 28 January 2014. Available online 10 February 2014