Bioresource Technology 139 (2013) 94–100

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Kinetic characterization of *Brocadia* spp.-dominated anammox cultures

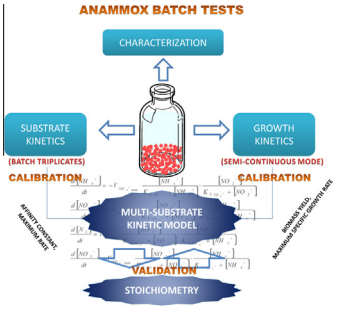
D. Puyol *, J.M. Carvajal-Arroyo, B. Garcia, R. Sierra-Alvarez, J.A. Field

Department of Chemical and Environmental Engineering, The University of Arizona, 1133 E. James E. Rogers Way, Harshbarger 108, Tucson, AZ, USA

HIGHLIGHTS

- Two *Brocadia*-dominated anammox enrichment cultures were kinetically analyzed.
- A kinetic model and modeling procedure were applied with high statistical support.
- Model parameters were validated by a very good stoichiometrical support.
- Kinetic conclusions can be extracted and applied for improving anammox applications.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 March 2013
Received in revised form 31 March 2013
Accepted 1 April 2013
Available online 8 April 2013

Keywords:
Anammox
Brocadia sp.
Kinetics
Modeling
Growth

ABSTRACT

In this study, kinetic analyses were conducted for two *Brocadia*-dominated enrichment cultures, granular and flocculent, retrieved from lab-scale anaerobic ammonium oxidation (anammox) reactors. Substrate K_S ranged from 0.35 to 0.69 mM N and V_{Smax} ranged from 0.67 to 0.88 mmol N g^{-1} VSS h^{-1}. The model respected the experimentally measured stoichiometry of N-compounds, serving as an independent validation. Growth kinetics of the flocculent sludge was also studied, which indicates a μ_{max} of 0.0984 d^{-1} and a $Y_{X/S}$ of 0.105 mol C-biomass mol^{-1} NH_4^+. The flocculent enrichment culture was used to determine the stoichiometric equation. The kinetic parameters of the *Brocadia* spp. cultures measured here can be used for optimizing applications of the anammox process.

1. Introduction

Recent advances in the understanding of the microbiology of the nitrogen cycle have opened the door to new possibilities for treating high-nitrogen bearing wastewater. Among them, the anaerobic ammonium oxidation (anammox) is the most promising to become a wide-spread technological option for high-ammonia and low-organic matter waste streams (Kuenen, 2008; Kartal et al., 2010). The anammox process is catalyzed by chemolitho-autotrophic bacteria of the phylum planctomycetes. Several genera have been identified being that are able to perform this process: *Brocadia*, *Kuenenia*, *Scalindua*, *Anammoxoglobus* and *Jetenia* (Kuenen, 2008). This mesophilic process involves the anoxic oxidation of ammonia with nitrite as the main electron acceptor in a thermodynamically favorable fashion ($\Delta G^{0\prime} = -357$ kJ mol^{-1} NH_4^+) (Jetten et al., 2001). The experimentally calculated stoichiometry from chemostat experiments has been established as follows (Strous et al., 1998):

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.256NO_3^- + 2.03H_2O + 0.066CH_2O_{0.5}N_{0.15} \quad (1)$$

Aside from functioning as electron acceptor, nitrite also functions as an electron donor for CO_2 fixation to support biomass growth (Kuenen, 2008). Consequently there is some formation of NO_3^- and a molar relationship of NO_2^- to NH_4^+-consumption greater than 1.

Modeling biological processes is critical for the proper control of full-scale plants. A multi-variable control system is often used

* Corresponding author. Tel.: +1 520 626 2896.
E-mail addresses: daniel.puyol@uam.es, danielpuyol@email.arizona.edu (D. Puyol).

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http://dx.doi.org/10.1016/j.biortech.2013.04.001

(such as supervisory control and data acquisition-SCADA-type), in which all the biological and abiotic processes are governed by differential–algebraic equations with dynamic state variables (Olsson, 2012). In anaerobic conditions, biological processes are commonly rate-limiting, so there is a great need to elucidate the kinetics of the process in order to optimize the subsequent step of modeling and control (Pavlostathis, 2011). The International Water Association (IWA) has developed different simulation models for activated sludge (Activated Sludge Models, ASM). In these models, all the biological processes are derived from Monod kinetics (Henze et al., 2000). These models include the nitrogen removal steps (Zhou et al., 2013), so they have been recently extended to cover the anammox process (Dapena-Mora et al., 2004).

There is a need to have reliable estimates of kinetic parameters that can improve the understanding and the modeling of the anammox process, which enable an increased predictability and better design of the biological process, facilitating its scale-up. Currently there is no fully developed and consensual kinetic anammox model. Early approaches consisted of simple models used for estimating maximum growth rate and affinity constants. Substrate anammox kinetics has been often explained by Monod (Strous et al., 1999; van der Star et al., 2008; Chen et al., 2011b; Oshiki et al., 2011; Ni et al., 2012), Haldane-type (Chen et al., 2011a) or even pseudo-first order models (Strous et al., 1998). An enormous variability in the values of the saturation constants (0.003–13.7 mM) and maximum specific activities (0.09–3.74 mmol N g^{-1} VSS h^{-1}), combined with the lack of a common anammox modeling approach, makes the modeling a major concern point for scaling-up. Moreover, multi-substrate biological processes are complex, requiring a more comprehensive approach to enable more accurate estimates of the kinetic parameters. A previous study recognized the necessity of improving the estimation of parameters for anammox kinetics to optimize the simulation of substrate consumption and biomass growth (Dapena-Mora et al., 2004).

The objective of this study is to apply a multi-substrate modeling approach towards fitting and calculating the kinetic parameters of the anammox process using measurements from *Brocadia* spp. dominated anammox enrichment cultures. These cultures were grown under batch conditions favoring either the measurement of activity or growth. The model is calibrated with experimental data and validated by comparing the experimentally measured with the model-predicted stoichiometries.

2. Methods

2.1. Anammox biomass

Biomass used in this work was collected from two sources: a lab-scale expanded granular sludge bed (EGSB) reactor operated in continuous mode for 250 d (granular sludge, GS), and a lab-scale membrane bioreactor (MBR) with a culture enriched in anammox biomass for 360 d (flocculent sludge, FS). The GS was retrieved from the reactor while it was 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 sludge, consisted of red and medium size (2.4 ± 0.6 mm) granular sludge, had a specific anammox activity (SAA) of 0.424 ± 0.014 mmol N_2 g^{-1} volatile suspended solids (VSS) h^{-1}, a volatile to total solids (VS/TS) ratio of 0.89 ± 0.11 and a VS concentration of 0.043 ± 0.009 g g^{-1} wet sludge. The original inoculum was granular biomass from a full a full-scale anammox plant treating sludge centrate in The Netherlands, which was provided by Paques BV, The Netherlands. The FS was retrieved from the reactor treating the same synthetic wastewater than the EGSB reactor, but the N-loading rate was 0.35 g N L^{-1} d^{-1} and the SRT was 78 d. The flocculent sludge was visually well dispersed with an average flocs size of 0.22 ± 0.18 mm, and the sludge volumetric index (SVI) was around 22 mL g^{-1}, indicating very good settling characteristics. The SAA was 0.464 ± 0.008 mmol N_2 g^{-1} VSS h^{-1}, the total to volatile suspended solids (TSS/VSS) relationship was 0.83 ± 0.01 and the biomass concentration was 765 ± 0.03 mg VSS L^{-1}. 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).

Anammox bacteria in the GS and FS were microbiologically characterized by generating a clone library as described in the Supplementary data. The two different inocula were characterized by different anammox species. One unique anammox phylotype was found in each of biomass types, showing very high similarity with the 16s rRNA gene of the genus *Brocadia* (>99.5%). The anammox strain in the FS was most closely related to *Candidatus Brocadia caroliniensis* (Magrí et al., 2012), whereas the granular sludge was composed mainly by *Candidatus Brocadia fulgida* (Kartal et al., 2008) (see Supplementary data, Fig. S1).

2.2. Basal medium

The basal mineral medium was prepared using ultrapure water (Milli-Q system, Millipore) and contained the following compounds (mg L^{-1}): $NaH_2PO_4{\cdot}H_2O$ (57.5); $CaCl_2{\cdot}2H_2O$ (100); $MgSO_4{\cdot}7H_2O$ (200); and 1.0 mL L^{-1} of two trace element solutions. Trace element solution 1 contained (in mg L^{-1}) $FeSO_4$ (5000) and ethylenediamine-tetraacetic acid (EDTA) (5000). Trace element solution 2 contained (in mg L^{-1}) EDTA (1500); $ZnSO_4{\cdot}7H_2O$ (430); $CoCl_2{\cdot}6H_2O$ (240); $MnCl_2$ (629); $CuSO_4{\cdot}5H_2O$ (250); $Na_2MoO_4{\cdot}2H_2O$ (220); $NiCl_2{\cdot}6H_20$ (190); $Na_2SeO_4{\cdot}10H_2O$ (210); H_3BO_3 (14); $NaWO_4{\cdot}2H_2O$ (50).

Nitrite and ammonium were added to the basal medium using NH_4HCO_3 and $NaNO_2$ at different concentrations. $NaHCO_3$ was added at 4 g L^{-1} to provide alkalinity and carbon source.

2.3. Substrate kinetics experiments

Batch experiments were performed in 160 mL flask serum bottles hermetically closed and inoculated with 1.875 g VSS L^{-1} or 0.383 g VSS L^{-1} of the GS and FS, respectively. The useful volume was set at 100 mL. NO_2^- and NH_4^+ were supplied at two molar relationships (1.2 and 1.54 mol NO_2^- mol^{-1} NH_4^+), corresponding to values 10% lower and higher, respectively, than the 1.32 stoichiometric relationship between NH_4^+ and NO_2^- (Eq. (1)). Total N-concentration was 6.5 mM. Anaerobic conditions were achieved by flushing the media and headspace with He/CO_2 80/20%, which also served to adjust the pH to around 7.2. Temperature was fixed at 30 ± 0.1 °C. A mixing speed of 200 rpm was used in order to minimize the external diffusion contribution. Liquid sampling (1.5 mL) was performed periodically in order to measure the concentration of NO_2^-, NH_4^+ and the pH. The experiments were assayed in triplicate. In all cases, a parallel operated experimental treatment was conducted and used for refilling the triplicates in each liquid sampling in order to maintain the liquid and headspace volumes unchanged, as well as the biomass concentration (refill bottle).

2.4. Growth kinetics experiments

The growth kinetics were studied by inoculating reaction bottles with 2 mL of the FS in the same conditions described in the substrate kinetics experiments but adding successive substrate spikes of 2.71 and 3.57 mM of NH_4^+ and NO_2^-, respectively (semi-continuous mode operation). Each spike was performed once the N_2 production had ended and all the NO_2^- and NH_4^+ were consumed.