Chemosphere 72 (2008) 1687–1693

Contents lists available at ScienceDirect

Chemosphere

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

# Influence of gas flow-induced shear stress on the operation of the Anammox process in a SBR

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ARTICLE INFO

*Article history:*
Received 3 December 2007
Received in revised form 7 May 2008
Accepted 8 May 2008
Available online 20 June 2008

*Keywords:*
Anammox
Granule
Off-gas flow
Power input
Specific Anammox activity

ABSTRACT

The start up and performance of the Anammox process were tested in sequencing batch reactors with two different configurations: a bubble column (SBR-B) and a gas-lift reactor (SBR-G). Different off-gas upflow velocities were tested (3.53–12.3 cm $min^{-1}$) in order to expose the biomass to different shear conditions and to study their effects on both efficiency and physical properties of the Anammox granular biomass. For the SBR-B the minimum gas upflow velocity needed to achieve biomass suspension inside the reactor was 12.3 cm $min^{-1}$. Such velocity made impossible the stable operation of the process. The fluidization of biomass for the SBR-G was reached at a gas upflow velocity of 3.52 cm $min^{-1}$. This system maintained an efficiency of nitrite removal around 98% at values up to 5.29 cm $min^{-1}$ but when the gas upflow velocity was increased from 5.29 to 9.70 cm $min^{-1}$ a significant decrease of the specific Anammox activity of the biomass from 0.35 to 0.05 g N $g^{-1}$ VSS $d^{-1}$ was measured. The system lost 85% of its nitrogen removal efficiency which was not restored in spite of returning the gas upflow velocity to its initial value.



## 1. Introduction

The Anammox process (anaerobic ammonium oxidation) is a promising alternative to the conventional processes of nitrogen removal in wastewaters with a low C/N ratio, but it also presents some drawbacks. Due to the slow growth rate of the Anammox micro-organisms and toxicity of specially nitrite (Strous et al., 1999) reactors with sufficient mixing and efficient biomass retention are required for operation on laboratory scale and full scale (van der Star et al., 2007).

Initially the Anammox process was operated in biofilm reactors, e.g. fixed bed reactor and fluidised bed reactors working in continuous mode (Van de Graaf et al., 1996; Strous et al., 1997). In order to improve the biomass retention and the stability of the process, the sequencing batch reactors (SBR), where mixing is achieved either by mechanical stirring (Strous et al., 1999) or by gas flow stirring, were extensively used in different research works (Sliekers et al., 2003; Dapena-Mora et al., 2004a). In these systems, shear forces generated by both mechanical and gas flow stirring can cause important effects on the biomass activity and structure. They have been found to play an important role on the formation of well settling biofilm particles or granules in aerobic (van Benthum et al., 1996; Gjaltema et al., 1997; Liu and Tay, 2002) and anaerobic/anoxic conditions (Lettinga et al., 1980; Klapwijk et al., 1981; Franco et al., 2006). However, an excessively high shear stress has been found to be responsible for the biomass wash-out and the loss of its activity (Chisti, 2000; Sánchez-Mirón et al., 2003; Arrojo et al., 2006).

From these works it is inferred that to operate the system under appropriated values of shear stress forces is required in order to favour the formation of compact granules, avoiding the loss of the biomass activity.

Since the effects of the mechanical stirring in an Anammox SBR were studied in a previous work (Arrojo et al., 2006), the aim of this work was the study of the start up and operation of two Anammox SBR where the complete mixture of the liquid media was accomplished by the off-gas recirculation. The effects of different gas flow velocities were analyzed in terms of the Anammox process performance and the physical stability of the Anammox granular biomass.

## 2. Materials and methods

### 2.1. Experimental set-up

A SBR with a total volume of 2.5 l and a working volume of 1.5 l was used. Dimensions of the unit were: height of 465 mm and inner diameter of 85 mm, with a maximum level of the liquid of 264 mm, and the height to the diameter ratio being 5.5.

The reactor was operated in two different layouts: as a bubble column (SBR-B), and as a gas-lift reactor (SBR-G) after the introduction of

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0045-6535/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2008.05.017

a concentric tube acting as a riser. The riser dimensions were 60 mm diameter and 200 mm height (Fig. 1). Recirculated off-gas was sparged at the bottom of both reactors with a diaphragm pump (Laboport, KNF Neuberger) together with small volumes of a mixture gas comprising 95% Ar and 5% $CO_2$ which is flushed continuously, creating an overpressure in the system and avoiding incidental introduction of air via the off-gas. The flow of the recycled off-gas in each stage was fixed at the desired value by means of the actuation of a regulation valve.

Both SBRs were operated in cycles of 6 h (Dapena-Mora et al., 2004b) with a minimum settling time of 0.4 m $h^{-1}$, at a fixed temperature of 30±1 °C by means of a thermostated jacket. The pH was not controlled and ranged between 7.5 and 8.0.

The control of the actuations of the different devices was performed by a programmable logic controller (CPU224, Siemens).

### 2.2. Feeding media

The reactors were fed with the following synthetic media (g $l^{-1}$): $NaNO_2$ (0.24–0.71), $(NH_4)_2SO_4$ (0.25–0.74); $KHCO_3$ (1.250); $NaH_2PO_4$ (0.050); $CaCl_2 \cdot 2H_2O$ (0.300); $MgSO_4 \cdot 7H_2O$ (0.200); $FeSO_4$ (0.0063); EDTA (0.0063); trace elements solution (1.25 mL $l^{-1}$) as described by van de Graaf et al. (1996). The feeding was appropriately diluted during the first periods of operation until stable conditions were achieved.

### 2.3. Inoculum

Both SBRs were inoculated with Anammox biomass grown in the form of granules coming from a mature SBR which was operated for two years at different operational conditions (Dapena-Mora et al., 2004a). The Anammox granules presented an average feret diameter of 0.6–0.7 mm and a specific Anammox activity of 0.35 g N $g^{-1}$ VSS $d^{-1}$. The initial biomass concentration inside the SBR-B was of 1.0 and 2.0 g VSS $l^{-1}$ in the first and second inoculation, respectively; and in the SBR-G of 1.5 g VSS $l^{-1}$.

### 2.4. Operational conditions

In both configurations the SBR was operated at a hydraulic retention time of 1 d and the applied nitrogen loading rate (NLR) ranged from 0.06 to 0.30 g N $l^{-1}$ $d^{-1}$ by varying the inlet ammonium and nitrite concentrations between 25–150 mg $NH_4^+$–N $l^{-1}$ and 25–150 mg $NO_2^-$–N $l^{-1}$, respectively. Nitrite was the limiting substrate because of its toxic effect on the Anammox biomass.

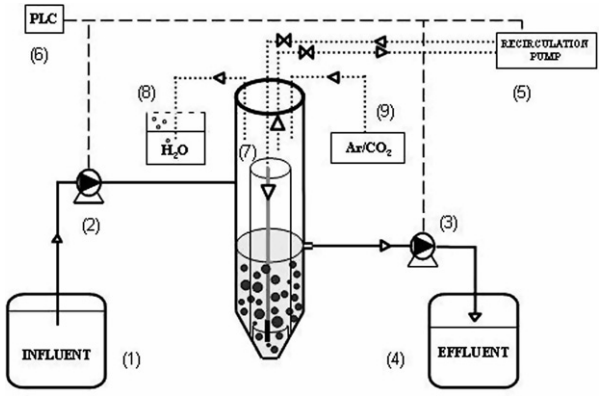


**Fig. 1.** Experimental set-up of the bubble column (SBR-B) and the gas-lift (SBR-G) reactors. (1) Feeding tank; (2) feeding pump; (3) effluent pump; (4) effluent tank; (5) recirculation pump; (6) PLC; (7) riser (only in the SBR-G); (8) water trap; (9) inlet gas flow.

When the steady state conditions were reached in the reactors, the operational strategy consisted of increasing shear forces applied to the reactor stepwise by changing the superficial off-gas upflow velocities ($U_g$) according to the data provided in Table 1. During the entire operational period the biomass in the effluent was settled and returned to the reactor in order to avoid its wash-out.

### 2.5. Analytical methods

The pH value, the concentrations of nitrate, nitrite, ammonium, volatile suspended solids (VSS) and total suspended solids (TSS) and the sludge volumetric index (SVI) were determined according to the Standard Methods (APHA, 1998). Total organic carbon (TOC) and inorganic carbon (IC) were analysed by using a total carbon analyser (Shimadzu TOC-5000).

Biomass density, in terms of biomass per volume occupied by the granules ((g VSS $(L_{granules})^{-1}$), was determined using dextran blue following the methodology proposed by Beun et al. (1999). The dimensions of the granules were measured regularly by using an image analysis procedure (Tijhuis et al., 1994) and described in Arrojo et al. (2006).

**Table 1**
Values of the off-gas flow rates and the corresponding upflow velocities

| Reactor | Days | Stage | Gas flow rate (l $min^{-1}$) | $U_g$ (cm $min^{-1}$) |
|---|---|---|---|---|
| SBRB | 0–130 | S1 | 0.70 | 12.35 |
| SBRG | 0–25 | S2 | 0.20 | 3.53 |
| | 26–33 | S3 | 0.30 | 5.29 |
| | 34–51 | S4 | 0.42 | 7.39 |
| | 52–79 | S5 | 0.55 | 9.70 |
| | 80–110 | S6 | 0.20 | 3.53 |

**Table 2**
Targeted organisms and the corresponding formamide (FA) percentages for the used oligonucleotide probes

| Probe | Probe sequence (5' → 3') | %FA | Targeted organisms | Ref. |
|---|---|---|---|---|
| EUB338I | GCT GCC TCC CGT AGG AGT | 20 | Bacteria domain | [1] |
| EUB338II | GCA GCC ACC CGT AGG TGT | 60 | Bacterial lineages not covered by probe EUB338. Planctomycetales | [2] |
| PLA46 | GAC TTG CAT GCC TAA TCC | 30 | Planctomycetales | [3] |
| Amx820 | AAA ACC CCT CTA CTT AGT GCC C | 40 | Anaerobic ammonium-oxidizing bacteria *Candidatus* "*Brocardia anammoxidans*" and *Candidatus* "*Kuenenia stuttgartiensis*" | [4] |
| Amx368 | CCT TTC GGG CAT TGC GAA | 15 | *All Anammox bacteria* | [5] |
| KST157 | GTT CCG ATT GCT CGA AAC | 25 | *Candidatus* "*Kuenenia stuttgartiensis*" | [4] |
| Ban162 | CGG TAG CCC CAA TTG CTT | 40 | *Candidatus* "*Brocadia anammoxidans*" | [4] |
| Bet42a | GCC TTC CCA CTT CGT TT | 35[a] | Betaproteobacteria | [6] |
| Gam42a | GCC TTC CCA CAT CGT TT | 35[a] | Gammaproteobacteria | [6] |
| NEU653 | CCC CTC TGC TGC ACT CTA | 40[a] | Most of the halophilic and halotolerant *Nitrosomonas* spp. | [7] |
| Ntspa712 | CGC CTT CGC CAC CGG CCT TCC | 50[a] | Most members of the phylum *Nitrospirae* | [8] |

[1] Amann et al., 1990; [2] Daims et al., 1999; [3] Neef et al., 1998; [4] Schmid et al., 2001; [5] Schmid et al., 2003; [6] Manz et al., 1992; [7] Wagner et al., 1995; [8] Daims et al., 2001.

[a] Used with an equimolar amount of corresponding unlabeled competitor oligonucleotide probe.