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# Improved anammox performance with a flow switched anaerobic baffled reactor (FSABR) modified from a common anaerobic baffled reactor (CABR)

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# ABSTRACT

An anaerobic ammonium oxidation (anammox) reactor was operated with two feeding regimes. In stage 1, the reactor was operated as a common anaerobic baffled reactor (CABR) (constant flow direction), whereas in stage 2, the reactor was modified as a flow switched anaerobic baffled reactor (FSABR) with the flow direction switching every 10 days. Adjusting the feeding regime resulted in an increase in the average nitrogen loading rate (from 1.62 to  $1.80 \text{ kgN m}^{-3} \text{ d}^{-1}$ ) and nitrogen removal efficiency (from 75.4 to 83.1%). In addition, a maximum nitrogen removal rate of  $3.49 \text{ kgN m}^{-3} \text{ d}^{-1}$  was obtained in stage 2, compared with  $3.00 \text{ kgN m}^{-3} \text{ d}^{-1}$  in stage 1. The sludge properties were also enhanced; from stage 1 to stage 2, the specific anammox activity increased by more than 5-fold, the settling velocity increased from 64.1 to  $71.9 \text{ m} \text{ h}^{-1}$ , and the average particle diameter increased from 0.8 to 2.3 mm. However, there was minimal variation in the heme c content. The maximum substrate removal rates in stages 1 and 2 were 4.28 and  $46.38 \text{ kgN m}^{-3} \text{ d}^{-1}$  when fitted by the Stover-Kincannon model, respectively. The results indicate that the FSABR performance was significantly enhanced compared with the CABR.

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# 1. Introduction

The anaerobic treatment of wastewater has been considered a promising process over aerobic treatment due to its many advantages, e.g., minimum operation and maintenance requirements/costs, and low excess sludge production. Anaerobic ammonium oxidation (anammox) is an anaerobic technique that converts ammonium into N<sub>2</sub> where nitrite plays as the electron acceptor (Eq. (1)) (Abbas et al., 2015; Carvajal-Arroyo et al., 2014; Strous et al., 1998; Tao et al., 2012). Compared with the traditional nitrification/denitrification process, exogenous oxygen or organic compounds are dispensable in the anammox process, making it more cost efficient.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+$$

$$\rightarrow 1.02N_2 + 0.256NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \tag{1}$$

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The anaerobic baffled reactor (ABR), which has been evolving since the early 1980s, is known as a highly efficient anaerobic reactor configuration that has many advantages compared with other reactors, e.g., longer retention times, less vulnerable to shock loadings, and the ability to separate the anaerobic metabolic phases (Barber and Stuckey, 1999; Shanmugam and Akunna, 2010). An ABR is a series of upflow anaerobic sludge blanket (UASB) reactors, and each compartment (C) is separated by a vertical baffle, which forces the wastewater flow up and down. The wastewater comes in close contact with the active biomass as it passes through the ABR Cs.

It has been recently reported that ABRs are appropriate for the treatment of nitrogenous compounds contained wastewater (Jin et al., 2013a; Uyanik, 2003; Wang et al., 2004; Wu et al., 2013). However, owing to the compartmentalized structure, the initial Cs of the ABR may be overloaded with substrate, whereas the final Cs would be nutrient limited (Uyanik, 2003). To obtain a robust operation, several researchers have attempted to find new ways to optimize the stability and capacity of ABRs, such as combination utilization and operational optimization. Some researchers have attempted to combine the anaerobic and aerobic processes, such as by making an ABR-aerobic biofilm reactor (Bodik et al., 2003) and incorporating an anaerobic stage followed by an aerobic one in a modified ABR





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(Barber and Stuckey, 2000). Lu et al. (2011)investigated the effectiveness of the combined technique with an ABR-sequencing batch reactor (SBR) system for hypersaline wastewater treatment and obtained satisfactory results. Uyanik (2003) employed two feeding regimes and found that a split-fed ABR has a number of potential advantages over the normally fed ABR during both the start-up period and steady operation period.

A flow switched anaerobic baffled reactor (FSABR) was proposed to eliminate the technical problems of substrate confusion in the Cs. Long-term substrate over-loading and nutrient-limited situations can be avoided by periodically switching the feeding direction. The objective of this study was to treat nitrogen-containing wastewater with an anammox FSABR with different feeding regimes and to test the superiority of a flow-switched feeding regime over the normal regime in terms of the reactor potential, robustness, and sludge characteristics.

# 2. Materials and methods

#### 2.1. Seed sludge and anammox reactor

An FSABR was used in this study and its working volume was 4 L (Fig. 1). The reactor was constructed with a height of 32 cm, width of 6.2 cm, bottom length of 36 cm and top length of 51.2 cm. The reactor was inoculated with mature anammox sludge from a sturdily operated anammox reactor in the laboratory. The suspended solids (SS) concentration of the sludge after inoculation was  $10.4 \, g L^{-1}$  with a volatile suspended solids (VSS) concentration of  $6.9 \, g L^{-1}$ . The reactor was observed for 354 days in the thermostatic room where the temperature was $35 \pm 1$  °C, and the research could be divided into two stages: a CABR from days 1 to 220 (Fig. 1A) and a FSABR from days 221 to 354, during which the feeding regime was alternated between the two feeding regimes every 10 days (Fig. 1A and B).

#### 2.2. Synthetic wastewater

The synthetic wastewater contained substrates, inorganic solution and trace elements.  $(NH_4)_2SO_4$  and  $NaNO_2$  were used as the source of ammonium and nitrite, respectively. Trace elements, including EDTA at  $15 \text{ g L}^{-1}$ ,  $CoCl_2 \cdot 6H_2O$  at  $0.24 \text{ g L}^{-1}$ ,  $CuSO_4 \cdot 5H_2O$  at  $0.25 \text{ g L}^{-1}$ ,  $ZnSO_4 \cdot 7H_2O$  at  $0.43 \text{ g L}^{-1}$ ,  $MnCl_2 \cdot 4H_2O$  at  $0.99 \text{ g L}^{-1}$ ,  $NiCl_2 \cdot 2H_2O$  at  $0.19 \text{ g L}^{-1}$ ,  $NaMOO_4 \cdot 2H_2O$  at  $0.22 \text{ g L}^{-1}$ ,  $H_3BO_4$  at  $0.014 \text{ g L}^{-1}$ ,  $NaSeO_4 \cdot 10H_2O$  at  $0.21 \text{ g L}^{-1}$ , and  $NaWO_4 \cdot 2H_2O$  at  $0.050 \text{ g L}^{-1}$ , were supplied at a dose of 1.25 mL per liter of wastewater (Yang and Jin, 2013). The inorganic solution consisted of  $NaH_2PO_4$  at  $10 \text{ mg L}^{-1}$ ,  $CaCl_2$  at  $5.65 \text{ mg L}^{-1}$ ,  $KHCO_3$  at  $1 \text{ g L}^{-1}$  and  $MgSO_4 \cdot 7H_2O$  at  $58.6 \text{ mg L}^{-1}$ .

## 2.3. Analytical methods

25 mL effluent were sampled with a small beaker every 2 days, and the samples were stored in a  $4 \circ C$  refrigerator for the measurement. The pH was measured with a pH meter (Mettler Toledo Delta 320, Switzerland), SS and VSS was measured by weight method, and NO<sub>3</sub><sup>--</sup>N, NO<sub>2</sub><sup>--</sup>N, and NH<sub>4</sub><sup>+</sup>-N were measured by spectrophotometry. The pH, SS, VSS, NO<sub>2</sub><sup>--</sup>N, NO<sub>3</sub><sup>--</sup>N, and NH<sub>4</sub><sup>+</sup>-N were determined with the methods described in standard methods (APHA, 2005). The particle diameter was measured by the method described by Jin et al. (2013b). The settling velocity (*V*<sub>S</sub>) was determined in a measuring cylinder (1 L), the height and internal diameter of the cylinder was 40.0 cm and 10.0 cm in order to minimize the wall effect. The sludge was slightly put into the middle of the water surface. When the sludge reach the line of 750 mL the timer starts and the time for the distance of 20 cm are recorded.

Extracellular polymeric substances (EPS) were extracted by 'heating' method, and the method described by Ma et al. (2012) was utilized to measure polysaccharide (PS) and extracellular protein (PN) concentrations. Heme c was measured by the method used by Berry and Trumpower (1987). Specific anammox activity (SAA) assays were measured in batch assays according to Yang and Jin (2013).

#### 2.4. Statistical analysis

Statistical comparison between variables was conducted using One-Way analysis of variance (ANOVA) and the Mann-Whitney Test by SPSS software (SPSS 13.0). A *p*-value of 0.05 or lower indicates that the difference between the variables under comparison is statistically significant.

#### 2.5. Stover-Kincannon model

The Stover-Kincannon model has been widely applied in immobilized systems in order to calculate the kinetic constants (Isik and Sponza, 2005; Jin and Zheng, 2009; Stover and Kincannon, 1982). In this study, this model was modified and used in the FSABR (Eq. (2)).

$$\left(\frac{dS}{dt}\right)^{-1} = \frac{V}{Q(S_0 - S)} = \frac{K_B}{U_{max}} \frac{V}{QS_0} + \frac{1}{U_{max}}$$
(2)

in which dS/dt is the substrate removal rate (kg m<sup>-3</sup> d<sup>-1</sup>);  $U_{max}$  is the maximum substrate removal rate (kg m<sup>-3</sup> d<sup>-1</sup>); Q is the inflow rate (Ld<sup>-1</sup>);  $S_0$  and S are influent and effluent total nitrogen concentrations (kg m<sup>-3</sup>); V is the reactor volume (L) and  $K_B$  is the saturation value constant (kg m<sup>-3</sup> d<sup>-1</sup>).

## 3. Results

#### 3.1. Anammox performance at different feeding regimes

#### 3.1.1. Stage 1 (days 1-220)

In stage 1, the reactor was operated as a CABR. The supplementation of  $NO_2^--N$  and  $NH_4^+-N$  was in a ratio of 1:1 which was demonstrated appropriate for the anammox reactor according to our previous studies (Jin et al., 2013a,b,c; Ma et al., 2012). The hydraulic retention time (HRT) was initially set at 21.3 h, and the initial concentrations of  $NO_2^--N$  and  $NH_4^+-N$  were 70 mg  $L^{-1}$ , and high nitrogen removal efficiency (NRE) was achieved. Thus, the HRT was shortened to 4.1 h within 10 days for a given substrate concentration. The NRE consistently remained above 99.0% during these days.

As shown in Fig. 2, from days 8 to 75, the HRT was consistently maintained at 4.1 h, and the concentration of the substrate gradually increased. On day 37, the concentration was  $308 \text{ mg L}^{-1}$ , and the effluent NO<sub>2</sub><sup>-</sup>-N started to ascend gradually over  $100 \text{ mg L}^{-1}$  (day 41), which may lead to the risk of nitrite inhibition to the anammox system (Strous et al., 1999). Hence, the concentration was decreased until the anammox system recovered at 70 mg L<sup>-1</sup> on day 73. During days 8–75, a maximum nitrogen removal rate (NRR) of 3.00 kgN m<sup>-3</sup> d<sup>-1</sup> was obtained.

After the recovery of the anammox system, the influent substrate concentrations were fixed, and an exploration of the minimum HRT that could be achieved by the CABR was conducted. The HRT was gradually shortened until the HRT reached 3.3 h. The nitrogen conversion decreased due to the accumulation of nitrite. To obtain a maximum NRR in the rest time of stage 1, both the HRT and substrate concentrations were adjusted. However, no NRR greater than 3.00 kgN m<sup>-3</sup> d<sup>-1</sup> was achieved.

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