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A low volumetric exchange ratio allows high autotrophic nitrogen removal in a sequencing batch reactor

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ABSTRACT

Sequencing batch reactors (SBRs) have several advantages, such as a lower footprint and a higher flexibility, compared to biofilm based reactors, such as rotating biological contactors. However, the critical parameters for a fast start-up of the nitrogen removal by oxygen-limited autotrophic nitrification/denitrification (OLAND) in a SBR are not available. In this study, a low critical minimum settling velocity ($0.7\ m\ h^{-1}$) and a low volumetric exchange ratio (25%) were found to be essential to ensure a fast start-up, in contrast to a high critical minimum settling velocity ($2\ m\ h^{-1}$) and a high volumetric exchange ratio (40%) which yielded no successful start-up. To prevent nitrite accumulation, two effective actions were found to restore the microbial activity balance between aerobic and anoxic ammonium-oxidizing bacteria (AerAOB and AnAOB). A daily biomass washout at a critical minimum settling velocity of $5\ m\ h^{-1}$ removed small aggregates rich in AerAOB activity, and the inclusion of an anoxic phase enhanced the AnAOB to convert the excess nitrite. This study showed that stable physicochemical conditions were needed to obtain a competitive nitrogen removal rate of $1.1\ g\ N\ L^{-1}\ d^{-1}$.

1. Introduction

Nitrogen removal during wastewater treatment is often accomplished by the conventional nitrification/heterotrophic denitrification process. This process is suitable for the treatment of ammonium rich wastewaters with high biodegradable carbon content. However, it becomes more expensive for wastewaters with low carbon to nitrogen ratios (C/N) due to the required addition of an external organic carbon source for the denitrification step. In the latter case, the combination of partial nitritation and anammox can decrease the operational costs by almost 90% (Mulder, 2003). Consequently, several processes have been developed in the last years with different names, such as oxygen-limited autotrophic nitrification/denitrification (OLAND) (Kuai and Verstraete, 1998), completely autotrophic nitrogen removal over nitrite (CANON) (Third et al., 2001) and deammonification process (DEMON) (Wett, 2006). All these processes are based on the cooperation between aerobic ammonium-oxidizing bacteria (AerAOB), oxidizing ammonium to nitrite, and anoxic ammonium-oxidizing bacteria (AnAOB), oxidizing nitrite and ammonium to nitrogen gas.

Despite the economical advantage of these AnAOB based processes in comparison with the conventional nitrification/denitrification, these processes are hindered by the long start-up period due to the slow growth rate of the AnAOB, which have a doubling time of 7–14 days (Strous et al., 1999). Therefore, biomass washout has to be minimized, e.g. by biofilm formation or granulation. Several biofilm based reactors, such as a rotating biological contactor (RBC) (Siegrist et al., 1998; Pynaert et al., 2004), a moving bed reactor (Cema et al., 2006) or a fixed bed reactor (Furukawa et al., 2006) have already been successfully applied. High biomass retention can also be obtained in a sequencing batch reactor (SBR) operated at a critical minimum biomass settling velocity. The latter is defined as the ratio between the settling time and the vertical distance of the water volume decanted per cycle, and it can also be expressed as the volumetric exchange ratio, i.e. the ratio of the decanted to the total water volume. Reported minimum biomass settling velocities for OLAND type SBRs are in the range of $0.3–0.7\ m\ h^{-1}$ (Third et al., 2001; Sliekers et al., 2002; Wett, 2006; Vlaeminck et al., 2009). Although SBRs have advantages, such as a lower footprint and a higher flexibility, compared to biofilm based reactors, such as RBC, so far the nitrogen removal rates obtained in these reactors are almost five times lower (Table 1).

Not only efficient biomass retention is required for a successful OLAND process, a good balance between the AerAOB and AnAOB is needed as well. A higher activity of the AerAOB in comparison to the AnAOB results in nitrite accumulation in the reactor, which can inhibit the AnAOB activity at nitrite concentrations of 98–350 mg NO_2^-–N L^{-1} (Strous et al., 1999; Dapena-Mora et al., 2007). While in RBCs the microbial balance is equilibrated spontaneously due to the limited penetration depth of oxygen in the biofilm, the control of this microbial balance in SBRs is not

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Table 1
Overview of the volumetric nitrogen removal rates in OLAND type rotating biological contactors (RBC) and sequencing batch reactors (SBR).

Reactor type	Volume (m^3)	Nitrogen removal rate ($kg\ N\ m^{-3}\ d^{-1}$)	Reference
RBC	33	0.4	Siegrist et al. (1998)
RBC	0.044	1.1	Pynaert et al. (2003)
RBC	240	1.7	Schmid et al. (2003)
RBC	0.005	1.8	Pynaert et al. (2004)
SBR	0.002	0.1	Third et al. (2001)
SBR	0.002	0.3	Sliekers et al. (2002)
SBR	0.002	0.5	Vlaeminck et al. (2009)
SBR	500	0.6	Wett (2006)
SBR	0.002	1.1	This study
Gas-lift	0.002	1.5	Sliekers et al. (2003)

straightforward. Two kinds of biomass morphologies, flocs and granules, were mainly present in suspended growth systems (Innerebner et al., 2007; Vlaeminck, 2009; Vlaeminck et al., 2009). Granules can be described as compact and dense aggregates with a high macroscopic circularity that do not coagulate under reduced hydrodynamic shear and settle significantly faster than flocs (Lemaire et al., 2008). Flocs were found to be enriched in AerAOB, while AnAOB were dominant in the granules (Nielsen et al., 2005; Vlaeminck, 2009; Vlaeminck et al., 2009). Therefore, the overall balance between the AerAOB and AnAOB is dependent on the biomass morphology distribution in the reactor. Morphology selection on the basis of the settling velocity could therefore improve the microbial balance.

In this study, the microbial balance between the AerAOB and AnAOB was evaluated in an OLAND SBR. The critical parameters for a fast start-up were determined and strategies to control the microbial balance and enhance the biomass retention in the reactor were evaluated.

2. Methods

2.1. OLAND SBR

The lab-scale OLAND SBR consisted of a cylindrical vessel with an internal diameter of 14 cm (working volume of 2.5 L). The reactor was inoculated with OLAND biomass harvested from the reactor described by Pynaert et al. (2003) at an initial biomass concentration of 2.3 g VSS L^{-1}. The reactor was fed with synthetic wastewater containing an initial ammonium concentration of 100 mg N L^{-1}, 10 mg KH_2PO_4–P L^{-1} and 2 mL L^{-1} of a trace elements solution (Kuai and Verstraete, 1998). To provide both buffering capacity and inorganic carbon, 1 mol of bicarbonate was added per mol of nitrogen. If necessary, the latter ratio was increased temporarily to ensure that the reactor pH did not drop below 7.4. In addition, the influent ammonium concentration was gradually increased whenever the effluent concentration was below ca. 25 mg N L^{-1} up to 300 mg N L^{-1} on day 162. The reactor was mixed with a magnetic stirrer at 245 rpm and aerated at an airflow rate of 40 L h^{-1}. The temperature and the dissolved oxygen (DO) concentration were controlled automatically at 33 ± 1 °C (temperature controlled room) and 0.3 to 0.7 mg O_2 L^{-1} (Oxymax W COS31 probe with Liquisis M COM 223 controller; Endress and Hauser, Reinach, Switzerland), respectively. Three different phases of operation were carried out: phase 1 with high volumetric exchange ratio (40%) and high critical minimum settling velocity (2 m h^{-1}); phase 2 with high volumetric exchange ratio (40%) and low critical minimum settling velocity (0.7 m h^{-1}); and, phase 3 with low volumetric exchange ratio (25%) and low critical minimum settling velocity (0.7 m h^{-1}). During the first and the second phases, the exchangeable volume was fixed at 1 L, resulting in a volumetric exchange ratio of 40% (1/2.5). During the third phase, the working volume was lowered to 2 L in order to obtain an exchangeable volume of 0.5 L, and consequently, the volumetric exchange ratio decreased to 25% (0.5/2). The nitrogen compounds (ammonium, nitrite, nitrate), DO concentrations and pH were monitored during the whole experiment.

2.2. SBR cycle

The SBR was operated with 1 h cycles during the whole experimental period. During the first phase, 1 L of synthetic medium was fed to the reactor during a 5 min filling period. The reactor was mixed and the DO was controlled both during the feeding and the reaction phase. Subsequently, the biomass was allowed to settle for 2 min, so that the minimum biomass settling velocity was 2 m h^{-1}. Finally, an effluent pump removed the supernatant. During the second and third phases, the settling time was increased to 6 and 3 min, respectively, resulting in a lower selection pressure (critical minimum settling velocity of 0.7 m h^{-1}). To control the nitrite production, an anoxic phase of 10 min was included at the end of the reaction period from day 122 on. During this phase only mixing took place.

2.3. Aerobic and anoxic batch tests

The specific activities of AerAOB and AnAOB were determined in aerobic and anoxic batch tests, respectively, as described in detail by Vlaeminck et al. (2007). Prior to the activity tests, the biomass was washed with a phosphate buffer (100 mg P L^{-1}; pH 8) on a sieve (pore size 50 μm) to remove residual dissolved reactor compounds. The aerobic tests were performed in open Erlenmeyer with ammonium as substrate. For the anoxic tests, biomass incubation occurred in a gas-tight anoxic serum flask with ammonium and nitrite as substrates. Both tests were performed on a shaker at 34 ± 1 °C.

2.4. Chemical analyses

Nitrite and nitrate were determined on a Metrohm 761 Compact Ion Chromatograph (Zofingen, Switzerland) equipped with a conductivity detector. Ammonium (Nessler method) was measured according to standard methods (Greenberg et al., 1992). The pH was measured with a Consort C532 pH meter (Turnhout, Belgium).

2.5. Physical aggregate characteristics

A mixed liquor sample obtained in a Petri dish was photographed with a high resolution (10 megapixels) digital camera for particle analyses. The Feret diameter (largest diameter in an irregularly shaped particle) and the circularity of the biomass aggregates were calculated from the digital photographs using the ImageJ software (open source). The settling velocity was determined as described by Vlaeminck et al. (2009).

3. Results

3.1. OLAND SBR performance

During phase 1 the strategy of the OLAND SBR operation was based on a high critical minimum settling velocity of 2 m h^{-1} to induce granulation. These conditions resulted in an average total nitrogen removal rate of only 20 mg N L^{-1} d^{-1}, which was attributed to complete conversion of ammonium to nitrite (Fig. 1B). Moreover, no anammox activity was observed during this phase. Therefore, it was concluded that a critical minimum settling velocity of 2 m h^{-1} was too high.