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Long-term performance and microbial characteristics of the anammox-enriched granular sludge cultivated in a bench-scale sequencing batch reactor

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ABSTRACT

The anammox-enriched granular sludge was successfully formed during the long-term biogranulation experiment lasting over 330 days. The cultivation was conducted at 30 °C in a 10-L sequencing batch reactor (SBR) fed with synthetic medium containing ammonia, nitrite and trace elements. The properties of the developed granules were investigated in terms of the biomass activity (including the growth rate of anammox bacteria), size distribution of the granules as well as nitrogen removal performance and pathways. The compositions of the microbial communities in the inoculum sludge and ultimate granules were compared using the metagenomic analysis. The mean particle size of the biomass increased from 290 μm (inoculum sludge) to 728 μm (ultimate granules). The overall nitrogen removal rate (NRR) and specific anammox activity (SAA) reached the maximum value of 5.3 kg Nm^{-3} d^{-1} and 1.6 kg Nkg VSS^{-1} d^{-1}, respectively. In the matured granules, *Planctomycetes* were the most abundant phylum (aprox. 44% of total 16S rRNA reads), exclusively represented by *Candidatus Brocadia*. Based on the 16S rRNA reads frequency derived from *Planctomycetes* in the total metagenomic library and volatile suspended solids (VSS) concentrations of the inoculum sludge and ultimate granules, the observed specific growth rate of anammox bacteria was estimated at 0.14 d^{-1} over the entire study period.

1. Introduction

The anammox (anaerobic ammonium oxidation) process has widely been recognized as the efficient nitrogen removal process from high strength ammonia wastewater streams. The principal advantages of the anammox process over conventional nitrification-denitrification are substantially lower oxygen consumption and sludge production as well as no need for external carbon sources. However, anammox bacteria grow very slowly and they are vulnerable to several specific inhibitors, including dissolved oxygen (DO), pH, organic compounds, temperature and nitrite [1].

The critical point with regard to the enrichment of anammox bacteria is their low specific growth rate constant, e.g. $\mu_{max} = 0.065\ d^{-1}$ [2]. This makes the start-up period longer compared to the conventional nitrogen removal systems based on nitrification-denitrification. Furthermore, the anammox reactors have to be operated at a long solids retention time (SRT) in order to accumulate the necessary biomass in the system [3–5]. Sludge immobilization techniques include the growth on particulate carriers, hydrocyclon separation or biogranulation. The latter technique (biogranulation) involves changing the inoculum sludge to compact aggregates, next to irregular granular sludge and ultimately to spherically shaped granules. The anaerobic/anoxic biogranulation of anammox biomass may thus constitute an effective strategy

Abbreviations: Anammox, anaerobic ammonium oxidation; AOB, ammonia oxidation bacteria; DO, dissolved oxygen; FISH, fluorescence in situ hybridization; H index, Shannon index of general diversity; HRT, hydraulic retention time; MLSS, mixed liquor suspended solids; MLVSS, mixed liquor volatile suspended solids; NLR, ntrogen loading rate; NOB, nitrite oxidation bacteria; NRR, nitrogen (sum of ammonium and nitrite) removal rate; OTU, operational taxonomic unit; PCR, polymerase chain reaction; SAA, specific anammox activity; SBR, sequencing batch reactor; SRT, solids retention time; TSS, total suspended solids; UASB, upflow anaerobic sludge blanket; VSS, volatile suspended solids; $\mu_{ANAM,obs}$, observed specific growth rate of anammox bacteria d^{-1}.

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for both stable nitrogen removal with high loading rates [6] and retaining the biomass due to its good settling ability [3,7].

Granules can generally be shaped by several mechanisms, including hydraulics, gas flow and mechanical shear stress [8]. The biogranulation process has been studied in upflow anaerobic sludge blanket (UASB) reactors [9–12], gas lift reactors [13] and mechanically stirred sequencing batch reactors (SBR) [4,7]. The biogranulation has also been successfully achieved in a hybrid reactor [8] and membrane reactor [14].

The most frequently analyzed physical parameters of the anammox sludge comprised the settling properties, diameter distribution and substrate diffusion [1,7,10,14–16]. In some studies, a morphological aspect of the granular sludge was analyzed using a visual inspection stereomicroscope or scanning electron microscope (SEM) [7,10,15].

Molecular tools that are now commonly used for identification of specific microorganisms comprise fingerprinting methods such as denaturing/temperature gradient gel electrophoresis (DGGE/TGGE), random amplified polymorphic DNA (RAPD), amplified ribosomal DNA restriction analysis (ARDRA) and fluorescence in situ hybridization (FISH). However, those methods are of limited use in the identification and characterization of microbial communities. The fingerprint methods can show differences between communities but do not provide direct taxonomic identities, whereas the FISH is not able to identify unknown microorganisms [17–19]. These drawbacks may be overcome with novel, high resolution molecular techniques, such as metagenomic analyses. Until now, the latter techniques have been used only in a few anammox studies focusing on determination of the current microbial compositions in various anammox reactors [20,21].

In the present study, the use of the metagenomic analysis was aimed at comparing a shift in the composition of the microbial community during the long-term biogranulation of the anammox-enriched sludge in a SBR. With these results, it was also possible to address two new issues not found in the previous studies on granular anammox systems. First, the composition and abundance of the entire microbial population in the SBR was determined. Secondly, the observed growth rate of anammox bacteria could be accurately estimated. Furthermore, the properties of the developed granules were investigated in terms of the biomass activity, size distribution of the granules, nitrogen removal performance and pathways.

2. Materials and methods

2.1. SBR operation and evaluation of the anammox activity

The long-term biogranulation experiment was conducted for 330 days in a laboratory scale plexiglass SBR with a working volume of 10 L (Fig. 1). The reactor was inoculated with anaerobic sludge originated from a full-scale sidestream treatment system in Switzerland. The concentration of volatile suspended solids (VSS) in the inoculum sludge was 0.51 g L^{-1}.

The temperature, pH and DO concentration in the reactor were continuously measured and recorded (data not shown). During the entire study period, the SBR was operated at a constant temperature of 30 (±1) °C. The desired temperature set point was kept with a thermostatic water bath (F32-ME Refrigerated/Heating Circulator, JULABO GmbH). The pH was controlled in the range of 7.5–7.8 by automatically adding 6 M hydrochloric acid (HCl). The DO concentration in the non-aerated SBR did not exceed 0.2 mg L^{-1}

The SBR was fed with synthetic medium as originally descried in [9] and subsequently used in other studies, e.g. [13,22]. The most important components, i.e. nitrite and ammonium, were supplied in the form of NH_4Cl and $NaNO_2$, respectively. The detailed composition of the medium is presented in Table 1.

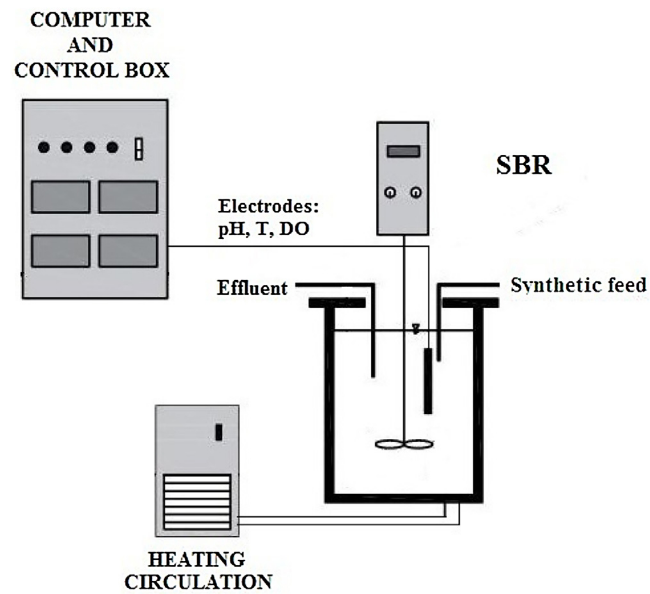

Fig. 1. Laboratory set-up, including the 10 L SBR, used for the long-term biogranulation experiment.

The entire study period was divided into four phases characterized by different operating conditions as shown in Table 2. The initial values of the operating cycles were based on the previous publications [23]. During the course of the study, the duration of the reaction phase was reduced, leaving unchanged the duration of the other phases. The nitrogen loading rates (NLRs) were adjusted in response to the actual anammox activity by changing either the total nitrogen (sum of ammonia and nitrite) concentrations in the synthetic feed or hydraulic retention times (HRTs) in the studied SBR. In the course of the biogranulation process, the nitrogen loads were temporarily increased to determine their maximum values without deteriorating the system performance.

Concentrations of ammonia, nitrite and nitrate were determined on a regular basis (every 1–7 d) at the beginning of the reaction phase and at the end of the denitrification phase. In addition, the nitrogen removal rates (NRR) and specific anammox activities (SAAs) were determined in situ once or twice a week. The NRR was defined as the nitrogen removal rate (sum of ammonia and nitrite) on the daily basis under non-aerated conditions and non-limited concentrations of ammonia and nitrite. The SAA was defined as a ratio between the observed NRR and VSS concentration. The consumption rates of ammonia and nitrite were calculated by a linear regression of the measured concentrations. The sampling frequency was dependent on the actual NRR and varied in the range of 15–30 min.

2.2. Particle size distribution of the granular sludge

The size distribution of the granules was measured using a laser particle size analyzer Mastersizer 2000 with Hydro 2000MU unit (Malvern Instruments Ltd, United Kingdom). The particle diameters measured by the laser apparatus (using red and blue laser beams) ranged from 0.2 to 2000 μm. The analyzer results are consistent with the ISO 13320 requirements concerning the particle size analysis by laser diffraction. Determination of the particle size occurs indirectly by calculating its volume. A mathematical description of this phenomenon was provided by McCave and Syvitski [24]. The Mastersizer software enabled calculation of the specific diameters (d(0.1); d(0.5); d(0.9)), defined as follows:

d(0.1) – the diameter of particle which is larger than 10% of the particles in the analyzed sample,