# Start-up of the Nitrogen Removal Process in an Anaerobic Up-Flow Reactor Inoculated with Aeration Tank Sludge

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**Abstract**
Anaerobic ammonium oxidation (anammox) has been considered a promising alternative to the traditional nitrification/denitrification process to remove nitrogen without using external carbon source. The start-up of the anammox process was performed in a stirred anaerobic up-flow reactor seeded with aeration tank sludge, and fed with medium containing ammonium and nitrite (1:1). The reactor was running consecutively for 369 days, with an initial instability period that lasted 117 days, resulting in nitrite removal and nitrate accumulation. In view of this, the reactor stopped being fed with nitrite, which led to a nitrate decrease in the effluent. Afterwards, a 140 days transition period was followed by a 112 days stable period. In the end, the average ammonium removal was 53.8 % in the transition stage, slighting decreasing to 45.6 % in the stable period, while there was no nitrite in the effluent. Preliminary microbial assessment showed the presence of an anammox community in the reactor related to *Candidatus* 'Brocadia fulgida'. The next challenge will be the assessment of eventual shifts on the microbial community structure and composition during the entire process.
**Keywords:** Nitrogen removal; Upflow Bioreactors; Anaerobic processes; Anammox; Wastewater

## 1. Introduction

In the last two decades, a particular emphasis has been placed on reducing the discharge of nitrogen-containing compounds during wastewater treatment. These compounds can strongly stimulate the growth of algae and other photosynthetic aquatic life, which leads to increased eutrophication, oxygen depletion in water bodies, and undesirable changes in aquatic population (Chatterjee et al. 2016). Several treatment systems have been developed for improving nitrogen removal from wastewater, including anaerobic ammonium oxidation (anammox), which has been considered a promising biological process to remove nitrogen. Anammox is a process where anammox bacteria convert ammonium to $N_2$ gas with nitrite as electron acceptor in the absence of $O_2$ (Mulder et al. 1995; van de Graaf et al. 1995). The anaerobic and autotrophic nature of anammox bacteria allows the total reduction of aeration, eliminates the need of organic carbon, and reduces sludge production (Ma et al. 2018). Theoretically, the growth rate of anammox bacteria is low, with estimated doubling time of 10-25 days, being the metabolism affected by environmental factors, such as pH, temperature, and dissolved oxygen (Ibrahim et al. 2016). Besides, the start-up of the anammox phase, as well as the selection of the treatment unit is crucial to improve the efficiency of the anammox process.
In this context, the aim of this study was the development of the start-up of an up-flow reactor focusing in the anammox process.

## 2. Method

A laboratorial scale up-flow reactor was used and seeded with aeration tank sludge from an urban wastewater treatment plant. The working volume of the reactor was 4.8 L with an internal diameter 12 cm and a height of 45 cm. The reactor was continuously fed, during 369 days with synthetic medium containing ammonium ($NH_4^+$), and nitrite ($NO_2^-$) at the ratio of 1:1, with a pH ranging between 7.8 and 9. Several floating bioballs were placed in the reactor as biomass carriers. The reactor was stirred, and dinitrogen gas was purged regularly to maintain anaerobic conditions. The nitrogen removal performance was evaluated by calculating the nitrogen loading rate (NLR), nitrogen removal rate (NRR), and nitrogen removal efficiency (NRE).
The reactor was running consecutively for 369 days, and the identification of the anammox bacteria in the system was carried out after 299 days by sequencing of positive PCR amplicons. The 16S rRNA gene of anammox bacteria (amx) was amplified by the primer set Amx368F (5'-TTCGCAATGCCCGAAAG-3') and Amx820R (5'-AAAACCCCTCTACTTAGTGCCC-3') (Teixeira 2012). The obtained sequences were identified by comparison with 16S rRNA gene sequences in GenBank, using the Basic Local Alignment Search Tool.

## 3. Results and Discussion

The start-up of the reactor could be divided into three stages according to the $NH_4^+$ removal and nitrate ($NO_3^-$) build-up performance (Figure 1). The first stage was an instability period, which happened during 117 days with

constant NLR 34 mg-N $L^{-1}$ $day^{-1}$. During this period, nitrite decreased and was totally removed at the end, followed by nitrate accumulation. The average of ammonium removal in this period was 57.4% while nitrite removal was 94 %. The NRR and NRE during the instability period was 0.3 mg-N $L^{-1}$ $day^{-1}$ and 6 %, respectively.

A second stage was identified afterwards, lasting 140 days. During this period, the reactor was not fed with nitrite, which led to a nitrate degression in the effluent. The NLR of this period was 17 mg-N $L^{-1}$ $day^{-1}$. During this transition period, the process yielded higher NRR and NRE than during the initial period, reaching 0.40 mg-N $L^{-1}$ $day^{-1}$ and 17 %, respectively. Afterwards, it is possible to identify a more stable period (Stage III), where the concentration of nitrate decreased progressively. Indeed, under anaerobic conditions, nitrate could be reduced to nitrite by denitrifying bacteria.

Since there was no nitrite available in the effluent, we hypothesized the nitrogen species was totally used as an electron acceptor during the ammonium oxidation. Average ammonium removal of 54 % was observed in the transition period, decreasing to 46 % in the stable period, probably caused by the less electron acceptor availability. The average of final NRR of the reactor was 2 mg-N $L^{-1}$ $day^{-1}$, while the NRE was 34 %.

Preliminary assessment of the microbial community showed the presence of anammox in the reactor of *Candidatus* 'Brocadia fulgida'.

## 4. Conclusion

In this study, the start-up of an anammox process was carried out in a continuous stirrer anaerobic up-flow reactor. A stable ammonium removal above 30 % could be maintained in the reactor right after the start-up. The presence of bacteria closely related *Candidatus* 'Brocadia fulgida' (an anammox bacteria) was confirmed by sequencing analysis.

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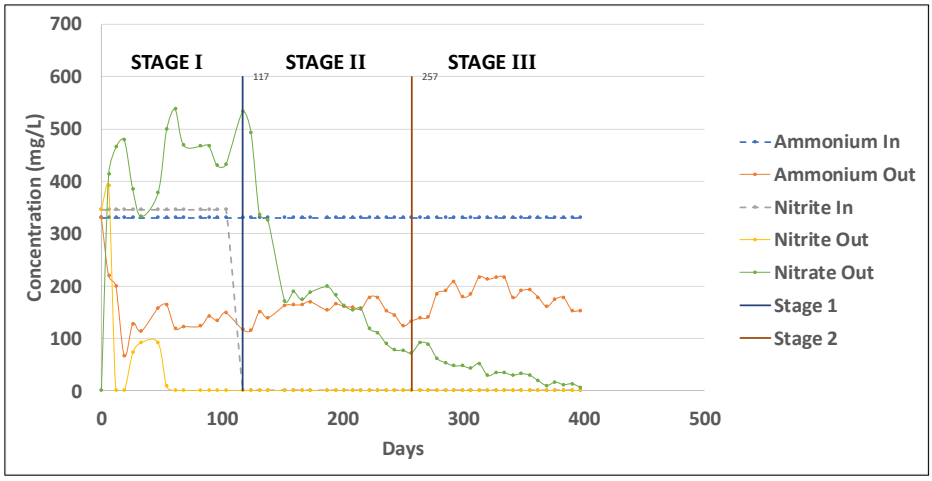


**Figure 1.** Up-Flow reactor performance during the startup period.

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