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# Long-term starvation and subsequent reactivation of anaerobic ammonium oxidation (anammox) granules



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#### HIGHLIGHTS

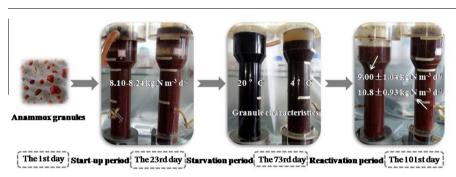
- The effect of long-term starvation on anammox granules properties was evaluated.
- The influence of starvation temperature was examined.
- The reactivation characterization of anammox granules was investigated.
- The properties of anammox granules can be restored after starvation up to 50 days.
- Starvation at 4 °C was more suitable for the reactivation of anammox granules than that at 20 °C.

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#### G R A P H I C A L A B S T R A C T



#### ABSTRACT

Starvation of biomass is common in the biological wastewater industries. To investigate the influence of starvation on the sludge characteristics and reactivation performance of the anaerobic ammonium oxidation (anammox) process, the starvation process of high-activity anammox granules over 50 days and the subsequent reactivation characterization of the granules were evaluated in two upflow anaerobic sludge blanket (UASB) reactors at 20 °C and 4 °C, respectively. The results showed that the starvation temperature had a significant impact on the morphological and physical properties. Anammox granules stored at 4 °C achieved a relatively low decay rate, excellent nitrogen removal capacity, and a low protein-topolysaccharide (PN/PS) ratio after reactivation. Those results demonstrated that 4 °C was more suitable for the maintenance of structural integrity and granule stability during long-term starvation. The nitrogen removal performance of anammox granules after starvation could be recovered after 4 days of operation, and the anammox activity could be fully restored within 8 days. Furthermore, anammox granules preserved at 4 °C obtained a better recovery performance than those at 20 °C. In summary, anammox granules could be stored up to 50 days without running the risk of losing the integrity of the granules and metabolic potentials. And anammox performance after prolonged starvation could be fully revived.

#### 1. Introduction

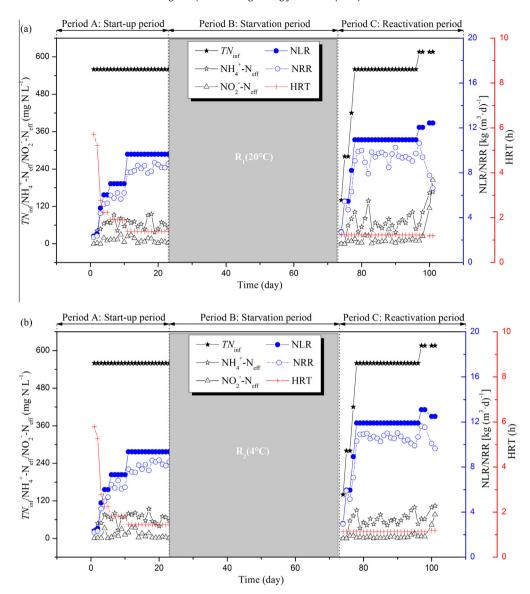
The anaerobic ammonium oxidation (anammox) bacteria catalyze the oxidation of ammonium using nitrite as the electron acceptor to produce dinitrogen gas under anoxic conditions [1]. After its discovery in the early 1990s, the anammox process has

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**Fig. 1.** Time course of the influent nitrogen concentration (*TN*<sub>inf</sub>), hydraulic retention time (HRT), nitrogen loading rate (NLR) and nitrogen removal rate (NRR) for R<sub>1</sub> (a) and R<sub>2</sub> (b) over the entire experimental period.

become a promising biotechnology for nitrogen removal from wastewaters. In 2002, the first full-scale anammox reactor was established for the treatment of reject water at the sludge treatment plant in Rotterdam, Netherlands [2]. It was stably operated, achieving a nitrogen removal rate (NRR) of up to 9.5 kg N m<sup>-3</sup> d<sup>-1</sup>. In recent years, the anammox process has been applied in practice for nitrogen removal from wastewaters, especially for the treatment of wastewater with a low C/N ratio, such as landfill leachate [3], digester liquid [4], and dry-spun acrylic fiber wastewater [5].

**Table 1**Decay kinetic parameters of anammox bacteria found in the literature.

Temperature (°C)	$b_{AN}(d^{-1})$	$t_{\rm HL}\left({ m d}\right)$	Refs.
20	0.001	693	[30]
30	0.003	231	[31]
35	0.0021	330	[30]
35	0.0011	630	[32]
35	0.0048	145	[24]
4	0.0075	93	This study
20	0.0086	81	This study

To date, more than 100 full-scale partial nitritation—anammox installations have been operated or are under construction worldwide [6]. However, the biological treatment of industrial wastewater is often challenged by its operation under transient states with respect to the flow pattern or variable influent characteristics [7,8]. In terms of the working mode, the wastewater flow could be completely stopped for several weeks or even months due to seasonal closure of the industry and annual maintenance or vacation periods, which are common in some of the aforementioned industries [9]. Theoretically, starvation is not beneficial to the structure, stability and microbial activity of the granules of the bacterial community, which has a close relationship with the starvation conditions (starvation phase, time and pattern) according to the published literatures [10–12].

The anammox granules have several advantages over flocculent sludge, such as a denser and stronger aggregate structure, better settleability, more ensured solid-effluent separation, higher biomass concentration and greater ability to withstand shock loadings and toxicity in industrial wastewaters [13]. The granule-based process is applied in more than 20% of all full-scale partial nitritation—anammox systems [6]. However, the properties of anammox

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