



# Restoration of real sewage partial nitrification-anammox process from nitrate accumulation using free nitrous acid treatment



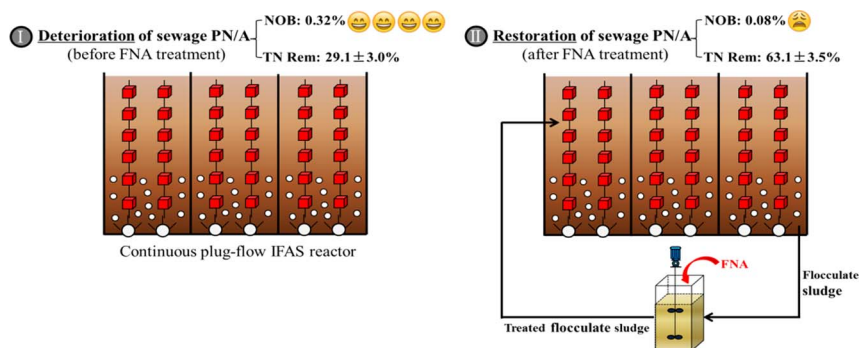
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## GRAPHICAL ABSTRACT



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## ABSTRACT

This study presented a strategy for recovering partial nitrification-anammox (PN/A) of real sewage from nitrate accumulation using free nitrous acid (FNA) treatment. Sewage PN/A was successfully achieved in an integrated fixed-film activated sludge (IFAS) reactor but effluent nitrate gradually increased. For recovering the system performance, flocculent sludge of the reactor was collected and treated with FNA of 1.35 mg/L for 24 h. After FNA treatment, effluent nitrate decreased from 17.6 to 6.1 mg/L with an increase of total nitrogen removal efficiency from 29.1% to 63.1% within 32 days. The improvement of nitrogen removal was mainly due to the selective suppression of FNA on nitrite-oxidizing bacteria. Its relative abundance decreased from 0.32% to 0.08% and the activity declined from 9.05 to 2.42 mg N/(g MLSS·h). Meanwhile, ammonium-oxidizing bacteria and anammox bacteria were barely affected. Overall, IFAS reactor combined with FNA treatment potentially provided a promising technology for stable operation of one-stage sewage PN/A.

## 1. Introduction

Partial nitrification-anammox (PN/A) is a novel and sustainable process for biological nitrogen removal. Compared to the conventional

nitrification and denitrification process, PN/A process could save 60% of the aeration consumption and nearly 100% of the organic carbon demand (Mulder, 2003; Ma et al., 2016). So far, the one-stage PN/A process has been successfully applied in ammonium-rich wastewater

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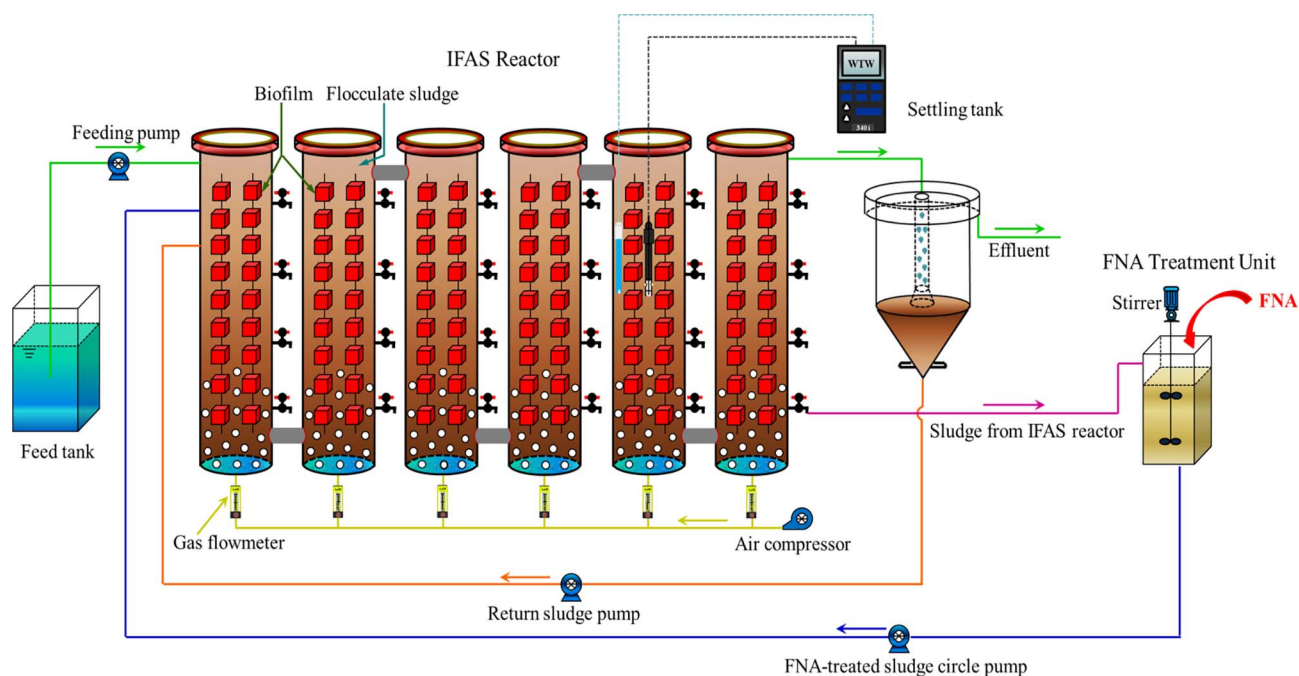


Fig. 1. Schematic diagram of the continuous-flow IFAS reactor and FNA treatment unit used in the experiment.

treatment (Lackner et al., 2014; Cao et al., 2017), such as reject water (Abma et al., 2010; Schaubroeck et al., 2015) and landfill leachate (Wang et al., 2010). For sewage treatment, the application of sewage PN/A process is also of great significance since it bears the potential to bring wastewater treatment plants close to energy autarky (Siegrist et al., 2008).

Despite of increasing experimental evidence, full-scale application of PN/A for the direct treatment of real sewage has not yet fully succeeded (Xu et al., 2015). For sewage anammox treatment, one of the most critical and difficult bottlenecks is the continuous suppression of nitrite-oxidizing bacteria (NOB) (Pérez et al., 2014). The enrichment of NOB would increase the effluent nitrate and reduce the nitrogen removal efficiency. Moreover, NOB growth has been widely reported in sewage PN/A process, seriously affecting the nitrogen removal efficiency. Miao et al. (2016) reported that PN/A reactor was deteriorated by the increase of NOB population and its activity. Yang et al. (2017a) also reported the stability of sewage PN/A system was seriously affected by NOB enrichment, as evidenced by the effluent nitrate accumulating rapidly to 12.9 mg/L. Thus, for sewage anammox process, effective suppression of NOB is important for the stable operation and full-scale application (Pérez et al., 2014).

In previous studies, different strategies have been proposed to enhance the suppression of NOB over ammonium oxidizing bacteria (AOB) during long-term operation. These strategies included applying intermittent aeration (Ma et al., 2015a,b), keeping a suitable residual ammonium concentration (Poot et al., 2016), physical methods to control (Han et al., 2016) and keeping a low DO concentration (Blackburne et al., 2008; Ma et al., 2015a,b). However, when sewage anammox process has been deteriorated by nitrate accumulations, these strategies tested above might fail to recover the PN/A. Rapid and effective strategies are still required to in-situ restore the deteriorated PN/A process caused by NOB proliferation. To date, several in-situ restoration strategies have been reported, such as applying intermittent aeration (Jardin and Hennerkes, 2012), re-inoculation of the anammox biomass (Joss et al., 2011), elevating substrates level (Wang and Gao, 2016) and adding external chemicals (Wang et al., 2015; Xiao et al., 2015). But the application of these strategies was limited due to the long restoration period and the high cost of operation and chemicals. Therefore, effective in-situ strategies for the restoration of deteriorated

sewage PN/A process are still required.

Previous studies have demonstrated that NOB is more sensitive to free nitrite acid (FNA) than AOB (Vadivelu et al., 2006a,b; Kim et al., 2012; Ma et al., 2017), which provides an alternative to selectively wash-out NOB from the sewage treatment system. Building upon this theory, Wang et al. (2014, 2016a) established a FNA-based side-stream sludge treatment and successfully achieved nitrite pathway of sewage treatment. However, FNA treatment is unfavorable to directly apply in the PN/A process since FNA also presented a high inhibitive effect on anammox bacteria (Strous et al., 1999; Zhou et al., 2011). Recently, the integrated fixed-film activated sludge (IFAS) reactor which combines flocculent sludge and biofilm, has been used for PN/A (Veuillet et al., 2014; Malovanyy et al., 2015a; Zhang et al., 2015a,b; Yang et al., 2017a). In the combined PN/A system, anammox bacteria mainly grows in the biofilm while AOB and NOB dominate in the flocculent sludge (Veuillet et al., 2014; Zhang et al., 2015b; Yang et al., 2017a). The segregation of microbial population allows the separate FNA treatment on AOB and NOB, without significant inhibition on anammox bacteria. To the best of our knowledge, there are few reports on applying FNA treatment strategy to enhance nitrogen removal of sewage PN/A process.

The purpose of this study was to explore the feasibility of FNA-based sides-stream sludge treatment strategy for NOB inhibition and restoration of the deteriorated PN/A process. In this study, the one-stage PN/A process of real sewage was established in an IFAS reactor and deteriorated by the accumulation of effluent nitrate. Flocculent sludge of the IFAS reactor was collected and treated using FNA. The feasibility of the FNA-based sides-stream sludge treatment was investigated and its potential for application was discussed.

## 2. Materials and methods

### 2.1. IFAS reactor setup

Fig. 1 shows a schematic diagram of a lab-scale continuous-flow IFAS reactor. The reactor has a total working volume of 150 L, which was made of plexiglass and consisted of six identical cylinders. The cylinders were connected with alternate top and bottom in series and aerated by a compressor with fine diffusers located at the bottom of the

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