A novel ZVI assembled anammox reactor for strengthening anammox process to adapt extreme circumstance

L. F. Ren, S.Q. Ni

Shandong Provincial Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, Jinan250100, P. R. China (E-mail: sqni@sdu.edu.cn)

Abstract
To further expand the application of anammox biotechnology, a novel zero-valent iron assembled upflow anaerobic sludge bed reactor was employed for strengthening anammox performance under extreme circumstance, i.e. low temperature and shock load. Packed with sponge iron and polyester sponge, this novel reactor could speed up the reboot of anammox activity in 12 days, and improve the adaptability of anammox bacteria at the temperature of 10-15 °C. The high nitrogen loading rate of 1109.2 mg N/L/d could be accommodated in 27 days and the new nitrogen pathway under the effect of sponge iron was clarified using batch tests. Moreover, the real-time quantitative PCR enumeration and Illumina MiSeq sequencing verified the dominant status of Candidatus Kuenenia stuttgartiensis and planctomycete KSU-1, which also demonstrated the positive role of sponge iron on proliferation of anammox microorganisms. The findings might be beneficial to popularize anammox-related processes in municipal and industrial wastewater engineering.

Keywords
Anammox; sponge iron; qPCR; Illumina MiSeq sequencing; shock load; low temperature

INTRODUCTION
Anaerobic ammonium oxidation (anammox) process, which optimal temperature is approximately 33-37°C, has been successfully implemented in lab-scale and full-scale studies, mainly focusing on ammonium-rich wastewater treatment (van der Star et al., 2007). If sufficient activity is possible at low temperatures (i.e., temperatures below 25°C) through appropriate manner, anammox process would be expected to be applicable in kinds of industrial wastewater treatments during winter in North China. Zero-valent iron has been proved as one of effective bioaugmentation agent for anammox activity (Ren et al., 2015), so adding ZVI is a probable method to resist low temperature and sudden increased nitrogen loading rate (NLR).

This study focused on investigating anammox activity reboot in the novel reactor and the anammox performance under low temperature and shock NLR coupling with the addition of sponge iron. Sponge iron batch experiment was employed to explore the new nitrogen turnover pathway.

MATERIAL AND METHODS
A 48 cm height cylindrical column gas tight UASB was designed and fabricated for promoting the reproduction of anammox bacteria and accelerating the start-up of the anammox process. The bottom of this UASB was immobilized with thick pristine sponge iron (ca. 200 g, 1 cm diameter) for providing iron ions under the function of dissolution. Three groups of 50 ml centrifugal tubes containing 250 mg/L nitrate that were prepared to test the reduction capacity of sponge iron from nitrate to ammonium by adding 0, 0.2, and 0.4 g ball milled sponge iron successively.

A couple primer set (Amx809F and Amx1066R) was employed for the amplification and quantization of 16S rRNA genes of anammox bacteria. Amplicon sequencing of bacterial 16S rRNA genes V4 region was amplified using specific anammox primers Anhzs1597f and
The purified PCR products were performed on the Illumina MiSeq platform and Qiime v 1.7.0 were employed to process the sequencing data.

RESULTS AND DISCUSSIONS
A batch experiment was designed to confirm the conversion function and dosage of sponge iron. Nearly none ammonium was produced and none nitrate was consumed in control sample (1#) while about 25 and 50 mg/L nitrate was converted into ammonium in 2# and 3# after 48 h. It was clear that sponge iron could transform part nitrate into ammonium without the participation of microorganisms.

Reboot period of R1 was 12 days, whereas that for R0 was about 16 days with slight lower removal efficiencies of ammonium and nitrite (R1: 96.8%, 98.3%; R0: 92.6%, 95.0%), proving the promoting effect of sponge iron on anammox start-up and nitrogen removal. Fifteen days was consumed for R1 to get rid of the deterioration status caused by the increase of NLR until removal efficiencies picked up to 90.8% (ammonium) and 92.2% (nitrite) on day 88 with a NRR of 252 mg N/L/d, while R0 could not realize the activity recovery until day 100.

To simulate the occasion of sudden increased pollutant levels in wastewater treatments, the concentrations of ammonium and nitrite in influent increased to 252 and 302.6 mg/L respectively from day 137. The HRT was also halved to 12 h, lifting the NLR from 344.5 to 1109.4 mg N/L/d. It could be seen that the ammonium and nitrite removal efficiencies of both reactors in the beginning were rather poor, only about 25-37% (Fig. 1), which reflected the inhibition of shock load on the reactor's nitrogen removal stability and efficiency. In the following days (from day 144), the removal of ammonium and nitrite both improved gradually and the recovery rate of R1 surpassed R0 obviously on account of the addition of sponge iron. After day 164, the ammonium and nitrite removal in R1 all climbed over 90% (ammonium: 90.7%, nitrite: 94.7%) indicating the successful recovery of anammox activity, while the removal efficiencies in R0 were still below 80% on day 168.

![Figure 1](image_url)

**Figure 1.** The nitrogen removal performance of R1 and R0 under sudden increased NLR.

REFERENCES