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# Chemoautotrophic denitrification based on ferrous iron oxidation: Reactor performance and sludge characteristics

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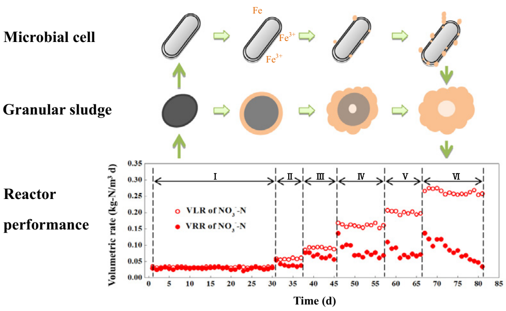
## HIGHLIGHTS

- VLR and VRR for Fe-CAD reactor were $0.26 \pm 0.01$ kg-N/($m^3$·d) and $0.09 \pm 0.03$ kg-N/($m^3$·d).
- Fe-CAD sludge was dominated by *Rhodanobacter*, *Mizugakiibacter*, and *Sulfuricella*.
- Maximum removal rate of $NO_3^-$ by Fe-CAD sludge was 4.68 mg-N/(L·gVSS·h).
- Iron-encrustation caused sludge activity dropping and reactor deterioration.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Aiming at treatment of wastewaters with low C/N ratio, a novel ferrous iron-based chemoautotrophic denitrification (Fe-CAD) reactor was developed with inoculum sludge from a municipal sewage plant in Hangzhou, China. The efficiency of the Fe-CAD reactor was remarkable. The volumetric loading rate (VLR) and volumetric removal rate (VRR) of $NO_3^-$ were $0.26 \pm 0.01$ kg-N/($m^3$·d) and $0.09 \pm 0.03$ kg-N/($m^3$·d), while the VLR and VRR of $Fe^{2+}$ were $3.10 \pm 0.24$ kg-Fe/($m^3$·d) and $1.69 \pm 0.26$ kg-Fe/($m^3$·d), respectively. By means of next generation sequencing, the Fe-CAD sludge was found to be rich in ferrous iron-oxidizing nitrate-reducing bacteria including *Rhodanobacter*, *Mizugakiibacter*, *Sulfuricella*, *Comamonas* and *Gallionella*. Reaction dynamics of the Fe-CAD sludge were determined by batch experiments. After fitted by Haldane Model, the maximum specific activity ($\mu_{max}$), the saturation concentration ($K_i$) and the half inhibition concentration ($K_s$) of $NO_3^-$ to the Fe-CAD sludge were calculated as 0.24 mg-N/(h·gVSS), 72.82 mg-N/L and 2722.97 mg-N/L, while the $\mu_{max}$, $K_i$, $K_s$ of $Fe^{2+}$ were 2.28 mg-Fe/(h·gVSS), 203.09 mg-Fe/L and 229159.38 mg-Fe/L, respectively. The produced ferric iron formed an brilliant yellow iron-encrustation with irregular shape around the functional microorganisms, and the iron-encrustation resulted in a dropped specific activity of the Fe-CAD sludge. Removal or prevention of the iron-encrustation around microbial cells were suggested to be the key to improve the performance of the Fe-CAD reactor.

## 1. Introduction

Nitrate is a worldwide pollutant in water bodies and is notoriously known for its role in eutrophication. Biological denitrification, especially heterotrophic denitrification, is widely used for the nitrogen removal from wastewaters [1]. However, heterotrophic denitrification for treating wastewaters with low C/N ratio

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is a challenge that remains unsolved [2]. The addition of organic electron donors, such as methanol and acetate, allows denitrification increase costs and bear risks of secondary pollution [3].

In 1996, Straub reported a new autotrophic denitrifier using ferrous iron as electron donor [4]. Thereafter the autotrophic denitrification has drawn wide attentions due to its microbial novelty and ecological significance [5–7]. The ferrous iron-based chemoautotrophic denitrification (Fe-CAD) is promising also to treat wastewaters with low C/N ratio because first, ferrous salt is much cheaper than methanol or acetate, and second, the produced ferric iron could serve as coagulants for phosphate removal from wastewaters [8].

Only a few studies on the application of Fe-CAD in wastewater treatment have been reported during the past 20 years [9–12]. Zhang operated a Fe-CAD reactor with inoculum from an IC reactor in a paper mill, the volumetric loading rate (VLR) and volumetric removal rate (VRR) of $NO_3^-$ were 0.16 ± 0.01 kg-N/($m^3$·d) and 0.07 ± 0.01 kg-N/($m^3$·d) respectively [10]. Zhou ran a continuous-upflow Fe-CAD biofilter with *Microbacterium* sp. W5 as inoculum, the maximum nitrogen removal efficiency was about 90% when the influent concentrations of $NO_3^-$-N and $Fe^{2+}$ were 30 mg/L and 800 mg/L respectively [11]. Besides, Li isolated a novel bacterial species able to oxidize ferrous iron and reduce nitrate, and tested its feasibility for *in situ* remediation of nitrate and metals in polluted groundwater [12].

In this work, an up-flow anaerobic sludge bed (UASB) reactor with inoculum sludge from a municipal sewage treatment plant was operated to test the feasibility and suitability of Fe-CAD for treating wastewaters with low C/N ratio. After cultivating for one month, the inoculum sludge in the Fe-CAD reactor showed a very different morphology and was thus entitled the Fe-CAD sludge. Here, characteristics (morphology, component, granularity, settleability, microbial diversity and specific activity) of the Fe-CAD sludge were studied in detail. Furthermore, the relationship between sludge characteristics and the reactor efficiency was revealed. All results obtained here will help to develop/improve the Fe-CAD technology further to treat practical wastewaters with low C/N ratio in future.

## 2. Materials and methods

### 2.1. Reactor setup

The Fe-CAD reactor was 700 mm high, had a volume of 1.3 L with a diameter of 65 mm. Activated sludge from a municipal sewage treatment plant in Hangzhou was inoculated into the Fe-CAD reactor as the inoculum sludge. The total solid (TS) and volatile solid (VS) of the inoculum sludge were 142.0 g/L and 61.1 g/L, respectively.

Synthetic wastewater was applied as influent for the Fe-CAD reactor, the detailed components of which were listed in Table 1. To avoid precipitation of ferrous iron, pH in influent was regulated at 6.2 ± 0.2 using NaOH (1.0 M) and HCl (1.0 M).

**Table 1**
Synthetic wastewater components.

| Components and concentrations of the macronutrient | | | |
|---|---|---|---|
| Chemical substance | Concentration | Chemical substance | Concentration |
| $NaNO_3$ | 0.73 g/L | $FeSO_4·7H_2O$ | 6.00 g/L |
| $NaHCO_3$ | 2.50 g/L | $MgSO_4·7H_2O$ | 0.50 g/L |
| $CaCl_2·2H_2O$ | 0.01 g/L | $(NH_4)_2SO_4$ | 0.28 g/L |
| $KH_2PO_4$ | 0.25 g/L | | |
| Components and concentrations of the micronutrient | | | |
| Chemical substance | Concentration | Chemical substance | Concentration |
| EDTA | 3.000 mg/L | $MnCl_2·2H_2O$ | 0.500 mg/L |
| $NiCl_3·6H_2O$ | 0.024 mg/L | $CoCl_2·6H_2O$ | 0.190 mg/L |
| $CuCl_2·2H_2O$ | 0.010 mg/L | $Na_2MoO_4·2H_2O$ | 0.036 mg/L |
| $H_3BO_3$ | 0.010 mg/L | $ZnCl_2$ | 0.070 mg/L |

### 2.2. Reactor operation

The Fe-CAD reactor was operated in the dark with a constant temperature of 35 °C. The hydraulic retention time (HRT) was 16 h. The initial VLRs of $NO_3^-$ and $Fe^{2+}$ were 0.03 kg-N/($m^3$·d) and 0.27 kg-Fe/($m^3$·d), respectively. Once the Fe-CAD reactor reached its steady state, the VLRs of $NO_3^-$ and $Fe^{2+}$ was increased stepwise for five times to 0.26 kg-N/($m^3$·d) and 1.67 kg-Fe/($m^3$·d). Concentrations of $NO_3^-$, $Fe^{2+}$ and total-iron in both influent and effluent were measured immediately after sampling according to the standard methods [13]. The pH was also determined in the samples.

According to the change of VLR, the reactor operation was divided into 6 stages (Fig. 1). Detailed descriptions were given in Section 3.1.

### 2.3. Sludge morphology

A digital single lens reflex camera (Nikon, Japan) and a stereo discovery stereoscope (Carl Zeiss, Germany) were used to study the morphology of the granular sludge. Transmission electron microscopy (TEM) (Gtontorn, USA) and field emission scanning electron microscope (FESEM) (Hitachi, Japan) were used to analyze the microbial cell structure. Energy dispersive spectrometer (EDS) (Oxford, UK) on the FESEM was used to analyze the elemental composition.

### 2.4. Sludge component

The wet sludge sample was weighed as $M_0$ after being drained by filter papers, and its volume was determined as $V_0$ in a graduated cylinder. Then the sludge was dried at 105 °C for 6 h to weight as $M_1$, and incinerated at 600 °C for 6 h to weight as $M_2$. The water content (WC/%), mineral matter content (MMC/%) and volatile matter content (VMC/%) were calculated as follows (Formula 1, 2, 3).

$$WC(\%) = \frac{100\% \times (M_0 - M_1)}{M_0} \tag{1}$$

$$MMC(\%) = \frac{100\% \cdot M_2}{M_0} \tag{2}$$

$$VMC(\%) = \frac{100\% \cdot (M_1 - M_2)}{M_0} \tag{3}$$

### 2.5. Sludge granularity

Granularities of the sludge samples, including diameter, aspect ratio, sphericity and convexity, were analyzed by the QICPIC system (Sympatec, Germany) [14].

### 2.6. Sludge settleability

Settling velocities ($V_s$) of the sludge samples were determined in a graduated cylinder (1L) according to Li et al. [15].