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# Characteristics of self-alkalization in high-rate denitrifying automatic circulation (DAC) reactor fed with methanol and sodium acetate

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## HIGHLIGHTS

- Self-alkalization of high-rate denitrifying reactor was remarkable.
- Self-alkalization with acetate as carbon source was stronger than that of methanol.
- Excellent self-adaptation to alkalinity was found in high-rate denitrifying reactor.
- The self-adaptation mechanism of high-rate denitrifying reactor was revealed.

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## ABSTRACT

Denitrification is a self-alkalization process. In this experiment, the characteristics of self-alkalization in high-rate heterotrophic denitrifying automatic circulation (DAC) reactor fed with methanol and sodium acetate were investigated, respectively. The results showed that, (1) The self-alkalization of high-rate denitrifying reactors was remarkably strong both with methanol and sodium acetate as carbon sources, while the effluent pH was much lower than the stoichiometric values and the malfunction from self-alkalization of denitrification was far less serious than expected. (2) The self-adaptation of the reactors was attributed to the neutralization of carbon dioxide (oxidization product of organic matter) and the self-adaptation of denitrifying sludge. The formation and discharge of calcium carbonate precipitates gave rise to lower effluent pH and alkalinity than the stoichiometric values.



## 1. Introduction

Simultaneous elimination of nitrogen and organic matters by biological denitrification has become increasingly important in wastewater treatment (Kapoor and Viraraghavan, 1997; Nancharaiah and Venugopalan, 2011; Hu et al., 2012). The development of denitrifying reactor can improve denitrification technology (Rabah and Dahab, 2004). The denitrifying granular sludge reactor was reported in 1975 for the first time (Miyaji and Kato, 1975) and it has been a focus in the field of denitrification technology due to its excellent performance and low cost. The maximum nitrogen removal rate (NRR) and COD removal rate (ORR) reported so far in literature are 25 kg N $m^{-3}$ $d^{-1}$ (Bode et al., 1987) and 67.5 kg COD $m^{-3}$ $d^{-1}$ (Franco et al., 2006), respectively.

Denitrifying bacteria played a key role in denitrifying reactor and the reactor performance was largely dependent on their growth and metabolism (Lew et al., 2012; Volcke et al., 2012). Like other species, denitrifying bacteria have their own pH or alkalinity range for growth. Drastic variations in environmental pH can harm microorganisms by inhibiting the activity of enzymes and membrane transport proteins. It has been reported that the suitable pH range for denitrifying bacteria growth is 7.5–9.0 (Glass and Silverstein, 1998; Lee and Rittmann, 2003). However, the denitrification is a self-alkalization process and it often leads to a pH of reaction solution over 9.7 at high substrate concentrations (Jin et al., 2012). Methanol is the most popular carbon source for denitrification both in experiments and engineering applications Eq. (1). If the alkalinity from denitrificaiton with methanol as carbon source was taken as a standard, the carbon sources can be divided into 3 types: weak alkalizer, moderate alkalizer and strong alkalizer. Methanol and sodium acetate Eq. (2) belong to

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moderate alkalizer (normal alkalizer because of its popularity) and strong alkalizer, respectively.

$$NO_3^- + 1.08CH_3OH \rightarrow 0.056C_5H_7NO_2 + 0.47N_2 + 0.52HCO_3^- + 0.24CO_3^{2-} + 1.68H_2O \quad (1)$$

$$NO_3^- + 1.06CH_3COO^- \rightarrow 0.15C_5H_7NO_2 + 0.42N_2 + 0.66HCO_3^- + 0.70CO_3^{2-} + 0.73H_2O \quad (2)$$

$$[H^+] = K_{a2}\frac{[HCO_3^-]}{[CO_3^{2-}]} \Longleftrightarrow pH = pK_{a2} - \lg\frac{[HCO_3^-]}{[CO_3^{2-}]} \quad (3)$$

where $K_{a2}$, $[HCO_3^-]$, $[CO_3^{2-}]$ and $[H^+]$ were secondary ionization constant of carbonate ($10^{-10.30}$, 30 °C), $HCO_3^-$ concentration, $CO_3^{2-}$ concentration and $H^+$ concentration, respectively.

Surprisingly, the effluent pH of high-rate denitrification reactor was much lower than the stoichiometric value and the malfunction from self-alkalization of denitrification was far less serious than expected. Here the self-adaptation to alkalinity of heterotrophic denitrification in a high-rate reactor and its mechanism were investigated to promote the development and application of high-rate denitrifying reactor.

## 2. Methods

### 2.1. Synthetic wastewater

In one reactor, $NaNO_3$ and $CH_3OH$ (carbon source) were added to get concentrations of 1 g $NO_3^-$–N $L^{-1}$ and 5 g COD $L^{-1}$, respectively. In the other reactor only $CH_3OH$ was replaced by $CH_3COONa$ (carbon source) with the same COD concentration. The other constituents of the mineral medium were (g $L^{-1}$): $KH_2PO_3$ 0.05, $CaCl_2$ 0.4, $MgSO_4{\cdot}7H_2O$ 0.1 and 1 ml $L^{-1}$ of trace elements solution. The trace elements solution contained (g $L^{-1}$): 5 EDTA, 5 $MnCl_2{\cdot}4H_2O$, 3 $FeSO_4{\cdot}7H_2O$, 0.05 $CoCl{\cdot}6H_2O$, 0.04 $NiCl_2{\cdot}6H_2O$, 0.02 $H_3BO_3$, 0.02 $(NH_4)_6Mo_7O_2{\cdot}4H_2O$, 0.01 $CuSO_4{\cdot}5H_2O$ and 0.003 $ZnSO_4$. The pH of synthetic wastewater was in the range of 7.1 ± 0.2.

### 2.2. Reactor operation

The experimental work was carried out in two parallel plexiglass-made denitrifying automatic circulation (DAC) reactors (one fed with $CH_3OH$, the other fed with $CH_3COONa$) with inner diameter of 0.06 m and height of 0.45 m (Fig. 1). The effective volume was 1.25 L (Li et al., 2013). 1 L denitrifying granular sludge obtained from other lab-scale reactor was used as seeding sludge. The VSS/SS and settling velocity (Vs) were 0.55 and 79.41 ± 13.77 m $h^{-1}$ (Li et al., 2013), respectively. The reactor was started up at high NRR of 25 kg $m^{-3}$ $d^{-1}$ (Bode et al., 1987) with a fixed effluent recycling ratio (recycling flow to inflow ratio) of 2.0. Both the synthetic wastewater and the recycling liquid were mixed in the manifold at the bottom of reactor. The loading rate was increased by shortening hydraulic retention time (HRT) (Tang et al., 2011). Experimental temperature was set at 30 ± 1 °C.

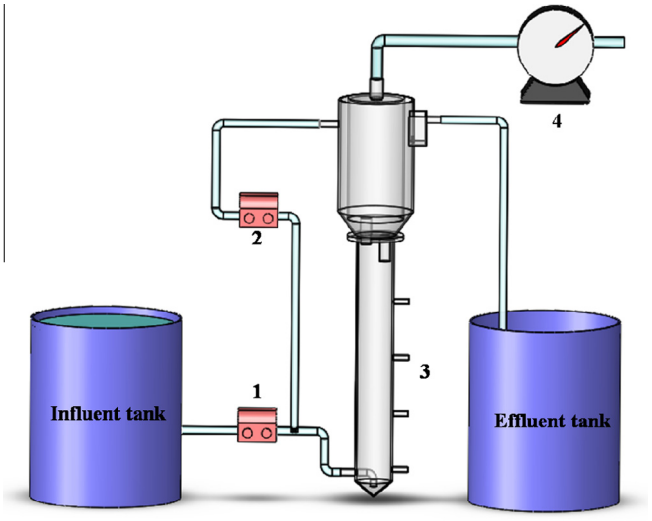


**Fig. 1.** DAC reactor system.

### 2.3. Analytical methods

The influent and effluent samples were taken using a syringe and filtrated with disposable Millipore filter units (0.45 μm pore size) for analyses of pH, alkalinity, calcium, nitrate, nitrite, ammonium and chemical oxygen demand (COD). The pH, alkalinity, calcium, nitrate, nitrite, ammonium, COD, suspended solids (SS) and volatile suspended solids (VSS) were determined according to the Standard Methods (APHA, 2005).

For analysis of total calcium in granules, samples of 20 ml were directly transferred into a digestion tube and 2.5 ml 65% $HNO_3$, 7.5 ml 37% HCl and 10 ml distilled water were added. Hereafter, the mixture was heated for two hours at 80 °C, subsequently cooled, and diluted with demineralized water in a volumetric flask of 100 ml. Then, calcium was analyzed according to the Standard Methods (APHA, 2005).

For scanning electron microscopy (SEM) analysis, granules were fixed for 2 h in 2.5% glutaraldehyde. After rinsing twice with sodium cacodylate buffer, the granules were fixed for 1.5 h in 1% osmium tetroxide. After rinsing with demineralized water, the aggregates were dehydrated in an ethanol series (10%, 30%, 50%, 70%, 90% and 100%, 20 min per step) and subsequently dried at critical point with $CO_2$. After gold/palladium sputter coating, the aggregates were examined by SEM. SEM was performed according to Zhang et al. (2009). Energy-dispersive X-ray spectroscopy (EDS) was used for element content analysis of the precipitates in granules. The samples tested were the same as in SEM analysis.

### 2.4. Specific denitrification activity (SDA) assays

The denitrification batch assays were performed in serum bottles with a volume of 120 ml. A synthetic wastewater with $NaNO_3$ concentration of 200 mg $L^{-1}$ was prepared. The $NO_3^-$–N/COD ratio was fixed at 5. The pH were adjusted as needed (glycine-NaOH buffer was used for pH of 9.3 ± 0.1 and pH of 9.7 ± 0.1, $Na_2HPO_4$–$NaH_2PO_4$ buffer was used for pH of 7.8 ± 0.1). The biomass concentration was about 1 g VSS $L^{-1}$. The temperature was 30 ± 1 °C. Gas and liquid phases were purged with 95% Ar-5% $CO_2$ for 20 min. The serum bottles were sealed tightly with butyl rubber caps. The $NO_3^-$–N concentration was monitored during the incubation. SDA was estimated from a decrease of substrate concentration and biomass concentration.

## 3. Results and discussion

### 3.1. Performance of the DAC reactor

#### 3.1.1. Performance of the DAC reactor fed with methanol

With the initial NLR of 25 kg $m^{-3}$ $d^{-1}$, the DAC reactor fed with methanol was operated by shortening HRT at fixed influent substrate concentration. The performance of DAC reactor is depicted in Fig. 2. During the whole operation (0–70 day), the NLR increased from 24.58 to 56.40 kg $m^{-3}$ $d^{-1}$, with an increase of NRR from