



Simultaneous enhancement of organics and nitrogen removal in drinking water biofilm pretreatment system with reed addition

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HIGHLIGHTS

- Nitrate removal efficiency was positively related with the influent C/N ratio.
- Nitrogen removal was enhanced via reed addition in drinking water biofilm pretreatment system.
- Combined with HRT regulation, low effluent nitrate and TOC concentrations were achieved.
- Bacteria diversity shifted significantly with the variation of the influent C/N ratio.

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ABSTRACT

A novel drinking water biofilm pretreatment process with reed addition was established for enhancement of simultaneously organics and nitrogen removal. Results showed that nitrate removal efficiency was positively related with the influent C/N ratio, reaching to $87.8 \pm 2.8\%$ at the C/N ratio of 4.7. However, the predicted trichloromethane (THM) levels based on total organic carbon (TOC) and UV_{254} were high with the increase of influent C/N ratio. Combined with the pollutants removal performance and microbial community variation, an appropriate C/N ratio via reed addition was determined at 2.2 for the continuous biofilm reactor. With adjustment of hydraulic retention time (HRT), the highest of nitrate removal efficiency ($74.2 \pm 1.4\%$) and organics utilization efficiency ($0.63 \text{ mg NO}_3^- \text{ -N mg}^{-1} \text{ TOC}$) were achieved at an optimum HRT of 18 h, with both low effluent $\text{NO}_3^- \text{ -N}$ ($0.88 \pm 0.03 \text{ mg l}^{-1}$) and TOC ($2.86 \pm 0.67 \text{ mg l}^{-1}$).

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1. Introduction

In recent decades, nitrogen pollution has frequently appeared in natural waters due to wastewater discharge and non-point source of runoff, which poses a threat to public health through drinking water (Camargo and Alonso, 2006). Much attention has been paid to the polluted source water remediation and drinking water treatment, and biofilm treatment with fillers is a popular approach due to its low maintenance cost and effective nitrogen and organics removal (Farhadian et al., 2008; Qin et al., 2007).

It is well known that electron donors are essential to biological denitrification. However, the deficiency of electron donor is very common during the biological denitrification in wastewater and natural water (Calderer et al., 2010; Warneke et al., 2011). Thus, many studies have been carried out in external carbon addition

for the treatment of polluted water with low C/N ratio. Previous studies have proved that organic compound type and level were important for determining the biofilm community and architecture. Srinandan et al. (2012) reported that different exogenous carbon sources (e.g. acetate, glucose and methanol) significantly affected the denitrifying activity, *nosZ* gene abundance and biofilm structure. What's more, several studies focusing on determination of nutrients limiting biofilm development in water treatment and distribution systems, have shown a positive relationship between biodegradable carbon source in water and bacterial growth in biofilm (Chandy and Angles, 2001; Vanderkooij, 1992). In order to enhance biofilm growth and denitrification efficiency for polluted water treatment, the addition of external carbon sources seems to be a very attractive way for nitrogen removal in oligotrophic environment.

Until now, using plant carbon source for denitrification has become more and more popular in the recent years because it is economic and effective (Park and Yoo, 2009). The agricultural by-products (wheat straw, rice husk, etc.) and plants in ecosystem (reed, *Typha latifolia*, *Elodea canadensis*, etc.) are very familiar plants used as extra carbon source (Gibert et al., 2008; Ovez

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et al., 2006; Warneke et al., 2011). The prices of these plants like giant reed and cotton were 0.65 \$/kg substrate (Ovez et al., 2006) and 0.53 \$/kg substrate (Soares, 2000), respectively, which were much lower than those of soluble carbon source (e.g. methanol, ethanol, acetic acid, acetate) (Park and Yoo, 2009) and biodegradable polymers (Boley et al., 2000). However, most of previous studies focused on the nitrogen removal efficiency or mechanism, and little attention has been but paid to the problem of the increased organics, which was vital in drinking water treatment. It has been proved that organics have been closely related to the formation of disinfection by-products (DBPs), which were classified into carcinogenic groups (Platikanov et al., 2010). Several empirical models showed DBP formation was positively related with total organic carbon (TOC) and UV_{254} levels, which were used as good indicators to determine the amount of DBP precursors present in drinking water (Ates et al., 2007; Hong et al., 2008).

In order to expand the application of adding external carbon source into drinking water treatment, it was essential to develop a biofilm process which could simultaneously controlling effluent organics and nitrogen at low levels. Thus, based on the external carbon source addition, carbon source utilization efficiency enhanced method should be taken into consideration. Previous studies showed that when C/N ratio was fixed, a change of organic load rate (OLR) achieved by changing hydraulic residence time (HRT) could affect nitrogen removal performance in a continuous biological reactor. In a submerged biological denitrification system for the treatment of contaminated water, nitrate concentration decreased when HRT was increased greater than 2.4 h (Aslan, 2005). Zhao et al. (2012) obtained an optimum C/N of 0.8 with an HRT of 8 h for heterotrophic denitrification in an intensified biofilm-electrode reactor for the treatment of nitrate-contaminated drinking water. Li and Chu (2003) developed a membrane bioreactor with a short HRT which could achieve sufficient organic degradation and complete nitrification in drinking water.

In this study, a novel drinking water biofilm pretreatment process with plant addition was developed for simultaneous enhancement of microbial growth, organics and nitrogen removal in oligotrophic niche. The objectives are to: (1) obtain an appropriate influent C/N ratio to control reed addition amount through observing nutrients removal performance, DBPs level prediction and microbial community dynamic; (2) achieve good performance of nitrate and organics removal by combining the appropriate reed addition with HRT regulation.

2. Methods

2.1. Contaminated source water quality and reed nutrition

The synthetic contaminated source water was similar to the quality characteristics of surface water in the Hang-jia-Hu area, China. The composition of synthetic source water was as follows ($mg\ l^{-1}$): 11.8 of CH_3OH (4.4 as C), 16 of KNO_3 (4 as N), and 0.9 of KH_2PO_4 (0.2 as P).

The wilted reed was collected in winter from a wetland at Zhejiang University. The reed samples were dried and ground to homogeneous fine powder using a cryogenic impact grinder. Then the powder was emerged into purified water and heated in autoclave ($120\ ^\circ C$ for 1 h). After that, the supernatant was collected as reed nutrition, and stored at $4\ ^\circ C$. TOC, ammonia, nitrite, nitrate, phosphate, total nitrogen and total phosphorus of reed nutrition were analyzed (Table 1).

2.2. Batch experiment for different influent C/N ratio study

The effect of reed nutrition amount on biological denitrification was conducted in eight Plexiglas reactors with cuboid shape

($12 \times 12 \times 30\ cm$). Each reactor (B1–B8) was filled with the same volumetric filling ratios of 2.18% (v/v) (TA-II elastic filler, purchased from Tianyu Environmental Protection Engineering Co., Ltd.), which had a diameter and surface area of 200 mm and $200\text{--}300\ m^2\ m^{-3}$, respectively. No aeration was supplied in the batch reactors, and the dissolve oxygen (DO) was maintained at the range of $0.01\text{--}0.45\ mg\ l^{-1}$. Reactor B1 was fed with only synthetic source water for control, the others (B2–B8) were not only fed with synthetic source water but also with various levels of reed nutrition, which is shown in Table 2. Other operational parameters were as follows: hydraulic retention time (HRT) of 24 h, water temperature at $(25 \pm 2)\ ^\circ C$, pH 7.2–7.8. When a steady state of performance was achieved, samples were analyzed every other day.

2.3. Continuous flow experiments

According to the results in batch experiments, an appropriate C/N ratio by adjusting reed addition was chosen in the continuous flow reactor. The continuous flow biofilm systems belonged to plug flow reactor with long length of 150 cm, with a working volume of 50 l (Feng et al., 2012). To further improve organics utilization and nitrogen removal efficiency simultaneously, three continuous flow reactors were built with different HRT (12, 18, and 24 h), which represented different OLR ($13.14, 9.86, \text{ and } 6.57\ mg\ TOC\ l^{-1}\ d^{-1}$). In the biofilm reactors, TA-II elastic filler was used with a volumetric filling ratio of 2.18% (v/v). During the whole experiments, the room temperature was controlled at $(25 \pm 2)\ ^\circ C$ and DO were all below $0.56\ mg\ l^{-1}$. When a steady performance of reactor was achieved, samples were analyzed every other day.

2.4. Analytical techniques

2.4.1. Regular chemical index analysis

The pH was determined with a digital pH meter (METTLER TOLEDO 320, Switzerland), and DO was measured with a DO meter (YSI Model52, USA).

Nitrate, nitrite and ammonia were analyzed according to standard analytical methods (Chinese Standard Methods for the Examination of Water, 2002).

TOC was determined by a catalyzed combustion TOC analyser (TOC-V CPH, Shimadzu). UV_{254} was UV absorbance at 254 nm wavelength using spectrophotometer (UV-2401PC, Shimadzu).

2.4.2. Biofilm analysis

Genomic DNA of biofilms samples was extracted using a soil DNA kit (OMEGA). PCR was performed with universal bacterial primers (P357f with GC-clamp and P518r) for denaturing gradient gel electrophoresis (DGGE) analysis of total bacteria community. DGGE was conducted according to the previous study (Xu et al., 2012).

DGGE profile was analyzed by the Quantity One software (Version 4.62). The analysis of DGGE community profile similarity used pair wise similarity coefficients (Cs) of unweighted pair group method with arithmetic mean (UPGMA) cluster analysis (Fromin et al., 2002). The Shannon–Wiener index of species diversity (H) was calculated to examine the microbial diversity (Fu et al., 2010).

2.4.3. Trihalomethanes (THMs) predication

THMs are the common primary DBPs in chlorination of drinking water, and there have been some empiric models used to simulate the formation of THMs according to the water quality. In the present study, two empiric THM formation models based UV_{254} (Eq. (1)) and TOC (Eq. (2)) (Hong et al., 2008) were used in raw water, respectively, which are expressed as:

$$\sum THM = 0.125(TOC)^{0.852}(pH)^{1.801}(t)^{0.246} \quad (1)$$

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