Chemosphere 182 (2017) 532–538

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Heterotrophic Ammonia and Nitrate Bio-removal Over Nitrite (Hanbon): Performance and microflora

Wei Li [a], Zhao-yang Cai [a], Zi-Jun Duo [a], Yao-Feng Lu [a], Ke-Xin Gao [a], Ghulam Abbas [a, b], Meng Zhang [a, *], Ping Zheng [a, c, d, **]

[a] Department of Environmental Engineering, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou, China
[b] Department of Chemical Engineering, University of Gujrat, Gujrat, Pakistan
[c] Zhejiang Province Key Lab Water Pollution Control & Environmental, Zhejiang University, Hangzhou, Zhejiang, China
[d] MOE Key Lab of Environmental Remediation and Ecosystem Health, College of Environmental and Resource Science, Zhejiang University, Hangzhou, China

HIGHLIGHTS

- A novel Hanbon was established for simultaneously treating NH_4^+-N and NO_3^--N.
- High nitrogen removal rate of 9.0 ± 0.1 kgN·m^{-3}·d^{-1} was achieved in Hanbon.
- Potential capacity of Hanbon process to treat municipal wastewaters was achieved.
- Microflora in Hanbon were revealed at both high and low nitrogen loading rate.

ARTICLE INFO

Article history:
Received 17 January 2017
Received in revised form
9 May 2017
Accepted 11 May 2017
Available online 13 May 2017

Handling Editor: Hyunook Kim

Keywords:
Wastewater nitrogen removal
Heterotrophic ammonia and nitrate bio-removal over nitrite (Hanbon)
Performance
Microflora

abstract

A novel Heterotrophic Ammonia and Nitrate Bio-removal Over Nitrite (Hanbon) process, combining Short Nitrate Reduction (SNR) with Anaerobic Ammonia Oxidation (Anammox), was developed in a lab-scale continuous up-flow reactor. The substrate effects were investigated to characterize the performance of Hanbon process, and the corresponding microflora information was also revealed. Our results showed that the optimal substrate ratio of NH_4^+-N:NO_3^--N:COD for the Hanbon process was 0.65:1:2.2. The volumetric nitrogen removal rate was up to 9.0 ± 0.1 kgN·m^{-3}·d^{-1} at high influent substrate concentrations of NH_4^+-N 375 mg L^{-1}, NO_3^--N 750 mg L^{-1} and COD 1875 mg L^{-1}, which was superior to the reported values of analogous processes. Moreover, the effluent total nitrogen concentration was able to meet the strict discharge standard (less than 10 mg L^{-1}) at low influent substrate concentration of NH_4^+-N 26 mg L^{-1}, NO_3^--N 40 mg·L^{-1}and COD 88 mg L^{-1}. Illumina-based 16S rRNA gene sequencing results showed that *Halomonas campisalis* and *Candidatus* Kuenenia stuttgartiensis were the dominant bacteria in the SNR section and Anammox section at high substrate concentration condition. However, *Halomonas campaniensis* and *Candidatus* Brocadia brasiliensis were raised significantly at low substrate concentration condition. Hanbon process provided in the present work was flexible of treating wastewater with various nitrogen concentrations, deserving further development.

1. Introduction

Ammonia and nitrate are the common nitrogen contaminants in wastewaters. Biological treatment was regarded as the mainstream technology for nitrogen pollution control. In the past 10 years, the DEnitrifying AMmonia OXidation (DEAMOX) process had been developed to mitigate nitrate and ammonia pollution (Kalyuzhnyi et al., 2006a; Kalyuzhnyi and Gladchenko, 2009).

The application of DEAMOX is dependent on the ability to effectively shunt the nitrite produced by denitrification towards anammox. Only a partial reduction of nitrate to nitrite (partial denitrification, denitratation) is required for the successful application of DEAMOX (Du et al., 2015, 2017). In DEAMOX route, sulfide usually acts as the electron donor to reduce nitrate to nitrite, and

* Corresponding author.
** Corresponding author. Department of Environmental Engineering, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou, China.
E-mail addresses: zhangm_environment@zju.edu.cn (M. Zhang), pzheng@zju.edu.cn (P. Zheng).

http://dx.doi.org/10.1016/j.chemosphere.2017.05.068

nitrite is then utilized as an electron acceptor to oxidize ammonia (Anammox). The total nitrogen (TN) removal efficiency of 90% can be achieved in the baker's yeast wastewater treatment with sulfide driven DEAMOX (Kalyuzhnyi et al., 2006b). However, in most cases the wastewaters were deficient in sulfide, constraining the implementation of the process. Organic matter is another choice for electron donor since it is a common pollutant in nitrogen-containing wastewaters (Cao et al., 2016a, 2016b). Using synthetic wastewater, Kalyuzhnyi et al. (2008) tested if volatile fatty acids (VFAs) could substitute for sulfide in denitratation. However, the nitrite yield from nitrate in this case was poor. Recently, Cao et al. (2013) successfully developed a highly efficient denitratation process with 80% of nitrate-to-nitrite conversion ratio using organic compounds as the electron source, thus overcoming the bottleneck of DEAMOX process. Unfortunately, the combined technology of denitratation and Anammox processes was not fully studied.

In the present work, we have developed a novel Heterotrophic Ammonia and Nitrate Bio-removal Over Nitrite (Hanbon) process, which combined Short Nitrate Reduction (SNR) (shown as equation (1)) (Li et al., 2016) with Anaerobic Ammonia Oxidation (Anammox) (shown as equation (2)) (Strous et al., 1998), for simultaneously removal of nitrate and ammonia in a single continuous upflow reactor. In SNR section, acetate was used to drive the nitrite production from nitrate through denitratation process, and in Anammox section, ammonia and nitrite were converted to nitrogen gas. Compared to traditional nitration/denitrification (N/DN) route, the Hanbon process has significant advantages due to its cost occupied area, energy savings (mainly due to aeration costs), low organic matter quantity requirement, (mainly due to partial denitrification) (Du et al., 2015; Regmi et al., 2016; Vlaeminck et al., 2009). Herein, Hanbon process has can be promising for ammonia and nitrate-containing wastewaters treatment.

$$NO_3^- + 0.465CH_3COO^- \rightarrow 0.069C_5H_7NO_2 + 0.931NO_2^- + 0.534HCO_3^- + 0.052H_2CO_3 + 0.172H_2O \quad (1)$$

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} \quad (2)$$

In previous research works, we successfully enriched the denitritating bacteria from a methylotrophic denitrifying culture with nitrite yield of over 91% (Li et al., 2016). This motivated us to study the balance between SNR and Anammox in Hanbon process. In this study, the effects of substrate (ammonia, nitrate and COD) on the performance of SNR and Anammox were investigated, respectively, so as to establish the balanced activity of different processes in Hanbon process. At the same time information about the microbial community has been investigated. Aside from the work, we hope our efforts would pave the way for the implementation of this technology to control nitrogen pollution in the wastewaters.

2. Material and methods

2.1. Synthetic wastewater

Throughout this study, synthetic wastewater was used for the Hanbon process, a constant pH of 7.4 was maintained, and detail composition of synthetic medium was presented in Table 1. The composition of the mineral medium was presented in Support Information (Tables S1 and S2).

Table 1
The composition of synthetic medium (/L).

Chemicals	Concentrations	Chemicals	Concentrations
$NaNO_3$	As required	$KHCO_3$	0.24 g
CH_3COONa	As required	$NaHCO_3$	0.8 g
$MgSO_4\cdot 7H_2O$	0.5 g	NH_4Cl	As required
$CaCl_2$	0.5 g	Mineral medium I	1 ml
KH_2PO_4	0.072 g	Mineral medium II	1 ml

2.2. Experimental set up

A drawing of the Hanbon reactor used in this study was presented in Fig. 1. Two identical reactors, made of plexiglass, were used here. And the inner diameter of reaction zone is 80 mm and height of 500 mm, with the working volume of 2.5 L. The upside of reactor was inoculated with 1.0 L Anammox granular sludge, and the downside was inoculated with 1.0 L SNR granular sludge. Sample collection ports were selected at the sidewall and outlet of the reactor. The reactor recycle ratio was 2.0, and operation temperature was set at 30 ± 1 °C.

The Anammox granular sludge was collected from a Anammox reactor in our lab with the nitrogen loading rate (NLR) of 5–10 kg $N\cdot m^{-3}\cdot d^{-1}$ (Wang et al., 2016), and the SNR granular sludge was collected from a denitritating reactor with nitrite yield of over 91% (Li et al., 2016). Sodium acetate was taken as carbon source and COD/NO_3^--N was fixed at 2.2 in the SNR section according to the previous experimental results (Li et al., 2016 and unpublished data).

Synthetic medium was pumped continuously into the reactor by a peristaltic pump, synthetic wastewater entering the reactor in the downside, and effluent out of the reactor through the upside. Through SNR section, nitrate was converted into nitrite, providing electron acceptor for Anammox reaction afterwards. In the Anammox section, nitrite and ammonia were converted to nitrogen gas.

2.3. Experimental operation

The reactor operation schedule was presented in Table 2, consisting 6 phases (I - VI). The effect of substrate ratio on Hanbon process performance was investigated during phase I to phase IV; After optimizing the substrate ratio, the effect of substrate concentration on Hanbon process performance was investigated during phase III, phase V and phase VI. To evaluate the effect of

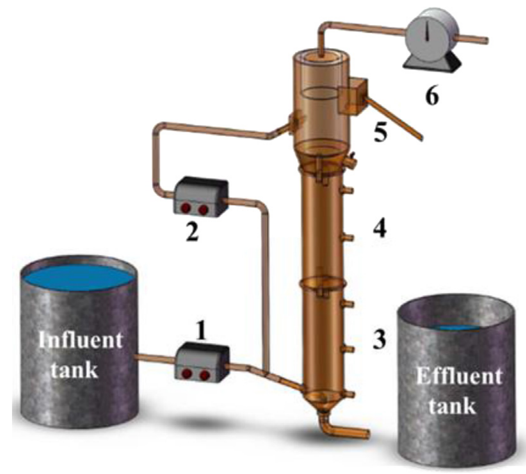

Fig. 1. The Hanbon reactor system: No.1 and No.2 are peristaltic pumps; No.3 is SNR section; No.4 is anammox section; No.5 is separator; No.6 is wet gas meter.