Chemical Engineering Journal 280 (2015) 549–557

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Effect of operational modes on nitrogen removal and nitrous oxide emission in the process of simultaneous nitrification and denitrification

Fang Zhang [a], Ping Li [a,*], Mengsi Chen [a], Jinhua Wu [a], Nengwu Zhu [a], Pingxiao Wu [a], Penchi Chiang [a], Zhiqiang Hu [b]

[a] *The Key Laboratory of Environmental Protection and Eco-Remediation of Guangdong Regular Higher Education Institutions, School of Environment and Energy, South China University of Technology, Guangzhou 510006, PR China*

[b] *Department of Civil and Environmental Engineering, University of Missouri-Columbia, 65211, USA*

HIGHLIGHTS

- Higher nitrogen removal efficiency was achieved in anoxic–aerobic SND system.
- More N_2O was emitted in fully aerobic SND system due to a lower denitrification rate.
- Most of the N_2O yield stemmed from nitrifier denitrification in both systems.
- The enrichment of *Nitrosomonas* sp. led to higher N_2O emission in fully aerobic system.
- *Mesorhizobium* sp. was only observed in fully aerobic SND linked to N_2O production.

ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form 2 June 2015
Accepted 4 June 2015
Available online 11 June 2015

Keywords:
Simultaneous nitrification and denitrification
Nitrogen removal
Nitrous oxide
Operational mode
Wastewater treatment

ABSTRACT

The effects of operational modes (fully aerobic mode and anoxic–aerobic mode) on simultaneous nitrification and denitrification (SND) process were investigated to reveal the different characteristics of nitrogen removal and nitrous oxide (N_2O) emission. Results indicated that total nitrogen (TN) removal and SND efficiencies under anoxic–aerobic mode increased by 17.8% and 10.1% in comparison with fully aerobic mode. Furthermore, N_2O emission in the anoxic–aerobic SND was much lower than in fully aerobic SND. The amount of N_2O emitted per cycle in the fully aerobic SND was 21.9 ± 7.1% of the removed TN, which was three times higher than 7.0 ± 1.6% observed in the anoxic–aerobic SND. Denitrification by nitrifiers was the main source of N_2O emission in both systems. High-throughput pyrosequencing analysis revealed a greater abundance of *Nitrosomonas* sp. and *Mesorhizobium* sp. in fully aerobic SND than anoxic–aerobic SND, which were closely associated with N_2O emissions.

1. Introduction

The stringent effluent quality standards require the development of biological nutrient removal (BNR) systems. BNR systems can be performed by adopting various process configurations, and simultaneous nitrification and denitrification (SND) systems have captured the increasing attention of researchers as it offers the potential for simplifying the operating procedures, reducing the oxygen supplied and energy consumption [1,2]. SND phenomena have already been confirmed in oxidation ditch [3], biofilm [4] and granular sequencing batch reactor [5] by far. Uncontrolled nitrous oxide (N_2O) emissions were observed during nutrient removal due to incomplete nitrification or denitrification under the suboptimal conditions [6]. It is well known that N_2O can be produced through nitrifier denitrification pathway carried out by ammonia oxidizing bacteria (AOB), aerobic hydroxylamine oxidation pathway in nitrification and heterotrophic denitrification pathway in denitrification [6–8]. Previous work had emphasized that nitrifier denitrification was the dominant pathway in nitrifying conditions [7,8].

N_2O is one of the crucial greenhouse gases with its global warming potential of 265 times that of carbon dioxide (CO_2) [9]. In addition, N_2O can be further converted into nitric oxide by photocatalysis, and nitric oxide could deplete the ozone layer [10]. Foley et al. provided an overview of N_2O emission from seven full-scale BNR wastewater treatment plants showing that N_2O emission ranged from 0.006 to 0.253 kg N_2O-N per kg N denitrified [11]. With the popularity of SND technology, N_2O generation from

* Corresponding author. Tel./fax: +86 20 39380568.
E-mail address: pli@scut.edu.cn (P. Li).

http://dx.doi.org/10.1016/j.cej.2015.06.016
1385-8947/

SND systems and methods of its reduction are of great interest to be investigated.

Various studies have been conducted to determine the factors controlling N_2O emission during nitrogen removal processes. These factors include COD/NH_4^+ [12], aeration rate [13,14], temperature [15,16], nitrite accumulation [13,17] and carbon source [18,19]. However, few studies have been performed with regard to the effect of different operational modes of SND on nitrogen removal and nitrous oxide emissions. The available literature shows that the most common operational strategies employed in SND systems are: fully aerobic mode, achieved by applying only aeration in the reaction stage [14,16] and anoxic–aerobic mode with adding a pre-anoxic stage prior to the aeration stage [12,20].

For the fully aerobic mode, the granular sludge cultivated under continuous aeration at fixed levels of dissolved oxygen (DO) had high nitrification activity and more than 60% of the influent nitrogen was denitrified [21]. However, it was also reported that the highest nitrogen removal efficiency only reached 36% when operated at an aeration rate of 0.2 L/min and COD/N ratio of 9.07 [14]. In the case of the anoxic–aerobic mode, the effect of cycle changes on anoxic and aerobic stages settings during the process has been studied [22]. Hu et al. reported nitrogen removal efficiencies ranged from 56.3% to 66.4% when anoxic/aerobic phase fraction changed [15]. Studies have suggested that pre-anoxic application technology had greater denitrification activity compared with other operational modes and the nitrogen removal efficiency reached 90% in high organic loading wastewater [20,23]. Reaching a consistent conclusion of nitrogen removal and N_2O emission data from previous papers is troublesome owing to the different operational and environmental parameters. For instance, the N_2O conversion rates showed tremendous variation in the fully aerobic mode, Kong et al. found the value was 0.6 ± 0.17% of the total nitrogen load [16]; Quan et al. observed the rate reached 2.2–8.2% during the varied COD/N and aeration rates [14]. In comparison with the fully aerobic mode, the N_2O conversion rate in the anoxic–aerobic system ranged from 0.4–27.5% of the incoming TN, depending on the extent of the anoxic and aeration periods [15]. The diverse microorganisms coexisted in activated sludge result in the removal of chemical oxygen demand (COD) and nitrogen. In recent years, researchers have been focused on the characteristics of nitrifying bacteria and denitrifying bacteria microorganisms, however, these papers were adopted molecular biological methods (PCR-DGGE, T-RFLP), methods that are capable of detecting the dominant microbial species, but lack sufficient gene sequence information to reveal the comprehensive community structure [12,16,24,25]. High-throughput pyrosequencing is a more sensitive method for sequencing analysis which can generate huge amount of DNA reads [26,27]. Information regarding the phylogeny, abundance and physiology based on *amoA* and *nosZ* genes involved in nitrification/denitrification can be elucidated by means of high-throughput pyrosequencing in the two SND systems, in order to clarify the N_2O emission.

This research provided a detailed investigation on the nitrogen removal efficiency and N_2O emission in the fully aerobic SND and anoxic–aerobic SND processes. We also identified sources of the N_2O in both SND processes and explored the relationship between N_2O production and microbial community structure, using high-throughput pyrosequencing techniques.

2. Material and methods

2.1. Laboratory-scale bioreactors

Two sequencing batch air-lift reactors (SBARs), each with an effective volume of 6 L were used to cultivate aerobic granule. The SBARs were made of transparent plexiglass, each with a height of 90 cm and internal diameter of 9 cm. The ring-shaped baffle with 80 cm in height, 6 cm internal diameter was vertically placed in the middle of the reactor, dividing the column into raiser zone and down comer zone. Both configurations were operated in successive cycles of 6 h, consisting of influent feeding (5 min), reaction (5 h), settling (1–10 min), withdrawal (4 min) and the 41–51 min idling. In the fully aerobic SND, the aeration stage lasted 5 h. For comparison, in the anoxic–aerobic SND, a pre-anoxic stage (lasting for 45 min) was ahead of aeration (255 min) stage. Aeration was provided by a porous stone diffuser located at the bottoms of the reactor with an airflow rate of 16 L/h. To keep the suspension of the sludge during the anoxic stage, nitrogen gas instead of air at the same gas flow rate was employed to provide the mixing power. Dissolved oxygen set-point of both reactors was adjusted at 0.8∼1.0 mg/L during aerobic periods and less than 0.3 mg/L during anoxic periods. The temperatures of two reactors were maintained at 28 ± 1 °C by using thermostatic heaters. The pH was kept at 7.5 ± 0.2 through the addition of $NaHCO_3$ and Na_2CO_3. 3 L synthetic wastewater was injected into the basin every cycle at a volume exchange ratio of 50%. The sludge retention time (SRT) was kept 25–30 days to ensure the growth of nitrifying bacteria.

2.2. Wastewater composition and seed sludge

The synthetic wastewater contained: COD 300 ± 50 mg L^{-1} (as glucose); NH_4^+-N 45 ± 5 mg L^{-1}; KH_2PO_4 31.5 mg L^{-1}; $MgSO_4{\cdot}7H_2O$ 100 mg L^{-1}; $FeSO_4{\cdot}7H_2O$ 15 mg L^{-1}; $CaCl_2$ 50 mg L^{-1}; $NaHCO_3$ 300 mg L^{-1}; Na_2CO_3 70 mg L^{-1} and 10 mL L^{-1} trace element solution.

The seed sludge was obtained from the secondary sedimentation tank of Yonghe wastewater treatment plant in Guangzhou. The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) of seed sludge were 6191 mg/L and 3135 mg/L, respectively. And the initial MLSS and mixed liquor were 1451 mg/L in the fully aerobic SND and 1538 mg/L in the anoxic–aerobic SND.

2.3. Nitrification inhibition experiments

For the purpose of investigating the characteristics of N_2O emission under different modes, inhibitors were used to differentiate between the N_2O emission contribution of autotrophic and heterotrophic according to the method described by Tallec et al. [13] and Jia et al. [24]. Allylthiourea (ATU) is the inhibitor of the first step of nitrification (NH_4^+-N to NO_2^--N) and chlorate ($NaClO_3$) is the most efficient inhibitor of the second step of nitrification (NO_2^--N to NO_3^--N) [28,29]. The activity of autotrophic nitrifying bacterium was obviously suppressed in the presence of ATU and $NaClO_3$, while the heterotrophic bacteria were not affected.

The experiments were conducted in three identical batch-scale bioreactors with a working volume of 1 L after the acclimation was accomplished. Each mini bioreactor was airtight and filled by 800 ml mixed liquor taken from the SBAR. Then three batch tests were simultaneously operated as follows: (1) without any nitrite or inhibitor addition, as the control group (2) with nitrite addition, and (3) with inhibitors and nitrite addition. The nitrite, inhibitors ATU and $NaClO_3$ were added at the beginning of experiment to reach final concentrations of 10 mg/L, 10 mg/L and 1 g/L, respectively [13]. Dissolved oxygen concentration was maintained 0.8∼1.0 mg/L by adjusting the air flux. Meanwhile, the magnetic stirrer was used for mixing the sludge.

Nitrogen species (NH_4^+–N, NO_3^-–N, NO_2^-–N) were detected every 30 min, N_2O was collected in 100 ml glass syringes and detected every 15 min. Each experiment was conducted three times.