Bioresource Technology 162 (2014) 30–37

Contents lists available at ScienceDirect

Bioresource Technology

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

Population dynamics of nitrifying bacteria for nitritation achieved in Johannesburg (JHB) process treating municipal wastewater

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HIGHLIGHTS

- Mechanism of nitritation startup in a continuous flow process was revealed.
- During nitritation establishing, *Nitrobacter* prior to *Nitrospira* was eliminated.
- Nitritation achieving depended on inhibiting and eliminating of NOB.
- *Nitrosomona-like* cluster and *Nitrosomonas oligotropha* were the dominant AOB.
- Control of aerobic hydraulic retention time did not change AOB communities.

ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form 18 March 2014
Accepted 20 March 2014
Available online 29 March 2014

Keywords:
Nitrite oxidizing bacteria (NOB)
Ammonia oxidizing bacteria (AOB)
Nitritation
Municipal wastewater
Johannesburg (JHB) process

ABSTRACT

Population dynamic of nitrifying bacteria was investigated for nitrogen removal from municipal wastewater. Nitritation was established with nitrite accumulation ratios above 85%. Quantitative PCR indicated that *Nitrospira* was dominant nitrite oxidizing bacteria (NOB) and *Nitrobacter* was few. During nitritation achieving, *Nitrobacter* was firstly eliminated, along with inhibition of *Nitrospira* bioactivities, then *Nitrospira* percentage declined and was finally washed out. Nitritation establishment depended on inhibiting and eliminating of NOB rather than ammonia oxidizing bacteria (AOB) enriching. This is the first study where population dynamics of *Nitrobacter* and *Nitrospira* were investigated to reveal mechanism of nitritation in a continuous-flow process. Phylogenetic analysis of AOB indicated that *Nitrosomonas-like* cluster and *Nitrosomonas oligotropha* were dominant AOB, accounting for 81.6% of *amoA* gene clone library. Community structure of AOB was similar to that of complete nitrification system with long hydraulic retention time, but different from that of nitritation reactor with low DO concentration.

1. Introduction

Traditional biological nitrogen removal (BNR) is accomplished by a two-stage treatment, i.e. nitrification and denitrification. In the first stage, ammonia is oxidized to nitrite by ammonia oxidizing bacteria (AOB), and then to nitrate by nitrite oxidizing bacteria (NOB). Thereafter, nitrate is reduced to nitrite, and then to nitrogen gas (N_2) in the second anoxic denitrification stage (Zhu et al., 2008). Nitrite is an intermediate in two stages. If ammonia is oxidized to nitrite (nitritation), and then directly reduced to N_2 gas (denitritation), the process will be largely shortened. Compared with traditional BNR, aeration costs can be reduced by 25% and demand of carbon source is decreased by 40% in nitritation/denitritation (Sun et al., 2010). For the treatment of carbon-limited municipal wastewater, nitritation/denitritation is particularly advantageous.

Based on the mechanism of nitritation/denitritation, the key to achieve nitritation is to control ammonia oxidizing to nitrite, namely nitrite oxidizing to nitrate is eliminated. From a microbiological point of view, NOB has to be inhibited or eliminated while AOB plays an important role to cause nitrite build-up. Previous studies found that several factors affecting the metabolic activity and growth rate of AOB and NOB, such as high free ammonia (FA) and free nitrous acid (FNA) concentration (Park et al., 2010), pH value (He et al., 2012), temperature (Tao et al., 2012), sludge retention time (SRT) (Hellinga et al., 1998), hydraulic retention time (HRT) (Zeng et al., 2010), dissolved oxygen (DO) (Blackburne et al., 2008; Guo et al., 2009) and inhibitor (Mosquera-Corral et al., 2005). The above selection factors can be used to inhibit or eliminate NOB.

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http://dx.doi.org/10.1016/j.biortech.2014.03.102
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Studies about nitritation/denitritation mainly focused on synthetic or industrial wastewater, such as high temperature wastewater and high nitrogen loaded wastewater. Temperature and FA are used as selection factors to achieve nitritation (Ganigué et al., 2012; Li et al., 2011; Wang et al., 2011). However, FA, FNA, pH value, temperature and inhibitor in municipal wastewater can hardly reach inhibitory level to NOB. Very limited studies on achieving nitritation in the treatment of municipal wastewater primarily focused on sequencing batch reactor (SBR). At sufficient oxygen supply (DO > 2 mg/L), aeration duration was usually controlled to eliminate NOB from SBR process (Fux et al., 2006; Ganigué et al., 2012; Zeng et al., 2009). Continuous-flow process is most commonly applied to treat municipal wastewater. The operational method for continuous-flow process is very limited, and different from SBR process (Wang et al., 2007). Nitritation was achieved in A^2/O and MUCT continuous-flow processes treating municipal wastewater through controlling low DO concentration (0.5 mg/L) and short HRT (6 h) to inhibit NOB (Zeng et al., 2010, 2013). However, the metabolic activity of AOB was inhibited at low DO concentration, and thus ammonia oxidizing rate and removal efficiency dramatically dropped. When DO concentration was up to 1.0 mg/L, nitritation quickly broke down. The above results demonstrated that achieving nitritation and steady nitrogen removal was difficult in a continuous-flow process treating municipal wastewater. The reason may be that the correlation of nitrifying bacteria (AOB and NOB) with process operation is not clear. The DO concentration and HRT are the main operational parameters in a continuous-flow process. Therefore, it is necessary to investigate the population dynamics of nitrifying bacteria at different DO and HRT to reveal the mechanism of nitritation, and set up an effective control strategy.

Presently, real-time quantitative polymerase chain reaction (QPCR) has become a popular method to quantify the abundance of functional bacteria in biological wastewater treatment (Harms et al., 2003). Application of QPCR in nitritation/denitritation was mainly related to quantification of AOB (Wang et al., 2012; Yapsakli et al., 2011), and very limited studies regarding quantification of NOB. However, the key to achieve nitritation is to inhibit or eliminate NOB. The NOB washed out of system is usually demonstrated through a fact that nitrite accumulation ratio (NAR) reaches a high level (>80%). Such indirect inference is not rigorous enough since it cannot distinguish between NOB inhibited and eliminated. The two situations will lead to different operational results. If the metabolic activity of NOB is just inhibited, nitritation will be unstable and even be destroyed when the conditions favor NOB growth. If NOB is washed out of system, nitritation will be stably performed and not be influenced by the short-term change of operational conditions. There is no report regarding the population dynamics of NOB during nitritation establishing. Due to lack of NOB detection in biological wastewater treatment, the correlation of community structure and population dynamics of NOB with operational conditions is not revealed. Therefore, the mechanism of nitritation cannot be clearly explained.

This study aims to (1) set up an effective method to achieve nitritation quickly in Johannesburg (JHB) process treating municipal wastewater at normal DO level, (2) investigate the correlation of population dynamics of AOB and NOB with operational conditions to reveal the mechanism of nitritation startup and (3) analyze the reasons causing the removal of ammonia and total nitrogen unstable during nitritation performance from a microbiological viewpoint.

2. Methods

2.1. Experimental set-up and operation

Fig. 1 shows the experimental system consisting of a Johannesburg (JHB) reactor with a working volume of 71 L and a secondary settler of 24 L. The JHB reactor was divided into seven chambers. The first chamber was a pre-anoxic zone for denitrification of returned sludge (external recycle, R_1) from secondary settler and for one-third of influent. The second chamber provided an anaerobic zone for phosphorus release and for two-thirds of influent. Therefore, organic matter in raw wastewater could be used as the carbon sources for denitrification and phosphorus release. The third and fourth chambers were anoxic zones for denitrification of nitrite/nitrate recirculation (internal recycle, R_2) from the last aerobic chamber. The last three chambers were aerobic zones for ammonia oxidation. The volume ratio of the pre-anoxic to anaerobic to anoxic to aerobic zone was 1.0:1.9:3.4:4.0. The flow rates of two feedings, returned sludge and nitrate recirculation were controlled by peristaltic pumps. Anaerobic zone was equipped with an ORP meter and each aerobic chamber was equipped with one DO probe. The air flow meter controlled the aeration rate to achieve the desired DO concentration. Temperature in the reactor was maintained at 25 ± 1 °C using a heater and thermostat. The sludge retention time (SRT) was controlled at 20 days by discharging an appropriate amount of settled sludge. The mixed liquor suspended solid (MLSS) concentration was about 3500 ± 500 mg/L.

2.2. Wastewater and sludge

The seed sludge was taken from a municipal wastewater treatment plant with a typical anoxic–aerobic process in Beijing.

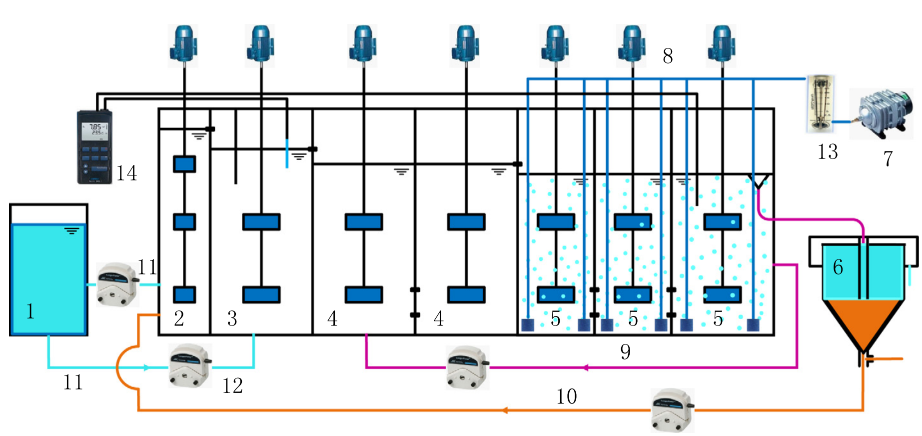

Fig. 1. Schematic diagram of JHB process (1. raw wastewater tank; 2. pre-anoxic zone; 3. anaerobic zone; 4. anoxic zone; 5. aerobic zone; 6. settler; 7. air pump; 8. mixer; 9. internal recycle; 10. external recycle; 11. influent; 12. pump; 13. airflow meter; 14. DO and ORT meter).