



# Integration of denitrifying phosphorus removal via nitrite pathway, simultaneous nitrification–denitrification and anammox treating carbon-limited municipal sewage

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## HIGHLIGHTS

- Achievement of nitrification was a prerequisite to improve nutrients removal.
- Denitrifying P removal via nitrite was the key pathway in anoxic zone.
- Simultaneous nitrification–denitrification contributed to N removal in aerobic zone.
- PCR verified presence of anammox bacteria with the largest of  $1.32 \times 10^6$  copies/g VSS.
- Combining denitrifying P removal, SND with anammox to solve carbon-limited problem.

## ARTICLE INFO

### Article history:

Received 24 July 2014

Received in revised form 11 September 2014

Accepted 14 September 2014

Available online 20 September 2014

### Keywords:

Municipal sewage

Nitrification

Denitrifying phosphorus removal

Simultaneous nitrification and denitrification

Anaerobic ammonia oxidation (anammox)

## ABSTRACT

High nutrients removal above 90% from carbon-limited municipal sewage was obtained without adding external carbon source. Achieving nitrification was a prerequisite to improve nutrients removal. Denitrifying phosphorus (P) removal using nitrite as electron acceptor was the key pathway in anoxic zone, where nitrogen removal reached above 60% and average denitrifying P removal was 88%. Simultaneous nitrification/denitrification and anaerobic ammonia oxidation (anammox) possibly contributed to nitrogen removal of 26–36% in aerobic zone. Quantitative PCR assays presented that the abundance of anammox bacteria under nitrification was more than that under complete nitrification. The largest amount of anammox bacteria was  $1.32 \times 10^6$  copies/g VSS, about 5.6 times increase over a period of 255 days. Nitrite concentration of 17 mg/L in aerobic zone inhibited anammox bacteria. Quantitative results suggested possible occurrence of anammox. Based on performance of nitrification, combining heterotrophic denitrification with autotrophic nitrogen removal is an effective strategy to improve nutrients removal from carbon-limited wastewater.

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## 1. Introduction

Biological nutrients removal is most commonly applied in wastewater treatment due to its economic and efficient characteristic. Recently, innovative processes incorporating both nitrogen and phosphorus (P) removal have been developed to improve treatment capacity and save energy and operational cost, such as short cut nitrification–denitrification, denitrifying P removal, simultaneous nitrification/denitrification (SND) and anaerobic ammonium

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oxidation (anammox). Short cut nitrification–denitrification implies that ammonia is oxidized to nitrite (nitrification), and then directly reduced to nitrogen gas (denitrification) (Zhu et al., 2008). Denitrifying P removal is defined that nitrate or nitrite is used as electron acceptor instead of oxygen for poly-β-hydroxyalkanoates (PHAs) oxidation under anoxic conditions, thereby performing P-uptake and denitrification simultaneously (Kuba et al., 1997). SND means that nitrification/denitrification or nitrification/denitrification occur concurrently in one reactor under aerobic conditions, using nitrate or nitrite as electron acceptor (Zeng et al., 2003). Anammox process converts ammonium to nitrogen gas using nitrite as the terminal electron acceptor (Strous et al., 2006). The novel processes lead to a considerable saving in energy cost and carbon source. Especially, anammox is an anaerobic–autotrophic biochemical reaction,

leading to 60% less oxygen and no extra carbon source in comparison with the traditional nitrification/denitrification (Kuenen, 2008).

Studies about the innovative processes focused on two aspects. Research on microbiological mechanism involving in these processes, such as the physiological characteristics of ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB) and anammox bacteria, and denitrifying capabilities of poly-phosphate accumulating organisms (PAOs) using different types of electron acceptors, established a good base for engineering application (Hellings et al., 1998; Kartal et al., 2007; Lanham et al., 2011). On the other hand, studies on regulating of operational conditions and setup of control method were carried out to achieve these novel processes in sewage treatment (Zeng et al., 2010; Carvalho et al., 2007; Kim et al., 2013a,b). With regard to research methods, these studies had the following three characteristics. First, sequencing batch reactor (SBR) was mainly used due to its flexible operation (Ganigue et al., 2012; Lanham et al., 2011; Kotay et al., 2013). However, continuous-flow process is most commonly used for municipal sewage treatment. Compared with SBR, continuous-flow system is more difficult to achieve the novel processes given above due to its operational restrictions. Very limited studies with continuous-flow system were related to anoxic-oxic (A/O) (Ma et al., 2009) and anaerobic–anoxic–aerobic (A<sup>2</sup>O) process (Kim et al., 2013a). Second, the treated wastewater mainly focused on synthetic wastewater (Liang et al., 2011; Lanham et al., 2011) and special industrial wastewater (Peng et al., 2008; Daverey et al., 2013). However, municipal sewage accounts for a large percentage in wastewater treatment, which is significantly different from synthetic and industrial wastewater in influent composition and microbial diversity. In general, composition of synthetic wastewater is simple, and industrial wastewater possibly contains certain special pollutant. Reactor with synthetic and industrial wastewater usually exhibits a lower microbial diversity than that with municipal sewage. Therefore, these differences may restrict a generalization of knowledge obtained from synthetic or industrial wastewater to municipal sewage. Lastly, previous studies were mostly related to a sole process, such as denitrifying P removal (Kim et al., 2013b) or anammox (Kartal et al., 2010). Integrative study combining shortcut nitrification, denitrifying P removal and anammox for incorporating both nitrogen and P removal is very limited (Yapsakli et al., 2011; Daverey et al., 2013).

Both denitrification and anaerobic P-release requires sufficient carbon source. Organic carbon source in real municipal sewage is typically limiting and can hardly satisfy a need for simultaneous nitrogen and P removal. In order to solve the problem of carbon source, study regarding the improvement of traditional process was conducted to fully utilize carbon source in raw wastewater, such as step-feeding anaerobic/anoxic/aerobic process (Ge et al., 2012). Moreover, innovative biochemical processes were

investigated to reduce the requirements of carbon source (Kartal et al., 2010). Sole process, e.g. partial nitrification, denitrifying P removal or anammox has been intensively studied. However, a systematic strategy for biological nutrients removal from carbon-limited municipal sewage should be further investigated.

Anaerobic/anoxic/aerobic process, as a representative of most full-scale wastewater treatment plants (WWTPs), was used in this study for incorporating both nitrogen and phosphorus removal from real municipal sewage without adding external carbon source. This study aims to (1) achieve denitrifying P removal via nitrite pathway in anoxic zone, simultaneous nitrification–denitrification in aerobic zone and anammox in anoxic/aerobic zone by regulating the operational conditions, (2) develop an integrative strategy combining the improvement of traditional process with establishment of innovative processes to solve the problem of limiting carbon source.

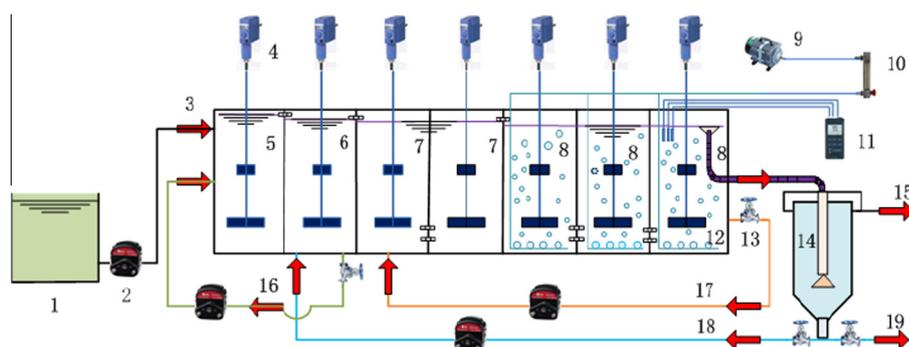
## 2. Methods

### 2.1. Experimental set-up and operation

Lab-scale system consisted of a MUCT reactor with a working volume of 70 L and a secondary settler with a working volume of 24 L (Fig. 1). The MUCT reactor was divided into seven chambers in series. The first four chambers with mechanical mixers were used as anaerobic or anoxic zones and the following three with air diffusers were used as aerobic zones. The first chamber provided an anaerobic zone for P-release and for influent. The second chamber was anoxic zone I for denitrification of returned sludge from secondary settler with a recycle ratio of  $R_1$ . The recycle ratio of mixed liquid from anoxic zone I to anaerobic zone was  $R_2$ . The third and fourth chambers were anoxic zone II for denitrification of recycled nitrification liquid from the last aerobic chamber with a recycle ratio of  $R_3$ . The volume ratio of the anaerobic zone, anoxic zone I, anoxic zone II and the aerobic zone was 1:1:2:3. The flow rates of feeding, returned sludge, nitrification liquid recycle and anoxic recycle were controlled by peristaltic pumps.

### 2.2. Wastewater and seed sludge

Characteristics of municipal sewage are given in Table 1. Raw wastewater from a campus sewer line was pumped into a storing tank for sedimentation, and then fed into the reactor. The average carbon to nitrogen ratio (C/N) was only about 3.0, indicating insufficient organic carbon source in raw wastewater for incorporating both nitrogen and P removal. Seed sludge was withdrawn from secondary settler of a local municipal wastewater treatment plant using an anaerobic–anoxic–aerobic process with good



**Fig. 1.** Schematic diagram of MUCT process. (1) raw wastewater tank; (2) pump; (3) influent; (4) mixer; (5) anaerobic zone; (6) anoxic zone I; (7) anoxic zone II; (8) aerobic zone; (9) air pump; (10) airflow meter; (11) DO and pH meter; (12) air diffuser; (13) valve; (14) settler; (15) effluent; (16) anoxic recycle; (17) nitrification liquid recycle; (18) sludge recycle; (19) waste sludge.

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