Chemosphere 109 (2014) 56–63

Contents lists available at ScienceDirect

Chemosphere

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

Biological nutrient removal with low nitrous oxide generation by cancelling the anaerobic phase and extending the idle phase in a sequencing batch reactor

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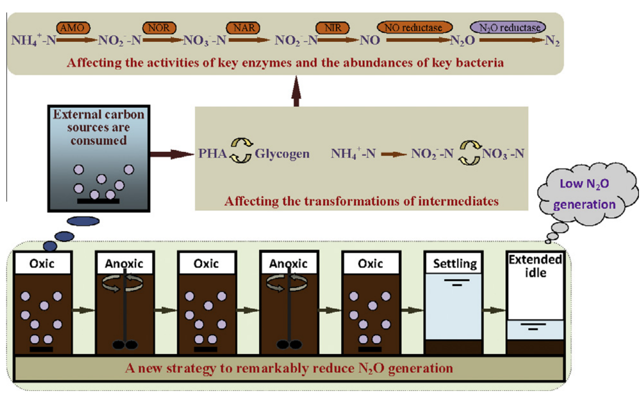
HIGHLIGHTS

- By cancelling anaerobic phase and extending idle phase the N_2O generation was reduced by 42.0%.
- The total nitrogen and phosphorus removals were unaffected.
- The strategy benefited heterotrophic denitrifier dominances.
- The strategy reduced the N_2O amount generated by the aerobic heterotrophic denitrifiers.
- The strategy decreased nitrite accumulation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 November 2013
Received in revised form 7 February 2014
Accepted 7 February 2014

Handling Editor: O. Hao

Keywords:
Nitrous oxide reduction
Biological nutrient removal
Ammonia-oxidizing bacteria
Aerobic/extended-idle phosphorus removal regime

ABSTRACT

Although wastewater biological nutrient removal can be achieved by alternating the anaerobic–oxic–anoxic phases, significant amount of nitrous oxide (N_2O) is generated in oxic phases, where ammonia-oxidizing bacteria (AOB) rather than heterotrophic denitrifiers are the main contributors. Here a new efficient strategy to remarkably reduce N_2O generation was reported. It was found that by cancelling the anaerobic phase and extending the idle phase the N_2O generation was reduced by 42% using synthetic wastewater, whereas the total nitrogen and phosphorus removals were unaffected. The mechanistic investigations revealed that the cancelling of anaerobic phase benefited heterotrophic denitrifiers instead of AOB to be responsible for nitrogen removal in the oxic phases, increased the ratio of total nitrogen removal driven by external carbon source, and decreased nitrite accumulation. Quantitative real-time polymerase chain reaction and fluorescence in situ hybridization analyses further showed that the new strategy increased the number of N_2O reducing bacteria but decreased the abundance of glycogen accumulating organisms, with N_2O as their primary denitrification product. It was also determined that the ratio of nitric oxide reductase activity to N_2O reductase activity was significantly decreased after anaerobic phase was cancelled. All these observations were in accord with the reduction of N_2O production. The feasibility of this strategy to minimize the generation of N_2O was finally confirmed for a real municipal wastewater. The results reported in this paper provide a new viewpoint to reduce N_2O generation from wastewater biological nutrient removal.

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http://dx.doi.org/10.1016/j.chemosphere.2014.02.011
0045-6535/

1. Introduction

Nitrous oxide (N_2O), which is a potent greenhouse gas and an important ozone-depleting substance, is increasing globally at an alarming rate (Kampschreur et al., 2009; Law et al., 2012). One significant source of N_2O production can be attributed to the wastewater treatment plants during biological nutrient removal (BNR) course (Yang et al., 2009), thus the control of N_2O generation in wastewater BNR processes has attracted increasing concerns. N_2O is usually produced in the reactions of microbial nitrification and denitrification under oxic and anoxic conditions. Considering the facts that N_2O has a serious impact on the environment, and BNR processes treat massive amounts of wastewater daily, any improvement for N_2O reduction in current BNR techniques will therefore have significant ecological consequence.

To minimize N_2O generation in wastewater BNR processes, many researchers have performed substantial work. For example, it was found that N_2O production could be reduced by the control of dissolved oxygen (DO) (Rassamee et al., 2011), pH (Pan et al., 2012), free nitrous acid concentration (Zhou et al., 2008), copper ion concentration (Chen et al., 2012; Zhu et al., 2013), wastewater composition (Zhu and Chen, 2011), and wastewater feed mode (Yang et al., 2009). Despite these recent meaningful progresses, the strategy for decreasing N_2O generation from the aspect of modifying BNR operational regime has seldom been reported. To remove nitrogen and phosphorus simultaneously, the conventional BNR process often requires wastewaters to be influent into the anaerobic zone, in which external carbon sources are mainly taken up and synthesized as intracellular polyhydroxyalkanoates (PHA) by polyphosphate accumulating organisms (PAO). As a result, the primary electron donor for subsequent denitrification is not external substrates but the accumulated intracellular PHA. It has been widely reported that the use of internal storage as the electron donor for denitrification will produce more N_2O than that of external substrate (Kampschreur et al., 2009; Law et al., 2012). Thus, if external carbon sources can appear in the period of denitrification or partial denitrification, the generation of N_2O might be reduced.

Apart from the conventional anaerobic/oxic regime, it has been recently reported that the oxic/extended-idle (OEI) regime can also obtain a satisfied biological phosphorus removal, and this regime enriches PAO not by performing the anaerobic phase but by extending the idle phase (Wang et al., 2012a,b, 2013b). Therefore, one method that can be employed to decrease N_2O production, we think, is to modify the conventional BNR regime via cancelling the anaerobic phase and extending the idle phase, because this method makes external carbon sources consumed in the oxic phase where nitrification and nitrifier denitrification will occur (Kampschreur et al., 2009). That is, this method provides a part of external carbon sources as electron donor for denitrification. Furthermore, it is observed that after cancelling the anaerobic phase the nitrifier denitrification pathways and transformations of metabolic intermediates as well as the activities of some key enzymes, such as nitric oxide (NO) reductase and N_2O reductase, are also affected, which decrease N_2O production significantly.

The aim of this paper was to report this efficient strategy for remarkably reducing N_2O generation. Firstly, the generation of N_2O and the performances of nitrogen and phosphorus removal in the reactors of conventional BNR and modified BNR (i.e., cancelling the anaerobic phase and meanwhile extending the idle phase), hereinafter referred respectively as the conventional reactor and anaerobic phase cancelled reactor, were compared. Secondly, the main source and contributor of N_2O generation in both reactors were identified by on-line microsensor measurements and batch experiments with specific nitrification inhibitor addition. Then, the mechanisms for anaerobic phase cancelled reactor causing lower N_2O generation were explored via analyzing the chief pathway of N_2O generation, metabolism of external carbon source, transformations of metabolic intermediates, structures of microbial communities, abundances of key microorganisms, and activities of key enzymes. Finally, mitigation of N_2O generation from wastewater BNR course through cancelling the anaerobic phase and extending the idle phase was tested for a real municipal wastewater.

2. Materials and methods

2.1. Comparison of the nutrient removal and N_2O generation in two reactors fed with synthetic wastewater

Two identical sequencing batch reactors with a working volume of 4 L each were operated and maintained at 21 ± 1 °C in a temperature controlled room. The seed sludge was withdrawn from the secondary sedimentation tank of a municipal wastewater treatment plant in Shanghai, China, and was inoculated into the two reactors simultaneously. Both bioreactors were carried out with three cycles daily.

The operation of conventional reactor was the same as described in the literature with minor revisions (Zhang and Chen, 2009; Tong and Chen, 2009). Each conventional reactor cycle consisted of approximately 90 min anaerobic, 80 min oxic, 50 min anoxic, 20 min oxic, 40 min anoxic, and 20 min oxic phases, followed by 55 min settling, 5 min decanting, and 120 min idle phases. By removing the 90 min anaerobic phase and adding this 90 min in the subsequent idle phase, each cycle of the anaerobic phase cancelled reactor therefore consisted of 80 min oxic, 50 min anoxic, 20 min oxic, 40 min anoxic, and 20 min oxic phases, followed by 55 min settling, 5 min decanting, and 210 min idle phases. It should be emphasized that the anaerobic phase cancelled reactor could also achieve a satisfied phosphorus removal, because it was reported that an extended-idle period (e.g., 210 min), in which polyphosphate was hydrolyzed substantially but the transformations of PHA and glycogen were negligible, could also enhance the role of polyphosphate in PAO metabolism which thereby induced the enrichment of PAO (Wang et al., 2012b).

After settling phase 3 L of the supernatant was discharged from both reactors and was replaced with 3 L of the identical synthetic wastewater (described below) during the initial 5 min of the first oxic period (the anaerobic phase cancelled reactor) and anaerobic period (the conventional reactor), respectively. During the oxic periods, air was supplied into both reactors at a flowrate of 4 L min^{-1}. The hydraulic retention time and sludge retention time in both reactors were maintained at approximately 11 h and 20 d, respectively. Both reactors, unless otherwise described, were generally mixed with a magnetic stirrer except for the settling, decanting, and idle periods. The effluent concentrations of NH_4^+, NO_2^-, NO_3^-, soluble orthophosphate (SOP), and the amount of N_2O production in both reactors were measured twice weekly during the acclimation period. It took about 84 d before these measured data reached relatively stable, then the cycle studies were performed, and the data were reported.

The synthetic wastewater contained 448 mg CH_3COONa L^{-1}, 120 mg NH_4Cl L^{-1}, and 44 mg KH_2PO_4 L^{-1}, yielding an influent chemical oxygen demand (COD), NH_4^+-N, and SOP of approximately 350, 31, and 10 mg L^{-1}, respectively. The concentrations of other nutrients in the synthetic medium were presented as below (g L^{-1}): 0.01 $MgSO_4·7H_2O$, 0.005 $CaCl_2$, and 0.5 mL L^{-1} of a trace metal solution. The trace metal solution was described in our previous publication (Wang et al., 2008).