Bioresource Technology 116 (2012) 379–385

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

N_2O and N_2 production during heterotrophic nitrification by *Alcaligenes faecalis* strain NR

Bin Zhao [a,*], Qiang An [a], Yi Liang He [b], Jin Song Guo [a]

[a] The Key Laboratory of Eco-environments in Three Gorges Reservoir Region, Chongqing University, Chongqing 400045, PR China
[b] School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, PR China

ARTICLE INFO

Article history:
Received 17 January 2012
Received in revised form 28 March 2012
Accepted 29 March 2012
Available online 6 April 2012

Keywords:
Heterotrophic nitrification
Biologic nitrogen removal
Enzyme activity
Hydroxylamine oxidase
Alcaligenes faecalis

ABSTRACT

A heterotrophic nitrifier, strain NR, was isolated from a membrane bioreactor. Strain NR was identified as *Alcaligenes faecalis* by Auto-Microbic system and16S rRNA gene sequence analysis. *A. faecalis* strain NR shows a capability of heterotrophic nitrification and N_2O and N_2 production as well under the aerobic condition. Further tests demonstrated that neither nitrite nor nitrate could be denitrified aerobically by strain NR. However, when hydroxylamine was used as the sole nitrogen source, nitrogenous gases were detected. With an enzyme assay, a 0.063 U activity of hydroxylamine oxidase was observed, while nitrate reductase and nitrite reductase were undetectable. Thus, nitrogenous gas was speculated to be produced via hydroxylamine. Therefore, two different metabolic pathways might exist in *A. faecalis* NR. One is heterotrophic nitrification by oxidizing ammonium to nitrite and nitrate. The other is oxidizing ammonium to nitrogenous gas directly via hydroxylamine.

1. Introduction

Nitrification is an aerobic microbial process by which specialized bacteria oxidize ammonium to nitrite and then to nitrate. Mainly autotrophic nitrifiers are believed to be responsible for this process. However, nitrification is also employed during heterotrophic growth of some bacteria, such as *Thiosphaera pantotropha*, *Alcaligenes faecalis*, *Pseudomonas stutzeri*, *Diaphorobacter* sp. and *Bacillus* sp. (Su et al., 2001; Joo et al., 2005; Kim et al., 2005; Khardenavis et al., 2007). Heterotrophic nitrification is thought to be performed in a similar fashion to the autotrophic process: NH_4^+ is firstly converted to NH_2OH by the enzyme ammonia monooxygenase, and followed by NH_2OH oxidation to NO_2^- by the enzyme hydroxylamine oxidoreductase (HAO), and then NO_2^- is further oxidized to NO_3^-.

Much attention has been paid to heterotrophic nitrifiers because many of them are found to denitrify their nitrification products (NO_2^- and/or NO_3^-) to nitrogenous gas simultaneously under aerobic conditions (Castignetti and Hollocher, 1984; Robertson et al., 1989b). In that case, nitrate reductase (NR) and nitrite reductase (NiR) are active to catalyze the reduction of NO_3^- to NO_2^- and NO_2^- to nitrogenous gas in the presence of oxygen respectively. The coupling of heterotrophic nitrification and aerobic nitrite/nitrate denitrification has been widely accepted as the result of nitrogenous gas production under aerobic conditions (Kim et al., 2005; Khardenavis et al., 2007; Wan et al., 2011; Zhang et al., 2011). However, a few researchers suggest that some heterotrophic nitrifiers proceeded heterotrophic nitrification and nitrogenous gas production simultaneously under aerobic conditions, while aerobic nitrite/nitrate denitrification did not occur. For *A. faecalis* No. 4, high ammonium removal efficiency and aerobic N_2 production were demonstrated. The N_2 production was speculated to be via hydroxylamine, for nitrite and nitrate could not be utilized as the nitrogen sources for denitrification (Joo et al., 2005). Recently, another bacterium, *Acinetobacter calcoaceticus* HNR, was reported to have the capability of heterotrophic nitrification and N_2 production. Also, aerobic denitrification of nitrate and nitrite was not responsible for this N_2 production, while the intermediate hydroxylamine likely contributed to N_2 production (Zhao et al., 2010b). Therefore, heterotrophic nitrification and its coupling to aerobic nitrite/nitrate denitrification may not be the sole metabolic pathway for nitrogenous gas production under aerobic conditions.

It is not yet clear whether the conversion of ammonium to nitrogenous gas only via intermediate hydroxylamine under aerobic conditions is a universal rule. However, in the light of the results presented, it is a fact that some, if not many, heterotrophic microorganisms aerobically convert ammonium into nitrogenous gas via hydroxylamine. Thus, the biological nitrogen removal process can be simplified to a large extent as $NH_4^+ \rightarrow NH_2OH \rightarrow N_2O \rightarrow N_2$, which is of great significance to nitrogen cycle and wastewater treatment application. To date, reports on this novel metabolic pathway are very rare (Joo et al., 2005; Zhao et al., 2010b). In the present work, a heterotrophic nitrifier strain NR

* Corresponding author. Tel./fax: +86 23 65128095.
E-mail address: binzhao11@cqu.edu.cn (B. Zhao).

0960-8524/$ - see front matter
http://dx.doi.org/10.1016/j.biortech.2012.03.113