Bioresource Technology 179 (2015) 421–428

Contents lists available at ScienceDirect

Bioresource Technology

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

Characterization of a halophilic heterotrophic nitrification–aerobic denitrification bacterium and its application on treatment of saline wastewater

Jinming Duan [a,b,*], Hongda Fang [a,b], Bing Su [b], Jinfang Chen [a,b], Jinmei Lin [a,b]

[a] Research Center of Water Treatment Engineering, Jimei University, Xiamen 361021, PR China
[b] School of Food and Biological Engineering, Jimei University, Xiamen 361021, PR China

HIGHLIGHTS

- Halophilic *Vibrio diabolicus* SF16 was isolated from marine sediment.
- It had good heterotrophic nitrifying–aerobic denitrifying ability at high salinity.
- It could utilize ammonium to generate primarily inert N_2 rather than N_2O.
- Strain SF16 improved nitrogen removal efficiency in the biological reactor system.

ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 11 December 2014
Accepted 15 December 2014
Available online 22 December 2014

Keywords:
Vibrio diabolicus
Halophilic bacterium
Heterotrophic nitrification
Aerobic denitrification
Aerated biological filter

ABSTRACT

A novel halophilic bacterium capable of heterotrophic nitrification–aerobic denitrification was isolated from marine sediments and identified as *Vibrio diabolicus* SF16. It had ability to remove 91.82% of NH_4^+-N (119.77 mg/L) and 99.71% of NO_3^--N (136.43 mg/L). The nitrogen balance showed that 35.83% of initial NH_4^+-N (119.77 mg/L) was changed to intracellular nitrogen, and 53.98% of the initial NH_4^+-N was converted to gaseous denitrification products. The existence of napA gene further proved the aerobic denitrification ability of strain SF16. The optimum culture conditions were salinity 1–5%, sodium acetate as carbon source, C/N 10, and pH 7.5–9.5. When an aerated biological filter system inoculated with strain SF16 was employed to treat saline wastewater, the average removal efficiency of NH_4^+-N and TN reached 97.14% and 73.92%, respectively, indicating great potential of strain SF16 for future full-scale applications.

1. Introduction

As the eutrophication of waters becomes more frequent, the removal of nitrogen nutrients gains greater importance in wastewater treatment. Biological treatment processes are widely used in removal of nitrogen and other pollutions because of its high efficiency and low cost. The conventional biological nitrogen removal processes include autotrophic nitrification, which oxidises ammonium via nitrite to nitrate by nitrifying bacteria under aerobic conditions, and heterotrophic denitrification, which converts nitrite and nitrate to N_2 gas by denitrifying bacteria under anaerobic conditions (Khardenavis et al., 2007; Joo et al., 2005). Recently, some bacteria capable of combined heterotrophic nitrification and aerobic denitrification abilities have been isolated and intensively studied as potential microorganisms in biological nitrogen removal systems (Joo et al., 2005; Padhi et al., 2013; Sarioglu et al., 2012; Shoda and Ishikawa, 2014; Zhang et al., 2012). As compared to conventional process, the utilization of these bacteria could not only reduce the size of the reactor required and energy costs, but also increase the nitrogen removal rates and decrease the treating time (Joo et al., 2005; Zhang et al., 2012).

However, the current heterotrophic nitrification–aerobic denitrification bacteria including those above mentioned were almost from fresh water or soil environments, and may not perform efficiently in high salinity (>1%) wastewater, which mainly results from various industrial activities such as seafood processing, tanning and petroleum production, and the use of sea water as a substitute for fresh water in municipal sanitation (Aslan and Simsek, 2012; Zheng et al., 2012; Zhuang et al., 2010). High salinity can cause cell plasmolysis due to the dramatic increase in osmotic pressure and changes on microbial metabolism (Vyrides and

* Corresponding author at: Research Center of Water Treatment Engineering, Jimei University, Xiamen 361021, PR China. Tel.: +86 592 6180385; fax: +86 592 6180470.
E-mail address: duanjinming@jmu.edu.cn (J. Duan).

http://dx.doi.org/10.1016/j.biortech.2014.12.057
0960-8524/

Stuckey, 2009), and the enzyme activity of common microorganism is often inhibited, which lead to poor treatment efficiency during treating saline wastewaters (Uygur and Karg, 2004). Consequently, the utilization of salt-tolerant halophilic microorganisms which can enhance wastewater biological treatment over a wide salinity range and improve the resistance or adaptability to salinity shock is of great significance. But the majority of the reports about isolated halophilic microorganisms have focused on degradation of organic substances (Gao et al., 2013; Zhuang et al., 2010), and halophilic strains capable of heterotrophic nitrification and aerobic denitrification have rarely been reported. Although some halophilic denitrifiers such as *Aeromonas* sp. (Chen et al., 2014), *Halomonas campisalis* (Guo et al., 2013) and *Marinobacter* sp. (Zheng et al., 2012), have been isolated and applied in the treatment of saline wastewater, these strains could remove nitrogen only under low salinity conditions.

In this study, a novel halophilic heterotrophic nitrification–aerobic denitrification bacterium, strain SF16, was isolated from marine sediments and identified. Its ability of heterotrophic nitrification and aerobic denitrification was examined, and the functional genes were amplified from genomic DNA and sequenced. Then key culture conditions affecting on heterotrophic nitrification characteristics of the isolated strain SF16 were investigated. Finally, the performance of strain SF16 as inocula on nitrogen and organics removal from saline wastewater in a biological aerated filter (BAF) system was examined.

2. Methods

2.1. Sampling and culture medium preparation

The marine sediment sample was obtained from Jimei harbour in Xiamen city, Fujian province, China, and used to isolate halophilic heterotrophic nitrification–aerobic denitrification bacteria.

To isolate aerobic denitrification bacteria, basic screening medium (BSM) was prepared as the following components (g/L of distilled water): $NaNO_2$, 0.5, $CH_3COONa{\cdot}3H_2O$ 3.9, $K_2HPO_4{\cdot}3H_2O$ 7.9, KH_2PO_4 1.5, $MgSO_4{\cdot}7H_2O$ 0.1, NaCl 30, agar 20, trace element solution 2 mL, pH 7.0–7.3, salinity 3% (w/v).

Nitrification medium (NM, g/L of distilled water): NH_4Cl 0.50 , $CH_3COONa{\cdot}3H_2O$ 7.50 , $K_2HPO_4{\cdot}3H_2O$ 7.90 , KH_2PO_4 1.50, $MgSO_4{\cdot}7H_2O$ 0.10, NaCl 30, trace element solution 2.00 mL, pH 7.0–7.3, salinity 3% (w/v). NM medium (inorganic medium) was used to determine the ammonium removal ability of the isolated strain.

Denitrification medium (DM, g/L of distilled water): KNO_3 1.00, $CH_3COONa{\cdot}3H_2O$ 7.85, $K_2HPO_4{\cdot}3H_2O$ 7.90 , KH_2PO_4 1.50, $MgSO_4{\cdot}7H_2O$ 0.10, NaCl 30.00, trace element solution 2.00 mL, pH 7.0–7.3, salinity 3% (w/v). The isolates were cultivated in DM medium to test their ability of denitrification.

Trace element solution (g/L of distilled water): Na_2EDTA 63.70, $CaCl_2$ 5.50, $ZnSO_4{\cdot}7H_2O$ 3.90, $MnCl_2{\cdot}4H_2O$ 5.06; $FeSO_4{\cdot}7H_2O$ 5.00, $Na_2MoO_4{\cdot}2H_2O$ 1.00, $CuSO_4$ 1.01, $CoCl_2{\cdot}6H_2O$ 1.61.

Luria–Bertani (LB) medium (g/L of distilled water): beef extract 5, tryptone 10, NaCl 5, pH 7.0–7.3. LB medium was used to extract genomic DNA of the isolated strain.

2.2. Isolation of bacterial strains

Ten grams of sediment samples were add to a beaker with 90 mL sterile water, and serial dilutions within the range of 10^{-2} to 10^{-7} were inculcated on solidified BSM medium. The inoculated media were incubated at 30 °C for about 2–3 days under aerobic conditions until visible colonies had formed. A total of 19 bacterial colonies were picked and streaked on fresh agar plates with the aim of obtaining pure colony. Then, the isolates were streaked onto DM medium and incubated with constant shaking (120 rpm) at 30 °C to test their ability of denitrification. The strain SF16 with the highest denitrification ability was selected for further study.

2.3. Identification of strain SF16

The physiological and biochemical tests were performed according to the methods of Dong and Cai (2001). Genomic DNA of the isloated bacterium SF16 after cultivation in LB medium for 24 h was extracted with MiniBEST Bacterial Genomic DNA Extraction Kit Ver.2.0 (Takara Biotechnology, China) according to the manufacturer's instructions. DNA extracts were stored at -20 °C. The amplification of the DNA was performed by PCR with universal primers (27f: 5′-AGAGTTTGATCCTGGCTCAG-3′, 1492r: 5′-GGTTACCTTGTTACGACTT-3′), which were designed by Sangon Biocompany (Shanghai, China). The PCR was performed under the following conditions: denaturing step of 95 °C for 5 min, followed by 35 cycles of denaturing for 40 s at 95 °C, annealing for 40 s at 51 °C, and extension for 90 s at 72 °C, and a final extension at 72 °C for 10 min. The PCR products were analyzed with electrophoresis on a 2% agarose gel, and were sequenced by Sangon Biocompany (Shanghai, China). The 16S rDNA sequence was compared with sequences in the GenBank database with online BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi), and submitted to GenBank. A phylogenetic tree was constructed using MEGA 4.0 by the neighbor-joining method.

2.4. Estimation of heterotrophic nitrification and aerobic denitrification of strain SF16

Three milliliter of pre-culture strain SF16 was transferred into 150 mL NM medium and 150 mL DM medium, respectively. The cultures were incubated aerobically for 48 h under following conditions: sodium acetate as carbon source; initial NH_4^+-N 119.77 mg/L; C/N 10; salinity 3%; initial pH 7.2; 30 °C; shaking speed 120 rpm. Samples were withdrawn periodically for measurements of bacterial growth (OD_{600}), dry cell weight (DCW), ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), and nitrate nitrogen (NO_3^--N). All experiments were carried out in triplicate.

2.5. Amplification of napA and narG gene

Genes encoding the periplasmic nitrate reductase (*napA*) and the membrane-bound nitrate reductase (*narG*) were used as functional markers. The *napA* gene is involved in aerobic nitrate respiration and denitrification, while *narG* is involved in anaerobic nitrate respiration and denitrification, being negatively regulated by O_2 and unaffected by ammonium (Philippot, 2002). The *napA* gene was amplified with the forward primer NAP1 (5′-TCTGGACCATGGGCTTCAACCA-3′) and the reverse primer NAP2 (5′-ACGACGACCGGCCAGCGCAG-3′) (Kong et al., 2006). The degenerate primer narGF (5′-GAYATGCAYCCGTT-3′) and the narGR (5′-AYCCARTCRTTRTC-3′) were used to amplify the *narG* gene (Roussel-Delif et al., 2005). The PCR protocol consisted of the following steps: denaturing step of 94 °C for 5 min, followed by 35 cycles of denaturing for 30 s at 94 °C, annealing for 40 s at 56 °C for *napA* and 50 °C for *narG*, extension for 60 s at 72 °C, and a final extension at 72 °C for 7 min. The PCR products were analyzed with electrophoresis on a 2% agarose gel and sequenced directly by Sangon Biocompany (Shanghai, China).

The amino acid sequences of *napA* gene were deduced using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and PFAM (http://pfam.sanger.ac.uk/), and compared with sequences in the GenBank database by BLAST program to confirm its identity as others amino acid sequences of *napA* gene. Phylogenetic trees