한국환경과학회 가을 학술발표회지 제15권(제2호), 436~438, 2006
Proceedings of the Korean Environmental Sciences Society Conference, Nov. 16~18, 2006

PB6)

Biological nitrogen removal under aerobic condition in continuous culture by a newly isolated *Pseudomonas* sp. AD-21

Su-Jeong Yoon*, Min-Ju Kim, Sun-Ja Cho, Hong-Joo Son[1], Jae-Lim Bahk[2] and Sang-Joon Lee
Dept. of Microbiology, Pusan National University, [1]School of Applied Life Science, Pusan National University, [2]Dept. of Environmental Engineering and Biotechnology, Silla University

1. Introduction

It is known that denitrification is facultative trait whose manifestation depends on environmental factors. Recently the existence and growth of denitrifiers have been reported to occur under various aerobic environments (1, 2). It is known that the rate-limiting parameters in aerobic denitrification may include the concentration of dissolved oxygen, C/N ratio (i.e. the concentration of available organic matters and nitrate), temperature and pH. Among these parameters, dissolved oxygen concentration and organic carbon concentration are suggested to be the two major factors affecting the denitrification reaction. Several studies have demonstrated that within the optimal range, the higher the carbon concentration as a energy source, the faster the denitrification reaction of the aerobic denitrifier (3, 4). However, the most suitable C/N ratio for aerobic denitrification is still unknown, especially for novel aerobic denitrifiers.

Under this background to search for best C/N ratio in aerobic denitrification we screened a new aerobic denitrifier and identified the effects of O_2 and NO_3^- on denitrification.

2. Materials and methods

2.1. Strains and media

Pseudomonas sp. AD-21 used in this study was isolated from soil. The following media were used: bromothymol blue (BTB) medium, screening medium (SM), denitrification medium (DM) and Luria-Bertani medium (1% trytone, 0.5% yeast extract, 0.5% NaCl) (6).

2.2. Nitrite reductase genes analysis from denitrifying isolate

Respiratory nitrite reduction to NO is catalyzed by a copper nitrite reductase (NirK) or a cytochrome cd_1 nitrite reductase (NirS). The nitrite reductase is a key enzyme of de-

nitrification process. We identified which type of *nir* gene the strain has. For this, the gene was amplified by PCR with well-known universal primers *nirK* 1F and *nirK* 5R for *nirK* gene, and with *nirS* 1F and *nirS* 6R for *nirS* gene from genomic DNA of the isolates (7). The PCR thermal cycling conditions were as follows: denaturation at 94℃ (5 min); 30 reaction cycles at 94, 60 and 72℃ (1 min each) for denaturation, anealing and extension, respectively.

2.3. Electron donor specificity in batch culture

To investigate their effects on denitrification, various carbon sources were added on DM instead of sodium citrate at a C/N ratio of 10.

2.4. Denitrification at different C/N ratios under aerobic condition in continuous culture

The cultures were harvested when cultures reached the logarithmic growth phase, and resuspended with fresh DM (C/N ratios to 2, 4, 6, 8, 10 and 20) adjust to O.D. 1.0 at 660 nm. The cultures (50 ml) were transferred to 150-ml bottles and incubated at 30℃ (pH 7.0). A continuous nutrient flow was continued at a dilution rate of 2.0-3.0 h^{-1} by fixing O.D. 1 approximately. The filtered air by 0.20 um glass filter (MILLIPORE) was supplied continuously to adjust DO 5.0 - 6.0 mg/L of the culture with air sparger. The culture broth was aseptically withdrawn through sampling tubes, and then NO_3^-, NO_2^- and bio-mass were analyzed.

2.5. Analysis of nitrogen converted to cell mass

Samples were harvested and washed twice with phosphate buffer (8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na_2HPO_4, 0.24 g/L KH_2PO_4 : pH 7.4) because of elimination the effects of ^{14}N in the medium. Then dry cells were prepared by vacuum freeze drying (FREEZE DRY SYSTEM SFDSM06, SAMWON). These dry cells were homogenized and analyzed by Stable Isotope Mass Spectrometer (GV Istrument, Isoprime and EuroVector, EA).

3. Results and discussion

We obtained a 890 bp PCR product amplified with *nirS* primers, but no product with *nirK* primers. So, both of *Pseudomonas* sp. AD-21 has cd_1 nitrite reductase. We inves-tigated the electron donor specificity of denitrification by *Pseudomonas* sp. AD-21 in flask culture under aerobic conditions at early stationary phase. Of the carbon sources tested, acetic acid supported denitrification most efficiently in AD-21. But AD-21 grown very slowly using acetic acid. Methanol as a carbon source was not suitable on denitrification. Because of its toxicity, methanol cannot supported cell growth efficiently either.

We developed the novel method for measurement of intracellular nitrogen. To be as-similated into cell mass, all types of nitrogen were converted glutamate through NH_4^+. We assumed that NH_4^+ in the medium was assimilated preferentially into cell mass rath-

er than other types of inorganic nitrogen. Intracellular nitrogen included 98% of ^{15}N atoms or above at different C/N ratio. From the results, we verified that the amount of removed NH_4^+ was converted to cell mass.

At different C/N ratios, the ammonium ions were removed at almost similar speeds on continuous culture by fixing cell density. The results had shown that C/N ratios hardly effect the amount of nitrogen assimilated into cell mass. In a comparison to assimilatory ammonification, denitrification rates were significantly different at different C/N ratios. In AD-21, it can be seen that the initial denitrification rate increases quickly with increasing C/N ratio. As the C/N ratio increases to about 8, the maximum denitrification rate were reached. Further increase in the C/N ratio results in a decreasing denitrification rate.

Reference

Robertson LA, Niel EWJ, Torremans RAM, Kuenen JG, 1988, Simultaneous nitrification and denitrification in aerobic chemostat culture of *Thiosphaera pantropha,* Appl Environ Microbiol, 54: pp. 2812-2818

Patureau D, Davision J, Bernet N, Moletta R, 1994, Denitrification under various aeration conditions in *Comamonas* sp., strain SGLY2, FEMS Microbiol Ecol, 14: pp. 71-78.

Koike I, Hattori A, 1975, Growth yield of a denitrifying bacterium, *Pseudomonas denitrificans*, under aerobic and denitrifying conditions, J. Gen Microbiol, 88: pp. 1-10

Lesley A, Robertson LA, Kuenen JG, 1983, *Thiosphaera pantropha* gen. nov. sp. nov., a facultative autotrophic sulpur bacterium, J Gen Microbiol, 129: pp. 2847-2855

ROB J. M. VAN SPANNING, EDITH HOUBEN, WILLEM N. M. REIJNDERS, STEPHEN SPIRO, HANS V. WESTERHOFF, AND NEIL SAUNDERS, 1999, Nitric Oxide Is a Signal for NNR-Mediated Transcription Activation in *Paracoccus denitrificans*, J. Bacteriology, pp. 4129-4231

Naoki Takaya, Maria Antonina B. Catalan-Sakairi, Yasushi Sakaguchi, Isao Kato, Zhemin Zhou and Hirofumi Shoun, 2003, Aerobic denitrifying bacteria that produce low levels of nitrous oxide, Appl. Environ. Microbiol., pp. 3152-3157

Claudia Etchebenhere and James Tiedje, 2005, Presence of Two Different Active *nirS* Nitrate Reductase Genes in a Denitrifying *Thauera* sp. from a High-NitrateR moval-Rate Reactor, Appl. Environ. Microbiol., pp. 5642-5645