한국환경과학회 봄 학술발표회지 제15권(제1호), 409~410, 2006
Proceedings of the Korean Environmental Sciences Society Conference, May, 19~20, 2006

PB4)

Efficiencies of denitrification at different C/N ratios under aerobic condition

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1. Introduction

Traditional denitrification is the anaerobic reduction of a fixed nitrogen oxide, coupled with respiratory generation of ATP and release of a gas. (Curtis A. *et al*, 1983) In recent reports, the existence and growth of denitrifiers have been reported to occur under various aerobic environments. The development of denitrifier in an aerobic environment has several advantages when compared to that of traditional denitrifiers in an anoxic system. First, the nitrification/denitrification reaction proceeds in a single reactor, thereby largely reducing the costs for both system space and construction. Second, aerobic denitrifiers can be easily controlled during the treatment operation. These advantages make it particularly attractive for application in tertiary wastewater plants(H. K. Huang *et al*, 2001). It is known that the rate-limiting parameters in aerobic denitrification may include dissolved oxygen concentration, C/N ratio, temperature and pH. However, the most suitable C/N ratio for aerobic denitrification is still unknown, especially for aerobic denitrifiers. In this study, we identified the optimal range of the C/N ratios for an aerobic denitrifier, *Pseudomonas stutzeri*.

2. Materials and methods

2.1. Strains and media

Pseudomonas stutzeri KCTC 2760 originated from Korean Collection for Type Cultures. The following media were used : 1% $NaNO_3$, 0.15% KH_2PO_4, 0.042% Na_2HPO_4, 0.5% CH_3CO_2-NH_4, 0.1% $MgSO_4 \cdot 7H_2O$, 2 ml of trace element solution [ethylenediamine tetra-acetic acid 50.0 g, $ZnSO_4 \cdot 7H_2O$ 22.0 g, $CaCl_2$ 5.54 g, $MnCl_2 \cdot 4H_2O$ 5.06 g, $FeSO_4 \cdot 7H_2O$ 4.99 g, $(NH_4)_5Mo_7O_{24} \cdot 4H_2O$ 1.10 g, $CuSO_4 \cdot 5H_2O$ 1.57 g, $CoCl_2 \cdot 6H_2O$ 1.61 g, H_2O 1000 ml. Adjusted to pH 6.0 with KOH.] (Naoki Takaya *et al*, 2003). For analysis of nitrogen content in cell mass, we used CH_3CO_2-$^{15}NH_4$ instead of CH_3CO_2-NH_4. To investigate the effect of C/N ratio, the carbon concentration was varied from 0.059 C · g/L to 2.547 C · g/L corresponding to C/N ratio of 2, 4, 6, 8, 10.

2.2. Batch culture

P. stutzeri inoculated to 100 ml of medium in a 500 ml Erlenmeter flask. The flask was sealed and rotary shaken at 200 rpm at 30℃.

2.3. Analytical methods

Nitrate, nitrite, ammonium ion were measured using auto ion analyzer. We analyze nitrogen content in cell mass using $^{15}NH_4^+$ by Stable Isotope Ratio Mass Spectrometer. The optical density was directly measured at 660 nm using a spectrophotometer.

3. Results and discussion

There were some differences in growth rate at different C/N ratios. By the results, we suggest that insufficiency of carbon source at low C/N ratios causes low level of cell yield. We assumed that bacteria used NH_4^+ preferentially to incorporate into the biomass. This was proved the culture with $^{15}NH_4$ and $^{14}NO_3$ as nitrogen sources by analysis of isotope in biomass. Analysis of ^{15}N ratio in cell mass by Stable Isotope Ratio Mass Spectrometer shows that most of the N atoms in cell mass were ^{15}N atoms. So we supposed that N content in cell mass is equal to NH_4^+ consumption. The other N atoms of the consumed NO_3^- should have been converted gaseous nitrogen compound. The rates of N consumption were significantly different for the various cultures. Efficiencies of denitrification at different C/N ratios under aerobic conditions are shown in table.

Table. Efficiencies of denitrification at different C/N ratios

	N removal (mg · N/L)	N contents of biomass(mg · N/L)
C/N 10	120.46	66.2
C/N 8	124.77	65.36
C/N 6	72.36	64.04
C/N 4	23.93	63.95
C/N 2	5.33	43.34

Reference

Curtis, A. *et al.*, 1983, Comparison of Denitrification by *Pseudomonas stutzeri, Pseudomonas aeruginosa, and Paracoccus denitrificans*, Appl. Env. Microbiol., 45(4), 1247-1253.

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