Characterization of *Bacillus* species occurring anaerobic denitrification in night soil treatment

박경주, 조경숙[2], 정은주, 염혜경[1], 이병헌[1], 이민규[2], 김중균
부경대학교 식품생명공학부 생물공학전공, [1]환경시스템공학부 환경공학 전공,
[2]화학공학부 화학공학 전공
전화 (051) 620-6186, FAX (051) 620-6180 (김중균)

Abstract

Bacillus species predominantly outgrown in a night soil treatment system were isolated and characterized. Cell interactions took place among them and cell population changed under various culture conditions. Maximum removal of NH_4^+-N and cell production occurred under the conditions of 30% DO and C/N ratio of 8. Additions of 0.8% peptone and 0.3% yeast extract to a basal medium influenced the growth of isolates and the removal of NH_4^+-N in flask culture, and metal ions such as Ca, Fe and Mg also did. During the flask experiment of nitrogen removal under an aerobic condition, active nitrification by the isolates occurred largely in 1 h with the decrease of COD and alkalinity destructed was only 74.6% of theoretical value. From the nitrogen balance, the percentage of nitrogen lost in the flask culture was estimated to be 29.0%. This conversion of ammonia to N_2 under an aerobic condition was confirmed by GC analysis. The B3 process using the *Bacillus* species seemed to have some economic advantage.

Introduction

Nitrogen removal is an important aspect of present day wastewater treatment processes, and biological nitrification-denitrification is one of the most economical processes for nitrogen removal from municipal wastewaters. It has commonly been accepted that denitrification requires anoxic conditions, but there have been periodic reports of aerobic denitrification[1)]. The nitrification- denitrification process has been also challenged by a one-step process in which ammonium is oxidized directly to N_2[2)]. Recently, researches focus on nitrite nitrification, which might be a short cut process for savings in oxygen for nitrification and carbon requirements for denitrification[3)].

In Korea, B3 (Bio Best Bacillus) process (Korean Patent No. 151928) has been known as an advanced wastewater treatment system in which *Bacillus* species predominantly outgrown. *Bacillus* species has known to occur heterotrophic nitrification, but other characteristics of the *Bacillus* species have not studied in detail so far. Understanding characteristics of microorganisms used in the biological nitrification- denitrification process is very important in order to remain high efficiency of treatment at all times, and the possible microbiological nitrogen conversions should be provided. The purpose of this study was to understand *Bacillus* species predominantly outgrown in a night soil treatment system.

Materials and Methods

Isolation of *Bacillus* species

Bacillus sludge were obtained from night soil treatment systems. The samples were first agitated to obtain homogeneous suspensions in sterile 0.2% NaCl. 1 ml of the suspended liquid was pipetted into a 10 ml tube that contained a 0.8% nutrient broth medium. After 2 days of incubation at 30℃ and 150 rpm, colonies were formed separately on 1.5% nutrient agar plates.

Characterization of isolates

To observe the change of cell population among *Bacillus* species under various culture conditions, a 5 l- bioreactor was used. The composition of a medium used in this experiment was (g/l): glucose, 1.05; NH_4Cl, 0.382; KH_2PO_4, 0.131; peptone, 0.05; yeast extract, 0.05; and 1 ml- mineral solution. In flask experiments to study effects of growth factors on growth of isolates, a basal synthetic medium was used, which contained (g/l): KNO_3, 1; K_2HPO_4, 1; EDTA, 3.5×10^{-3}; $ZnSO_4 \cdot 7H_2O$, 2×10^{-3}; $FeSO_4 \cdot 7H_2O$, 10×10^{-3}; $MnSO_4 \cdot 7H_2O$, 2×10^{-3}; $CuSO_4 \cdot 5H_2O$, 1×10^{-3}; $Co(NO_3)_2 \cdot 6H_2O$, 0.2×10^{-3}; and H_3BO_3, 1×10^{-3}.

Capacity of isolates for aerobic denitrification was tested in a tightly sealed 1l- branched flask. Pure oxygen was pressurized into the flask, and a septum was equipped on the mouth of the flask for gas analysis. The composition of the substrate was (mg/l): COD, 250; NH_4^+-N, 35; NO_2^--N, 0; NO_3^--N, 0; TN, 50; and TP, 7. 10% of active isolated *Bacillus* species were inoculated into the flask. The flask was incubated at 150 rpm and 30℃.

Analyses

Growth of isolates was determined spectrophotometrically at 510 nm. The concentrations of NH_4^+-N, NO_2^--N, NO_3^--N COD, TN, TP, alkalinity of medium were determined by procedures given in the Standard Methods. O_2 and N_2 gases were determined by GC/TCD analysis.

Results and discussion

Twenty-four species could be isolated. PK15 grew very well under aerobic conditions (more than 15% DO) and PK5 and PK16 did under the condition of 5% DO. Maximum removal of NH_4^+-N and cell production occurred under the conditions of 30% DO and C/N ratio of 8 (Fig. 1). When DO concentration maintained in a range of 15-30%, DO did not affect the removal efficiency of NH_4^+-N significantly, but the efficiency almost halved under the condition of 5% DO. The removal efficiency of NH_4^+-N by the isolates was higher at a C/N ratio of 8 than that at C/N ratio of 4 (Fig. 2). At a C/N ratio of 8, there was not any effect of the substrate composition on the removal efficiency of NH_4^+-N, but the effect of substrate composition was significant at the C/N ratio of 4.

The concentration of NH_4^+-N decreased significantly in 1 h, and that of TN also did (Fig. 3). The same trend could be seen in both the removal of COD and the destruction of alkalinity. After then, the concentrations of NH_4^+-N, TN, COD and alkalinity did not decrease significantly. The removal efficiencies of NH_4^+-N, TN, COD and TP for 4 h were found to be 72.0, 53.8, 89.5 and 21.4%, respectively. When the concentration of organic nitrogen decreased, cell production almost maintained and the substantial removals of COD and NH_4^+-N did not occur further.

From the nitrogen balance (Table 1), 14.5 mg/l (29% of initial TN) of nitrogen in the medium disappeared after 4h. From the above results, the mixed *Bacillus* cells were found to be able to occur aerobic denitrification indeed. This fact has not been reported yet.

Acknowledgements

This research has been supported by the Korea Science Engineering Foundation (KOSEF) (Grant No. R12-1996-009203-0) through the Institute for Environmental Technology and Industry at Pusan National University.

References

1. Su J-J, Liu B-Y, Lin J, Yang C-P. Isolation of an aerobic denitrifying bacterial strain NS-2 from the activated sludge of piggery wastewater treatment systems in Taiwan possessing denitrification under 92% oxygen atmosphere. (2001) J Appl Microbiol 91, 853-60.
2. Astrid A, van de Graaf AA, Mulder A, de Bruijn P, Jetten MS, Robertson LA, Kuenen JG. Anaerobic oxidation of ammonium is a biologically mediated process. (1995) Appl Environ Microbiol 61, 1246-51.

3. Eum y, Choi E. Optimization of nitrogen removal from piggery waste by nitrite nitrification. (2002) Wat Sci Technol 45, 89-96.

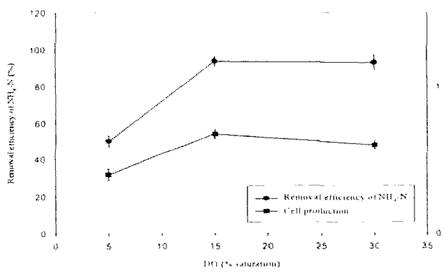

Fig. 1. The effect of DO on removal efficiency of NH_4-N and cell production. Error bar= mean±S.D.

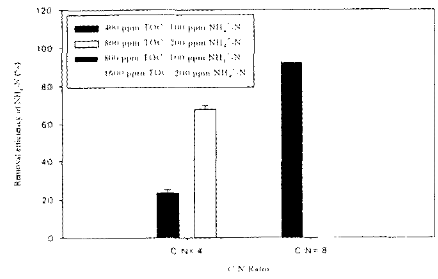

Fig. 2. The effect of substrate composition on removal efficiency of NH_4-N. Error bar= mean±S.D.

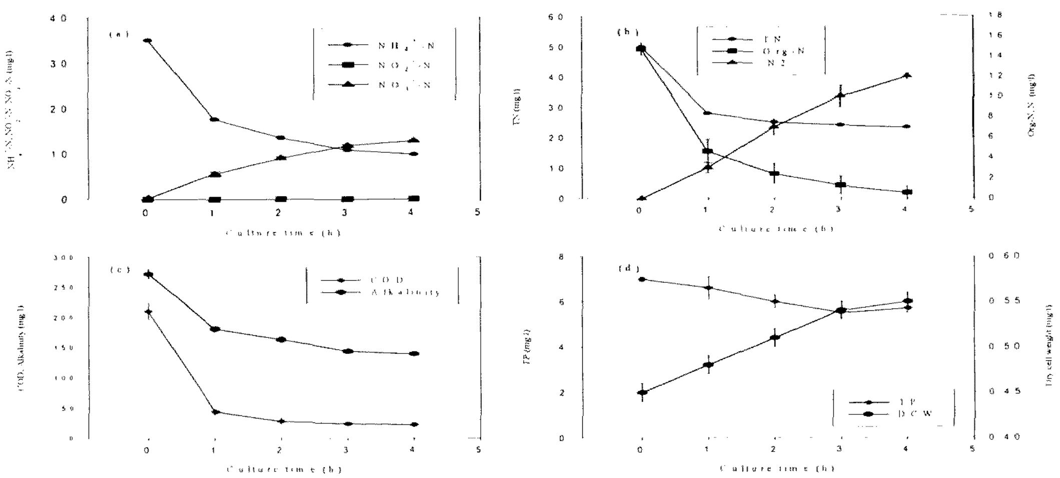

Fig. 3. Changes of NH_4^+-N, NO_2^--N and NO_3^--N (a), TN, Org-N and N_2 (b), COD and alkalinity (c) and TP and DCW (d) in the flask culture of mixed isolates. Error bars: mean±S.D.

Table 1. Nitrogen balance for the flask culture (units: mg/l)

Initial TN	Final NH_4^+-N	Final NO_2^--N	Final NO_3^--N	Final Org-N[1]	N in Biomass[2]	% N lost[3]
50.0	9.8	0	12.8	0.5	12.4	29.0

[1]Calculated value. [2]Biomass composition was assumed to be $C_5H_7O_2N$.

[3]% N lost = 100×{(initial TN)-(final NH_4^+-N)-(final NO_2^--N)-(final NO_3^--N)-(final Org-N) -(N in biomass)}/(initial TN)