Isolation, identification and immobilized-cell characteristics of a bacterium that produces N_2 from NH_4^+ under an aerobic condition

Kyoung Joo Park, Kyoung Sook Cho, Jeong Bo Kim, Min Gyu Lee*, Byunghun Lee**, Young-Ki Hong, Joong Kyun Kim†

Division of Food Science and Biotechnology, *Division of Applied Chemical Engineering, **Division of Environmental System Engineering, Pukyong National University, Pusan 608-737, Korea

Abstract

To treat wastewater efficiently by a one-step process of nitrogen removal, a new strain of N_2-producing bacteria from NH_4^+ under an aerobic condition was isolated and identified. By 16S-rDNA analysis, the isolate was identified as *Enterobacter asburiae* with 96% similarity. The isolate shows that the capacity of N_2 production under an oxic condition was approximately three times higher than that under an anoxic condition. The optimal conditions (pH, temperature and C/N ratio) of the immobilized isolate for N_2 production were found to be 7.0, 30°C and 5, respectively.

Under all the optimum reaction conditions, the removal efficiency of COD_{Cr} and TN reached 56.1 and 60.9%, respectively. The removal rates of COD_{Cr} and TN were highest for the first 2.5 hrs (with the removal COD_{Cr}/TN ratios of 32.1), and afterwards the rates decreased as reaction proceeded. For application of the immobilized isolate to a practical process of ammonium removal, a continuous bioreactor system exhibited a satisfactory performance at HRT of 12.1 hr, in which the effluent concentrations of NH_4^+-N was measured to be 15.4 mg/L with its removal efficiency of 56.0%. The maximum removal rate of NH_4^+-N reached 1.6 mg NH_4^+-N/L/hr at HRT of 12.1 hr (with N loading rate of 0.08 Kg-N/m^3-carrier/d). As a result, the application of the immobilized isolate appears a viable alternative to the nitrification-denitrification processes.

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. The nitrification -denitrification process has been challenged by a one-step process in which ammonium is oxidized directly to N_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.

A high cell concentration is possible with immobilization, and thus the volumetric efficiency is greatly increased. This can lead to relatively small reactors. The immobilized cells may afford protection from adverse conditions by creating micro-environments within the gel matrix, which would help maintain year round treatment. In response to the need for the development of a more compact and an efficient system for treatment of wastewater, immobilized-cells processes have been receiving increasing attention in the field of wastewater biodenitrification recently. As widely recognized, entrapment of cells in a proper support matrix is an effective means for cell immobilization. Compared to commonly used polymeric substances, polyvinyl alcohol (PVA) has some advantages: cheap chemical cost required for cell immobilization and strong gel strength. In addition, PVA gel beads would not float upward to the solution surface by N_2 production, due to their good gas permeability. For this reason, cell immobilization using PVA has been reported to be successfully applied to the immobilization of denitrifying sludge in the denitrification process.

In this study, microorganisms producing nitrogen gas from ammonium ion under an aerobic condition were isolated and identified, and the characteristic of the immobilized isolate was investigated in a five-neck flask. To apply the immobilized isolate to a practical process of nitrogen removal, a continuous stirred bioreactor was also executed with a synthetic medium that simulated municipal wastewater.

Materials and Methods

1. Bacterial isolation and identification

The sludge was obtained from a municipal sewage treatment plant in Busan, Korea. A sludge sample was first agitated to obtain homogeneous suspensions in sterile 0.2% NaCl. A purified isolate was then obtained by repeated streaking on the fresh agar plates. Identification of the isolate was done using 16S ribosomal DNA (rDNA) analysis. DNA was extracted from cells grown in the given medium with the DNA extraction kit. PCR amplification were performed, and its w products were electrophoresed. And then transfomation and selection were performed. These partial sequences were searched against GenBank (http://www.ncbi.nlm.nih.gov/).

2. Test of capacity for N_2 production from NH_4^+

Test of capacity for N_2 production from NH_4^+ was executed under an aseptic condition, 0.86 g (wet weight basis) of pure cells harvested at the end of the exponential growth phase was suspended in the syringe with 20 ml of the culture medium. Oxygen gas was supplied into the syringe with 40 ml at once. The syringe prepared in this way was incubated in a shaking incubator at 30℃ and 180 rpm. The gas produced by the isolate during incubation was analyzed by GC and the concentration of NH_4^+-N in liquid broth was analyzed by IC.

3. Cell immobilization and characteristics of the immobilized isolate

The isolated cells were harvested for cell immobilization. The cell was immobilized in phosphorylated PVA gel beads according to the method of *Chen et al*[1].

In order to determine optimum culture conditions of immobilized isolate, the 100 ml- syringes were incubated under various growth conditions of pH, temperatures and C/N ratios. Gel beads (10% packing) were suspended in the syringe with 20 ml medium. Oxygen gas was supplied into the syringe with 40 ml. The amounts of gases produced by beads at various conditions were measured, and the maximum N_2 production rates of beads were calculated after analyzing the gas by GC.

Under the optimum conditions, the characteristic of the immobilized N_2-producing isolate was examined in a five-neck flask of 1L (working volume). During reaction in the five-neck flask, the concentration of DO and pH were manually controlled between 1-3 mg/L and 6.5-7.0, respectively. Liquid broth was sampled and analyzed the concentrations of nitrate, nitrite and ammonium ions. The ability of N_2 production from NH_4^+ by the isolate was verified by measuring the removal of NH_4^+.

The application of the gel beads to a bioreactor system was executed in a 1L-continuous stirred bioreactor. Gel beads (10% packing) were suspended in the bioreactor with 700 ml of the reduced culture medium. The continuous operations at four different HRT were initiated at a stationary phase of batch culture. The concentrations of DO were controlled at a range of 1-3 ppm, and the feed medium was always maintained fresh.

Results and discussion

1. Isolation and identification

From the strain screening, only two isolates evolved N_2 gas under an aerobic condition(Table 1). By the 16S-rDNA analysis, it was known that only 4 bp were different between the two 16S-rDNA sequences, and the two isolate were identified as *Enterobacter asburiae* with 96% similarity. Interestingly, this species has not reported to produce N_2 from NH_4^+, yet.

Table 1. Characteristics of two isolated bacteria producing N_2 from NH_4^+ under an aerobic condition.

Isolates	Gram	Size (㎛)	Colony pigment		Chain forming	Motility	Agar-stab culture
			NH_4Cl medium	Selective medium			
KJ1	-	L: 2.0, W: 1.0	small, white	bigger, yellowish	pair is common, 4, 6, 8 chains also seen	very active	facultatively anaerobic with deep growth, strong gas production
KJ2	-	L: 1.5, W: 1.2	〃	〃	〃	〃	〃

2. Capacity of N_2 production from NH_4^+ by the free isolate

Test of capacity for N_2 production from NH_4^+ was executed with the free isolate in a 100 ml-syringe. Comparison of capacity for N_2 production from NH_4^+ between oxic and anoxic conditions is presented in Table 2.

Table 2. Comparison of capacity for N_2 production from NH_4^+ between oxic and anoxic conditions.

Measurement	Initial	Final	
		Oxic	Anoxic
pH	7.0±0.2	5.6±0.1	4.8±0.1
CODCr (mg/L)	2509.3±11.6	1969.6±22.8	2363.2±17.8
TKN (mg/L)	188.2±8.6	108.9±6.3	128.5±4.7
NH_4^+-N (mg/L)	121.1±2.5	92.8±4.8	111.7±3.1
NO_2^--N (mg/L)	0.0±0.0	0.6±0.3	0.2±0.1
NO_3^--N (mg/L)	0.0±0.0	0.3±0.2	0.1±0.1
N_2 (μmole)	0.0±0.0	171.3±7.6	57.8±2.6
DCW (mg/mL)	2.1±0.1	2.8±0.1	2.6±0.1

3. Characteristics and application of the immobilized isolate

Under all optimum reaction conditions with appropriate oxygen supplement, the characteristics of the immobilized isolate were investigated in a five-neck flask (Figure 1.), in batch operation (Figure 2.) and in continunous operation (Figure 3.).

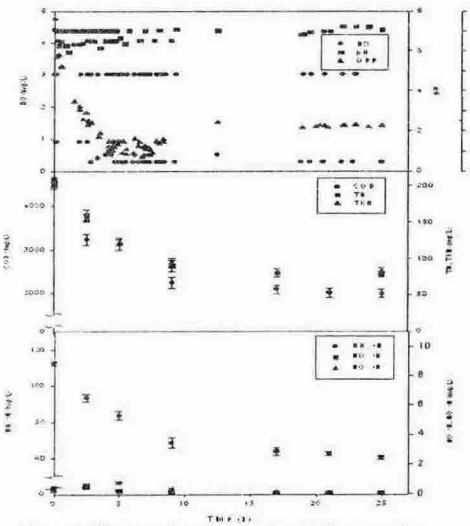

Figure 1. Changes of various parameters in ammonium removal by the immobilized isolate.
(A) DO, pH and ORP; (B) CODcr, TN, and TKN
(C) NH_4^+-N, NO_2^--N and NO_3^--N.

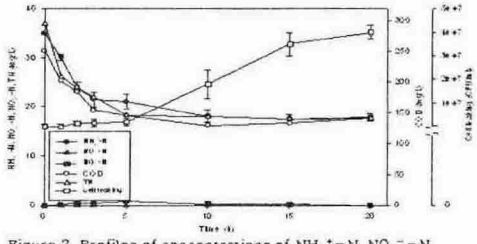

Figure 2. Profiles of concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, CODcr, TN and cell leaking during batch operation at 30℃ and pH 7.

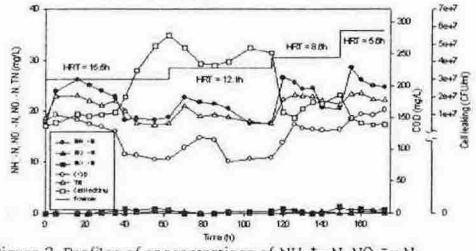

Figure 3. Profiles of concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, CODcr, TN and cell leaking during continuous operation at 30℃ and pH 7.

References

1. Chen, K-C, Lee, S.-C., Chin, S.-C. and Houng, J.-Y., "Simultaneous carbon-nitrogen removal in wastewater using phosphorylated PVA-immobilized microorganisms," *Enzyme Microb. Technol.*, **23, 311~320 (1998).**
2. Kim, J. K., Park, K. J., Cho, K. S., Nam, S.W. and Kim, Y. H., "Characteristics of a water-purification system using immobilized photosynthetic bacteria beads," *Envion. Eng. Res., Submitted.*