Bioelectrochemical denitrification using mediator

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In biological denitrification, certain bacteria use nitrate as the terminal electron acceptor in the absence of oxygen. Conventional biological denitrification needs excess amount of organic substrates such as ethanol and acetic acid. In this paper, we demonstrate a bioelectrochemical denitrification using a mediator, which supplies reducing equivalents to a denitrification bacterium.

Pseudomonas sp. IFO13302 was obtained from Institute for Fermentation, Osaka. Bacterium was pre-cultured at 30°C for a day in IFO702 medium (pH 7.0) composed of polypepton (10 g•l^{-1}), yeast extract (2 g•l^{-1}) and $MgSO_4$•$7H_2O$ (1 g•l^{-1}), with 50 mg-N•l^{-1} potassium nitrate. The cells were then harvested by centrifugation (1,500×g, 20 min) and washed twice with a 50 mM phosphate buffer solution (pH 7.0). Chronoamperometry was carried out using a potentiostat (HA-151, Hokuto Denko, Kanagawa, Japan). A carbon cloth (4 cm×2 cm, Model GF-20, Japan Carbon, Kanagawa, Japan), platinum mesh (80 mesh, BAS, Tokyo, Japan) and Ag/AgCl were used as the working, counter and reference electrodes, respectively. The two-compartment electrolysis cell was similar to that described previously (1). The working volume of both compartments is 10 ml. The anode and cathode compartments were separated by an ion-exchange membrane (Model Selemion CMV, Asahi Glass, Tokyo, Japan). The cathode solution contained bacterium (OD_{666} = 1.0) and 2-hydroxy-1,4-naphthoquinone (HNQ) of the concentration mentioned in the text in 50 mM phosphate buffer solution (pH7.0), and the anode solution was the phosphate buffer solution. The electrolysis cells were applied –0.7 V vs. Ag/AgCl reference electrode. The cathode solution was agitated with N_2 gas throughout the measurements. The concentrations of nitrate and nitrite ions were determined by an ion chromatography (LC-VP, Shimadzu, Kyoto) using a Shim-pack SCR-102H column (Shimadzu, Kyoto).

Figure 1 shows the reduction of 60 mg-N/l nitrate ion by *Pseudomonas* sp. with various concentrations of sodium acetate. At the concentration of more than 180 mg/l sodium acetate, 60 mg-N/l nitrate ion was removed in 6 hours. As the concentration of nitrate ion decreased, nitrite ion was produced. Nitrite ion was reduced after nitrate ion removal. When the cathode was applied –0.7 V vs. Ag/AgCl reference electrode, nitrate ion was reduced both with and without HNQ (Fig. 2). In the absence of HNQ, nitrate ion was gradually reduced. By the addition of HNQ, nitrate ion removal was accelerated and the concentration was less than 10 mg-N/l after 8 hours in the case of 0.5 mM HNQ. Denitrification activities of *Pseudomonas* sp. in the bioelectrochemical cell were almost the same as that in the case with excess amount of acetate. Figure 3 shows the behavior of current during bioelectrochemical denitrification. Since added HNQ was the oxidized form, substantial current was observed without bacterium. In the presence of bacterium, current increased to –0.2 mA at 0.1 mM HNQ and to more than –0.5 mA at 0.5 and 1.0 mM HNQ. The efficiency of electricity to nitrate removal was more than 80%.

Reference
1. T. Yagishita, S. Sawayama, K. Tsukahara and T. Ogi, *Bioelectrochem. Bioenerg*., **43**, 177-180 (1997).

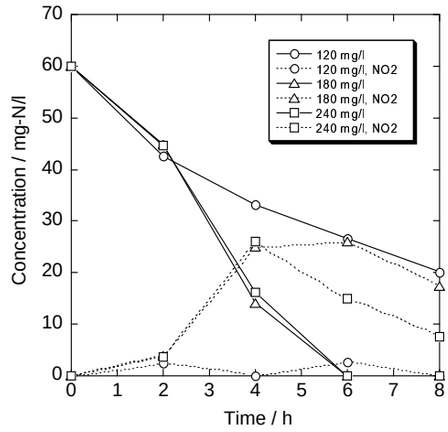

Fig. 1 Reduction of nitrate ion with acetic acid

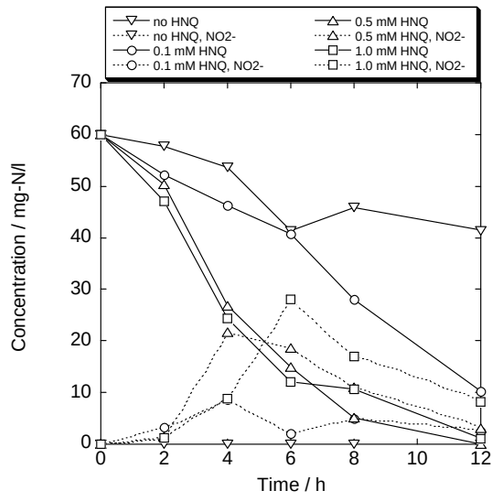

Fig. 2 Reduction of nitrate ion by bioelectrochemical system

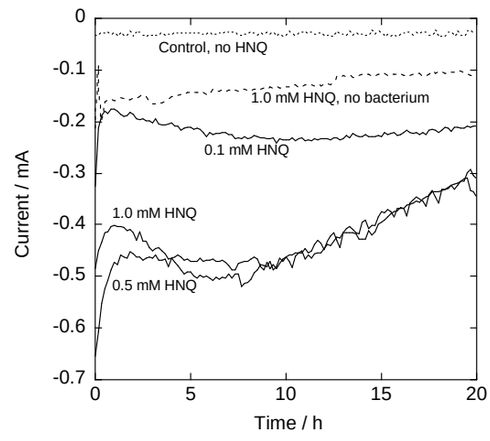

Fig. 3 Behavior of current during bioelectrochemical denitrification