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Nitrogen removal with a dual bag system capable of simultaneous nitrification and denitrification

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ABSTRACT

We tested the nitrogen removal ability of a dual bag system. The dual bag was constructed with an outer non-woven fabric bag and an inner non-porous polyethylene film bag. *Nitrosomonas europaea* and *Paracoccus pantotrophus* were immobilized on the outer bag and 23 mL of 99.5% ethanol was packed into the inner hermetic bag. The dual bag removes ammonia as follows: ammonia in the solution is oxidized to nitrite, and the produced nitrite is reduced to nitrogen gas by cooperation of the *N. europaea* and *P. pantotrophus* on the outer bag. Ethanol as an electron donor for denitrification is supplied to the *P. pantotrophus* from the inner bag. Ammonia solution (20 mg N L^{-1}) flowed into a reactor tank (200 mL volume) with three dual bags was continuously treated for a hydraulic retention time of 2.6 h at 25 °C. Total nitrogen in the outflow of the reactor was kept below 4.0 mg N L^{-1} for more than 90 days without accumulating organic carbons. The dual bags removed 80.0% of the nitrogen from the solution without additional operations. The inner bag containing 23 mL of ethanol probably releases ethanol for approximately 1000 days without refilling since the inner bag released only 0.022 mL ethanol per day. The dual bag system would effectively simplify the nitrogen removal system since it is easily installed in vacant spaces of various waterways and tanks.

1. Introduction

Excess nitrogen compounds cause environmental pollution, e.g., eutrophication in closed areas [1], nitrate contamination of groundwater [2], and nitrogen saturation in forest ecosystems [3]. Strict regulations have been applied for the nitrogen content of industrial effluents [4]. Biological treatment systems that involve a nitrification–denitrification process have already been applied to remove the nitrogen in the effluents [5]. Nitrogen compounds are also derived from a wide range of pollutant sources other than industrial effluents, e.g., excess fertilizer and livestock manure in agricultural areas, landfill leachates, leaky sewers, atmospheric deposition, and domestic horticulture in urban areas [6]. Various sources are also contaminating ground and surface waters despite industrial effluent control. Most nitrogen removal systems applicable to industrial effluents, however, are unsuitable for treatment of contaminated ground and surface waters since they require many facilities with complicated operations.

Sequential batch bioreactors and membrane-aerated biofilm reactors have been developed to improve treatment systems for nitrogen removal. Sequential batch reactors require strict operational controls despite comprising a single tank [7]. Although membrane bioreactors carry out simultaneous nitrification and denitrification in a single tank, they require the use of expensive and clog-susceptible hollow-fiber membranes [8,9]. A single-stage nitrogen removal process was evaluated by using a composite matrix of immobilizing, nitrifying, and sulfur-denitrifying bacteria [10]. The system released sulfate instead of nitrate into the treated groundwater although it could conduct simultaneous nitrification and denitrification without additional operations. Since the nitrogen removal system conducts only denitrification, simple systems have been proposed. Denitrification walls against nitrate-contaminated groundwater have already been constructed by injection of sawdust into the soil below the water table as a permeable wall perpendicular to the groundwater flow [11]. Other biodegradable polymers such as starch [12], cellulose [13], and fatty acid compounds [14] have been used in the denitrification of nitrate-contaminated groundwater, agricultural runoff, and urban runoff. These approaches are useful for removing nitrate from the waters since the buried sawdust serves as a continuous supply of electron donors to the denitrifiers without additional operations. These electron donors, however, are difficult to control their uncertain degradation in order to use the breakdown polymers as electron donors. The denitrifying activity resulting from use of breakdown polymers is certainly lower than that resulting from the use of methanol and ethanol, both of which are inexpensive and generally used in wastewater treatment. Such activity is easily

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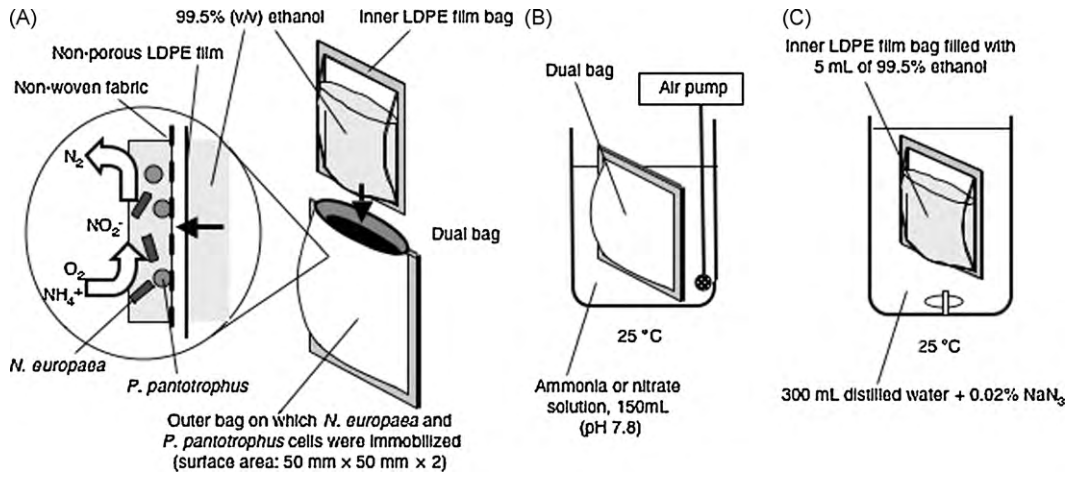

Fig. 1. Schematic diagram of the dual bag composed of an outer non-woven fabric bag and an inner ethanol-filled LDPE film bag (A) and the batch system established using the dual bag (B) or the inner bag only (C).

inhibited by oxygen dissolved in the waters since the anoxic zone for denitrification is fragile.

We have previously described the use of a novel electron donor for denitrification by using a non-porous low-density polyethylene (LDPE) film bag [15]. The ethanol-filled hermetic bag fabricated with LDPE film was able to continuously supply the ethanol needed to denitrify the bacteria since the LDPE film is permeable to ethanol. The permeability could be appropriately controlled by changing the film thickness. In addition, we studied the simultaneous nitrification and denitrification brought about by the cooperation of *Nitrosomonas europaea* and *Paracoccus pantotrophus* in a polymeric gel [16], demonstrating that a packed gel envelope on which *N. europaea* cells and *P. pantotrophus* cells were immobilized could remove ammonia in a single reactor tank. Thus, the combination of the two would enable the development of a simple system for the removal of both ammonia and nitrate from contaminated ground and surface waters.

In this study, we constructed a dual bag comprised of an outer non-woven fabric bag to which *N. europaea* and *P. pantotrophus* cells were attached and an ethanol-filled LDPE film inner bag. The efficacy of the dual bag for nitrogen removal was examined by using ammonia or nitrate solution.

2. Materials and methods

2.1. Bacterial strains and chemicals

Ammonia oxidizer strain *N. europaea* (NBRC-14298) and denitrifier strain *P. pantotrophus* (JCM-6892) were used in this study. Strains were aerobically cultured at 30 °C, as previously described [17]. After cultivation, *N. europaea* and *P. pantotrophus* cells were harvested by centrifugation (20,000 × *g*, 10 min, 4 °C) and washed 3 times with a phosphate buffer (9 g/L Na_2HPO_4, 1.5 g/L KH_2PO_4, pH 7.5). All chemicals used in this study were purchased from Wako Chemical Co. Ltd. (Japan).

2.2. Construction of the dual bag

The dual bag comprised an outer bag and an inner bag (Fig. 1A). To construct the outer bag with the immobilized *N. europaea* and *P. pantotrophus* cells, a non-woven polyethylene terephthalate fabric (G2260-1S; Toray Co. Ltd., Japan) was cut and heat-sealed. *N. europaea* (14 mg dry wt.) and *P. pantotrophus* cells (28 mg dry wt.) in 1.25 mL phosphate buffer were mixed with 3.75 mL of the photocrosslinkable polymer PVA-SbQ (SPP-H-13; Toyo Gosei Kogyo Corp., Japan). The cell mixture was spread on both the sides of the outer bag (50 mm × 50 mm × 2 sides of active area) as a 1-mm-thick layer and then solidified into a gel by metal halide light irradiation (1000 μmol m^{-2} s^{-1} light intensity) for 20 min [16].

To construct the inner bag, 0.1-mm-thick non-porous and non-stretch LDPE films (Miporon film; Mizwa Co., Ltd., Japan) were cut and heat-sealed to fabricate hermetic bags (50 mm × 50 mm × 2 sides of active area for the release of an electron donor). These LDPE film bags were subsequently filled with 5.0 or 23.0 mL volume of 99.5% (v/v) ethanol as an electron donor for denitrification and then inserted into the outer bag. After the insertion, the outer bag was incompletely closed to emit the N_2 gas produced by denirification.

2.3. Batch treatment with the dual bag

An ammonia or nitrate solution was treated with the dual bag comprised of the outer bag of immobilized *N. europaea* and *P. pantotrophus* cells and the inner LDPE film bag filled with 5 mL of ethanol (Fig. 1B). The batch system was composed of a tall beaker (63 mm in diameter and 134 mm in height) filled with 150 mL ammonia or nitrate solution, a water bath for temperature control at 25 °C, and a ventilator with air (150–200 mL/min). The ammonia solution (pH 7.8) contained (per liter) 0.094 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 9 g Na_2HPO_4, 1.5 g KH_2PO_4, 0.01 g $CaCl_2 \cdot 2H_2O$, 0.5 g $NaHCO_3$, 0.005 g EDTA–Fe, and 1 mL solution containing trace elements [18]. The nitrate solution (pH 7.8) contained the same components except that the $(NH_4)_2SO_4$ was replaced with 0.144 g KNO_3. After the dual bag was immersed in the ammonia or nitrate solution, the concentrations of ammonia, nitrite, nitrate, and total organic carbon (TOC) in the solution were periodically measured.

To confirm the controlled release of ethanol from the inner bag, the inner bag filled with 5 mL of ethanol was immersed in 300 mL of distilled water containing 0.02% (w/v) of sodium azide and then agitated with a stirrer (60 rpm) at 25 °C (Fig. 1C). Sodium azide was used to prevent consumption of released ethanol by germs. The amount of ethanol released from the inner bag was calculated as the TOC concentration in the water. The ethanol concentration in the inner bag was periodically measured.

2.4. Continuous treatment with the dual bag

The ammonia solution was continuously treated with the three dual bags comprising the outer bag and the inner LDPE film bag filled with 23 mL of ethanol (Fig. 2). The three dual bags were put into a beaker as a reactor tank (63 mm in diameter and 134 mm