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Controlling the process of denitrification in flooded rice soils by using microbial fuel cell applications

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ABSTRACT

Controlling the denitrification rate could help reduce the losses of applied nitrogen (N) fertilizer in fields. The applicability of microbial fuel cell (MFC) for controlling denitrification in flooded rice soils was investigated based on MFC theory coupled with redox changes. Because the soil about 10–20 cm beneath water and the soil near the water surface are anaerobic and aerobic, respectively, gradients of electric potentials could be generated between them, upon connecting them through insulated wires. Electrons released through the oxidation of organic matter during microbial metabolism can be utilized by this set-up, generating electricity. This can reduce the availability of electrons for reductive half-reactions of nitrate, to suppress denitrification. We studied the N losses in soil using planting pots with gas chamber experiment under three conditions: MFC systems, MFC systems with an externally applied voltage, and non-MFC systems as a control. Each system was set in triplicate, supplied with the same N fertilizer amounts, and flooded with automatic irrigation. Soil redox potential, N_2O flux, and inorganic nitrogen concentration in soil pore water were periodically monitored. The redox potentials of both MFC systems and MFC systems with externally applied voltage were significantly higher than that of non-MFC systems, while N_2O flux levels were significantly lower than that of non-MFC systems. The rice reproductive stage was the most effective on suppressing N_2O flux with MFC application. However, the effect of externally applied voltage on suppressing N_2O flux remains unclear. Inorganic nitrogen retention efficiencies in pore water were higher in MFC systems, which is consistent with the N_2O flux difference. While the proportion of denitrified N estimated for MFC systems was 2.3%, that of non-MFC systems was 6.6%. We confirmed the applicability of MFCs to control soil redox potential and thereby suppress the denitrification based on planting pot experiments.

1. Introduction

1.1. Problem of nitrogen deficiency

The global food industry is highly dependent on fossil fuels. Energy-efficient approaches to agriculture would offer a way to take advantage of the relationships between energy, food, and agriculture. A huge amount of energy is required for the fixation of nitrogen (N) fertilizer from the unlimited atmospheric nitrogen. To manufacture one metric ton of anhydrous ammonia (NH_3) which consists 82% of N, it is estimated that 3500 m^3 of natural gas is used (Olson and Halstead, 1974). It is also estimated that the field application of 150 kg/ha N fertilizer in the form of NH_3 involves the consumption of 645 m^3 of natural gas (Olson and Halstead, 1974). Moreover, despite the fact that rice is the staple food of half the world's population, N fertilizer is not used efficiently (Keeny and Sahrawat, 1986). Recognizing the fact that N fertilizer is absolutely essential for supporting the growing global population, there is an urgent need to explore efficient ways of using fertilizer to compensate for future food shortages. As conventional methods to improve N retention efficiency various methods such as leguminous crop production, crop rotation, and management of irrigation water have been proposed and implemented (Huang et al., 2007; Kaewpradit et al., 2008; Pramanik et al., 2014).Though these approaches are proven to be effective under specific conditions, sometimes these are not effective due to the uncontrollable factors such as climate conditions. Hence, if we can electrochemically control denitrification processes, that might be a more universally applicable technology.

The process of denitrification, involving the conversion of soil inorganic N to elemental N gas, is one of the main routes behind N deficiency in crop production. Here, denitrification refers to the process in which NO_3^- is converted to gaseous compounds such as NO, N_2O, and N_2 by microorganisms. In submerged soils, the denitrifying bacteria use NO_3^- in the absence of oxygen as the terminal electron acceptor in

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their process of respiration, which is reduced to NO_2^-, NO, N_2O, and finally N_2 (Reddy and Patrick, 1986). From the viewpoint of saving N fertilizer in agricultural fields, denitrification is undesirable. In addition, paddy fields have been recognized as one of the sources of atmospheric N_2O (Mosier et al., 1996; Akiyama et al., 2005), one of the greenhouse gases causing global warming. Thus, the extent of N loss via denitrification has attracted great interest among researchers as well as farmers (Iida et al., 2007).

1.2. Theory of microbial fuel cell for the control of soil redox potential

According to the definition given by Lovley (2006), a microbial fuel cell (MFC) is a device that converts chemical energy into electrical energy. Generating power in MFC depends on redox chemistry. The redox reaction involves a transfer of electrons between two chemical species. Oxidization involves losing electrons from one chemical species, whereas reduction involves gaining electrons by other chemical species. Microbes can gain energy through redox reactions between organic compounds and terminal electron acceptors such as O_2. This process can be interpreted as involving the oxidation of the organic compound and the reduction of the terminal electron acceptor. The principle of MFC involves the utilization of electrons liberated by the decomposition of organic matter by microbes to produce electricity.

The principle of MFC can also be applied to flooded soils. Fig. 1 illustrates the concept of an MFC applied to flooded soils. The electrons produced during the oxidation of organic matter are transferred directly to an anode, and the electrons travel through an electrical circuit to a cathode where oxygen is reduced to H_2O. While the region several centimeters below the flooded soil surface is anaerobic, near the soil surface, aerobic conditions prevail. Thus, potential gradients could be generated between these regions, when connected externally through insulated wires. It is therefore possible to generate electricity if anode respiring microbes have self-sustained extracellular electron transfer mechanisms (Lovley, 2008). In fact, some studies have already shown that electricity can be produced in flooded soils (Arends et al., 2014; Kaku et al., 2008; Kouzuma et al., 2014; Takenezawa et al., 2010).

In this context, the following question arises: How can the denitrification process be related to the principle of MFC? In the denitrification process of flooded rice fields, the electrons produced from

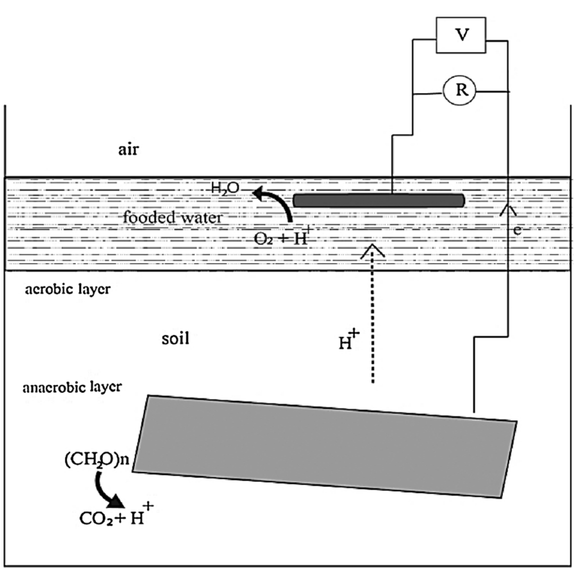

Fig. 1. Schematic explanation of Microbial Fuel Cell theory in ponded soil: A carbon graphite-felt mat is used as anode placed in the anaerobic soil layer, and a graphite rod is kept floating on the flooded water in contact with air. The anode and cathode are linked through insulated wires externally connected with a resister (R) and a voltmeter (V).

the oxidation of organic matter by microbes are gained by oxygen-containing inorganic N species such as NO_3^- and NO_2^-. Typical redox reactions related to NO_3^- respiration that are performed by microbes in the absence of oxygen are shown in the Eqs. (1) and (2).

$$5\,C_6H_{12}O_6 + 24\,NO_3^- + 24\,H^+ \rightarrow 30\,CO_2 + 12\,N_2 + 42\,H_2O \quad (1)$$

$$C_{12}H_{22}O_{11} + 9.6NO_3^- + 9.6H^+ \rightarrow 12CO_2 + 4.8N_2 + 15.8H_2O \quad (2)$$

Because these are redox reactions between organic carbon (C) and oxidized forms of N anions, the availability of organic C should be one of the factors determining whether denitrification occurs. The dynamics of organic C is difficult to control, whereas in contrast the electrons resulting from its oxidation through microbial metabolism can be controlled. Specifically, the electrons resulting from the oxidation of organic matter can be circulated in a chain, which is referred to as an electric chain, when the anaerobic layer is externally connected to the upper aerobic layer of soils. It is therefore hypothesized that denitrifying conditions could be controlled by redox potential changes in soils that are invoked by MFC. In other words, a competition for electrons is expected to invoke the generation of electricity, and to outcompete denitrification reactions at the same time. Thus, based on the above hypothesis, we studied on the control of denitrification – N losses in flooded rice soils by using MFC applications with pot experiments.

2. Materials and methods

2.1. Design of planting pots with gas chamber experiment

We conducted a planting pot with gas chamber experiment with three different conditions: MFC systems, MFC systems with an externally applied voltage, and non-MFC systems as a control. Each system was set in triplicate. The MFC systems with an externally applied voltage were aimed at increasing the efficiency of MFCs because it was expected that the rapid movement of electrons towards the anodic area would be enhanced by the externally applied voltage. An external voltage maintained at 25–50 mV was applied by a voltage stabilizer (AD-8735A; AND Company Ltd.) throughout rice growth. In the rest of this article, we refer to the MFC systems as MFCs, MFC systems with an externally applied voltage as MFC-extVs, and non-MFC systems as non-MFCs.

The experimental period included the entire period of rice growth after transplantation. Soil redox potential, N_2O gas flux, C/N ratio of soil, and NH_4^+, NO_2^-, and NO_3^- concentrations in soil pore water were periodically measured. Based on these measurements, the N retention efficiencies and denitrification loss rates were calculated and compared among these systems.

The designs of the planting pot and the gas chamber are shown in Fig. 2. For soil particle and pore water samplings, each planting pot had three holes (diameter of 2 cm) closed by rubber stoppers. In addition, each planting pot had a hole at the bottom, to inject acetylene gas in order to inhibit the reaction of nitrous oxide reductase enzymes, which further converts N_2O to N_2. A circular tube, which had holes in it at equal distances of 2.5 cm from each other, was fit snugly into the bottom hole and then inserted into soil. Because this tube was connected to a three-way stopcock, gas could be distributed at equal pressure in each pot. Gas flow pressure at the outflow valve was adjusted to 1.2 MPa. Each gas chamber also had three holes (diameter of 2 cm) closed by a butyl rubber septum for gas sampling.

The soil for the experiment was collected from the experimental paddy fields of the Field Science Center of Gifu University. According to the Japanese Soil inventory, it is Gleysol (https://soil-inventory.dc.affrc.go.jp/). Besides collecting the soil for experiments, the soil was also collected to soil samplers (100 cm^3) from about 20 cm below soil surface, and basic physical properties were measured by oven-drying at 110 °C for 24 h. Table 1 shows the basic soil physical properties. Except for in the non-MFCs, for an anode, a carbon graphite-felt mat (S-221;