

REDOX DISTRIBUTION PROFILES OF FLOODED PADDY SOILS WITH MICROBIAL FUEL CELL APPLICATIONS

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ABSTRACT: Microbial fuel cell is one of our considerations to control denitrification redox reactions in flooded rice soils. An independent study was conducted after a nitrogen behavior study in flooded rice soils accounted for microbial fuel cell systems and microbial fuel cell systems with an externally applied voltage. External voltage was applied in the aim of increasing microbial fuel cell efficiencies, for the better control of soil redox potential. But, their redox controlling potentials and denitrification controlling were lower than that of microbial fuel cell systems. It is hypothesized, that the lower controlling potentials were due to the redox distributions effected with the distance to the anode of the circuit where the external voltage was connected through. Therefore, soil redox distributions at three depth profiles were compared in both systems and controls over the period of flooding. In external voltage applied systems, the redox potential of the soil away from anode was not much effectively controlled as of microbial fuel cell systems, although the middle soil portions near anode area were of higher redox potentials. At depths of 10 and 20 cm away from the soil surface, the redox potential increased more with MFCs and moderately with MFC-extV, but reduced with non-MFCs over the period. Accordingly, most electrons from whole soils were not freely transferred to the anode, except that from near anode areas where the external voltage was connected through. Microbial fuel cell systems with externally applied voltage had a lower impact on controlling denitrification than that of microbial fuel cell systems alone.

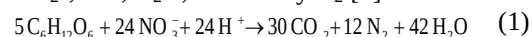
Keywords: Denitrification; Redox potential; microbial fuel cell; paddy soil; nitrogen fertilizer

1. INTRODUCTION

Redox, termed short for oxidation-reduction, denotes the transfer of electrons between two species. The reduction is the gain of electrons by a compound, whereas the oxidation is the loss of electrons by a compound. The electron donors are usually reduced carbon and the acceptors are usually oxygen in aerobic environments. Oxidation-reduction conditions are classically assessed by measuring the redox potential (Eh), expressed in volts. It is a measure of electron activity in a system. Microorganisms are probably the most important factor controlling the Eh of soils: as they reduce carbon to store energy, they oxidize it to release energy and they use oxygen or other molecules as electron acceptors, thus forming reduced species under oxygen-limiting conditions [2]. O₂ has the highest redox potential, followed by other alternative electron acceptors: NO₃⁻ > Mn (IV) > Fe (III) > SO₄²⁻.

However, redox potential (Eh) has received little attention in agronomy, unlike pH, which is regarded as a master variable [7]. This would be due to the fact that Eh measurements are difficult to reproduce and interpret [10]. In many disciplines, oxidation-reduction conditions and electron fluxes have not received the same attention as have pH and proton fluxes [7]. The control of redox process is a proper

niche for the unique capabilities of a bio-electrochemical system [1], since microbes participate in biochemical reactions to satisfy their energy needs and resource demands. Denitrification is one of the main routes leading to nitrogen deficiency in crop production. Here, 'denitrification' refers to the process in which NO₃⁻ is converted to gaseous compounds such as NO, N₂O, and N₂ by microorganisms. In submerged soils, the denitrifying bacteria use NO₃⁻ in the absence of oxygen as the terminal electron acceptor in their process of respiration, which is reduced to NO₂⁻, NO, N₂O, and finally N₂ [9].



Thus, reduction of NO₃⁻ accounts for denitrification problem in soils. Denitrification is a redox reaction between nitrogenous oxides and organic carbon as shown in equation (1). Therefore, we propose that redox potential-based controlling approaches of denitrification would be more promising. Microbial fuel cell (MFC) is one of our considerations in this regard. MFC could be a driver of change of electron activity in the system or redox potential changes. Figure 1 illustrates the concept of an MFC applied to flooded soils, which can then be referred to a paddy field microbial fuel cell. 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. Thus, a competition for electrons could be generated between the electronically active bacteria and denitrifying bacteria due to MFC. Further related MFC engineering approaches of microbe-electrode conversion of organic matter to electricity and that of direct electron transfer from electrodes to microbes are discussed by [3], [4], [5] and [5].

Denitrification rates were assessed from our previous study in summer, 2016, from flooded rice

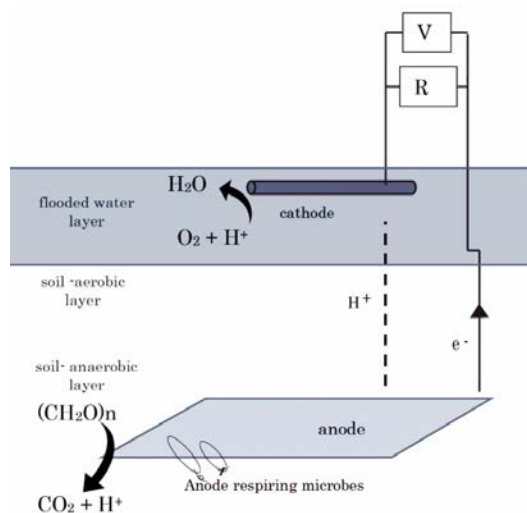


Fig 1 Scheme of a microbial fuel cell: A carbon graphite-felt mat is used as anode placed in the anaerobic soil layer, and a graphite rod is kept floating on the flooded water in contact with air. The anode and cathode are linked through insulated wires externally connected with a resistor (R) and a voltmeter (V).

soils treated with MFC systems, MFC systems with an externally applied voltage, and non-MFC systems as a control with applications of nitrogen fertilizers. In the rest of this article, we refer to the MFC systems as MFCs, MFC systems with an externally applied voltage as MFC-extV, and non-MFC systems as non-MFCs. An externally applied voltage, ranged at 25-50 mV, was aimed at conveying more electrons towards the anodic area for the efficiency of MFC. Therefore, the denitrification rates were expected to be lower, compared to that of MFCs. Denitrification rates were measured by N_2O flux rates with pot and chamber experiments by using acetylene inhibition technique. In that, the reason for lower redox and lower denitrification controlling potentials of MFC-extV systems was not clear. It was hypothesized that the redox distribution patterns with external voltage applications were not effected through whole soils, but were affected locally at the points of MFC anodes where they were connected through external voltage. In this study, we investigate the

effect of external voltage applied to MFCs on redox distributions at different soil depth profiles.

2. MATERIALS AND METHODS

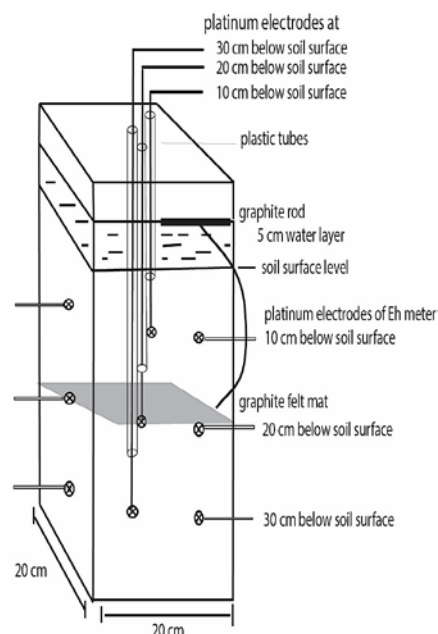


Fig 2 Design of the soil Eh-depth profile study with MFC treatments and controls

2.1 Design of the experiment

For studying the Eh - depth profiles of soils, pot experiment was conducted under the three conditions: MFC systems, MFC systems with an externally applied voltage, and non-MFC systems as a control. All the soil systems monitored were not plant-assisted in this experiment. The design of the soil Eh-depth profile study with potted soil is shown in Figure 2. Redox potential was measured by Redox (Eh) meters (PRN-41; Fujiwara Factory Co., Ltd.). Eh meters consist of counter electrodes made of Pt-coated with glass tubes, and reference electrodes made of AgCl with saturated KCl internal solution. While reference electrodes were put near the soil surface, counter electrodes were installed at the center, and at both sides 5 cm away from the center at each soil depth levels of 10 cm, 20 cm and 30 cm from the surface soil. Eh was continuously measured at 2 days' intervals. The voltage difference generated by the MFCs and the soil temperature (at 10 cm below the soil surface) were recorded on a data logger (CR-1000; Campbell Scientific Inc.) at 10-min intervals.

The soil for the experiment was collected from the experimental paddy fields of the Field Science Center of Gifu University and was filled into pots.

Except for in the non-MFCs, for an anode, a carbon graphite-felt mat (S-221; Osaka Gas Chemicals Co., Ltd.) of $20 \times 20 \times 0.5$ cm was introduced 20 cm below the soil surface. As a cathode, a graphite rod (C-072591; Nilaco Corporation) with a length of 10 cm and diameter of 0.5 cm was kept floating on the flooded water in contact with air. The anode and cathode were linked through insulated wires externally connected with a resistor of 330Ω . After the MFC configurations, soil was kept flooded at a depth of approximately 5 cm by an automatic water supply system throughout the experiment. For the systems of MFC-extV, an external voltage maintained at 25–50 mV was applied to the MFC circuit by a voltage stabilizer (AD-8735A; AND Company Ltd.) throughout the period of measurements.

2.1 Data analysis

Using the Eh distribution patterns in soils between the treatments were compared with depths and submerged periods. In addition, N_2O flux rates of the previous study is shown as supportive data, as for the basis of current study. All statistical analyses described in this article were performed using IBM SPSS Statistics version 24.

3. RESULTS AND DISCUSSION

3.1 Eh distributions with time and soil depth

Soil Eh distribution at depth profiles with period of submergence is shown for each treatment by Figure 3. The soils at 10 cm and 20 cm were comparatively of higher Eh values than that of 30 cm, especially with MFCs and are followed by

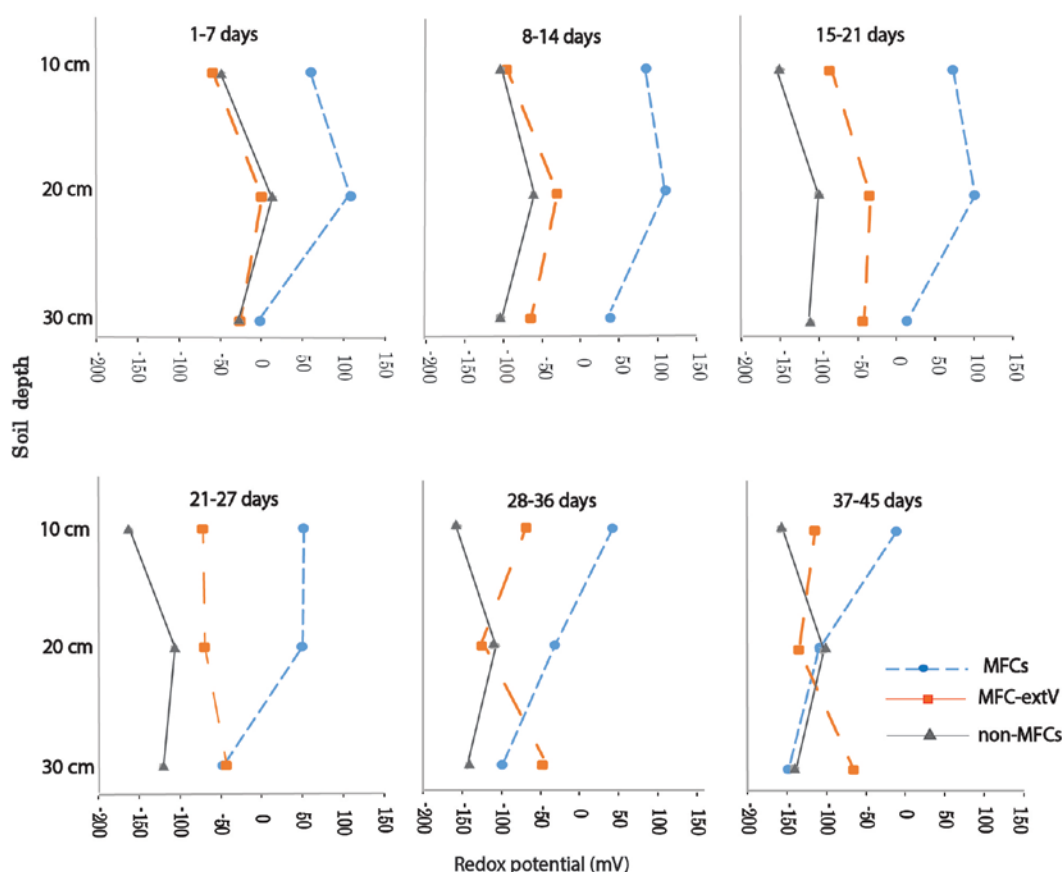


Fig 3 Soil Eh - depth profiles with period of submergence. Mean $\pm$ SD of Eh over the whole period are:
MFCs (52 ± 46), MFC-extV (-81 ± 97) at 10 cm.
MFCs (39 ± 82), MFC-extV (-64 ± 85) at 20 cm
MFCs (-39 ± 100), MFC-extV (-47 ± 143) at 30 cm

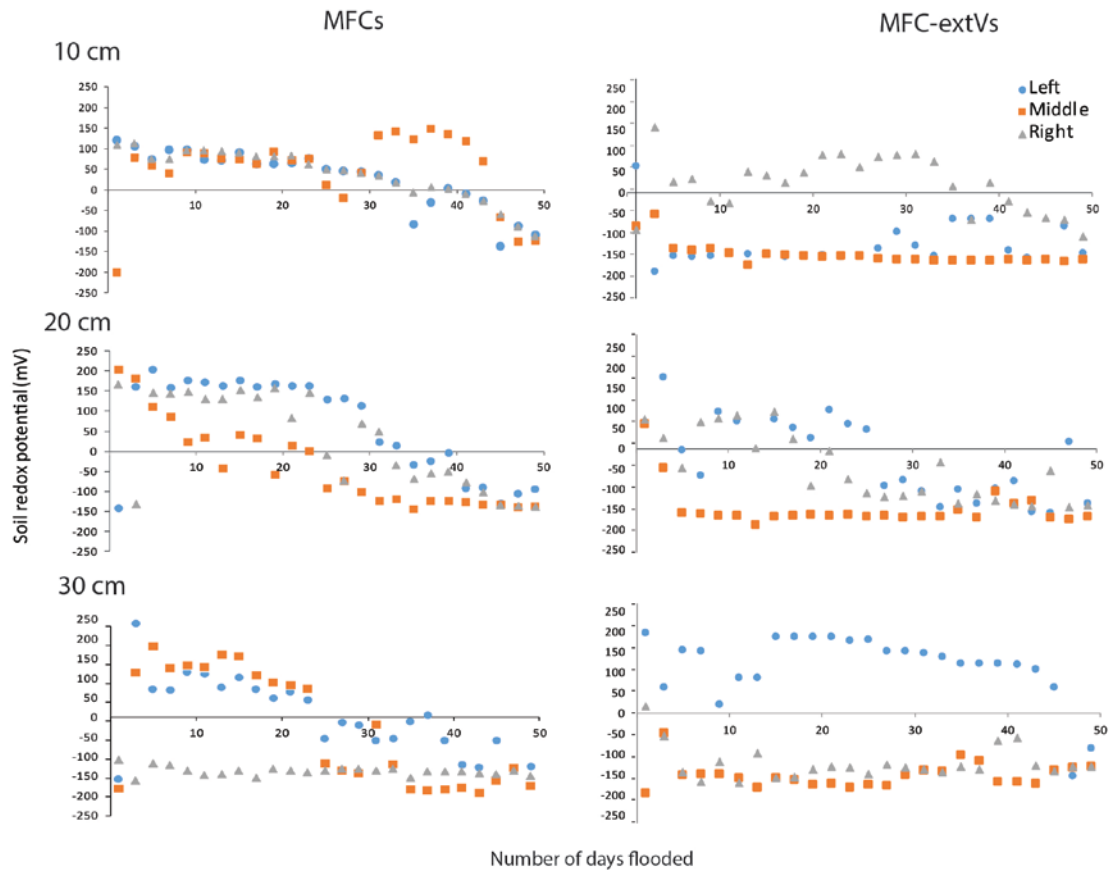


Figure 4: Soil Eh distributions with depth layers (Eh measured at left, middle and right side portions of each depth level)

MFC-extV. Non-MFCs didn't show much variations between oxidizing and reducing conditions with depth profiles, proving that more frequently they had reducing conditions. MFC-extV showed much variations of Eh with time and depths.

Univariate analysis of Variance was conducted for studying the variations of Eh between treatments and soil depth profiles. The soil redox data has a significant difference with treatments as well as depth profiles, and also there are significant interaction effects between depths and treatments, at $p=0.05$ level. However, MFC-extV treatment is less influencing on controlling redox potentials of soils, unlike that is of MFCs.

Further, the redox distribution profiles were compared with relative to soil portions (denoted as middle, left and right) at each depth, especially with MFCs and MFC-extV treatments as shown in Figure 4. External voltage applied soil portions showed Eh changes in an alternative pattern in soils between and near electrodes. Adjacent soil portions had opposite charges even at the same depth profiles which could be denoted as $+\Delta$ Eh and $-\Delta$ Eh. At 10 cm depths, right side soil portion was positively charged frequently, while at 30 cm depth, left side portion was positively charged. Middle soil portions

at all 3 depths, which were negatively charged frequently, were held by the attractions of those positively charged soil particles on its adjacent sides. However, it is not certain of reasons for such random distributions, the differently charged particles could be due to the electrolysis chemical reactions in the soils occurred non-spontaneously due to the DC supply of voltage. Other than microbial fuel cell, the systems with external voltage, could partially perform as an electrolytic cell, causing non-spontaneous reducing reactions. Therefore, negatively charged soil zones could have formed randomly as a result of the external voltage applied. These reducing reactions would be causative of not more electrons being transferred to the anode from the whole soil.

Also, distribution of the voltage gradients in the soil between the two electrodes might have complex patterns. The voltage gradients may vary from point to point between the two electrodes, and are not evenly distributed. They should be steep near the anodes, and spread-out in the middle between them. Near the electrodes where the current enters or leaves the soil could have voltage gradients become stronger. In the other area between the two

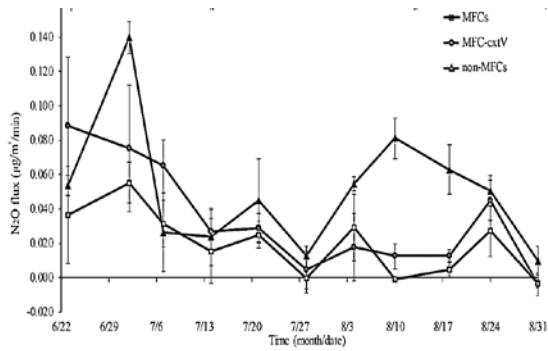


Fig 5 N₂O flux over the experimental period (summer, 2016). Mean flux for each treatment measured over the whole period is shown. Bars represent standard deviation of the mean

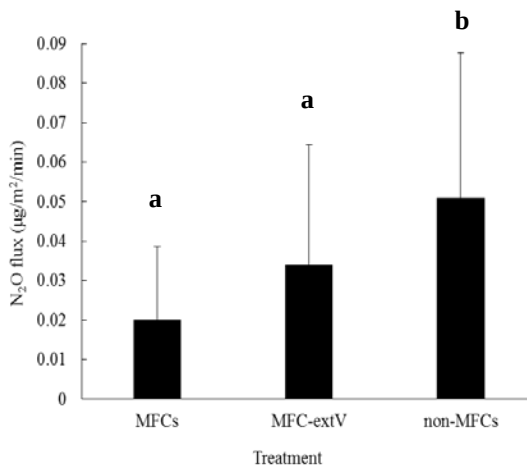


Fig 6 Results of multiple comparison of N₂O flux. Mean $\pm$ S.D. N₂O flux of soils in three different treatments are shown. Results of Tukey test are shown as letters below the columns, in that there are significant differences in the means of columns not sharing the same letter.

electrodes, the current becomes diffused to result the voltage gradients also be diffused. Thus, the transfer of electrons towards the anode for current generation was not totally covered by whole soil, but that was limited near the area of anode.

It is clear that the externally applied voltage was not in help with the efficiency of MFC-extV treatments, due to the voltage gradients and formation of soil zones that held together by electric charges. Thus, soil zoning could also be a barrier for free movement of electrons to the anode. Overall, effects of MFC-extV treatments on soil redox control were lower compared to that of MFCs treatments, but were higher compared to that of non-MFCs.

3.2 N₂O flux with MFC applied soils

The time series of N₂O flux of each treatment (summer, 2016 data) is shown in Figure 5. Both MFC and MFC-extV treatments showed lower flux rates than that of non-MFCs.

While N₂O flux showed a significant difference between MFCs and non-MFCs, there was no statistically significant difference between MFCs and MFC-extV. As a consequence of relatively high redox potential in MFCs and MFC-extV, denitrification processes were suppressed, and as a result N₂O flux was also suppressed. From the experiment data (summer, 2016) we investigated that MFC systems have the potential to control the denitrification process. But, it was not clear how externally applied voltage effected on its efficiency.

4. CONCLUSION

MFCs treatments had Eh controlling effects both spatially and temporary. But, with that of MFC-extV treatments, Eh controlling was not much effective as of MFCs. One possible reason could be due to the formation of charged soil zones that held adjacently together by their net electric charges. These random effects could have resulted through electrolysis chemical reactions, most of which are non-spontaneous reducing reactions in soils due to the externally applied DC voltage. The other reason could be due to the formation of voltage gradients, steeper at the electrodes and diffused away from them. Thus, most electrons in whole soils of MFC-extV treatments were not transferred freely to the anode, but were from near anode area where the external voltage was connected through. As a consequence of relatively high redox potential in MFCs treatments, denitrification processes were suppressed, and as a result N₂O flux was also suppressed, compared to that of MFC-extV treatments.

5. ACKNOWLEDGEMENTS

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