A novel sediment microbial fuel cell with a biocathode in the rice rhizosphere

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Abstract: Wetland plants possess the unique ability to release oxygen as well as organic matter into the rhizosphere. It is understood that microbial fuel cells (MFCs) can use organic matter from plants as key electron donors, but the effect of root excreted oxygen on MFCs is presently unknown. In this study, a novel biocathode was buried in the rice rhizosphere and found to be capable of delivering electrons to root excreted oxygen for oxygen reduction reactions. The voltages between electrodes in the rhizosphere and bulk soil were found to increase initially, but dissipate after approximately one month. Results from the MFC and oxygen microelectrode experiments indicated that the oxygen efflux rate from rice roots was dependent on the root maturity. Furthermore, the excreted oxygen from wetland plant roots could be used for the construction of highly efficient biocathodes.

Highlights

- The MFC cathode works well in soils where it can use oxygen excreted from wetland plant roots.
- A biocathode buried in the rice rhizosphere has comparable efficiency to an air cathode.
- Maximum oxygen efflux was 20 μmol·plant^{-1}·day^{-1} based on cell current calculations.

Keywords

Microbial fuel cells, wetland plant, radius oxygen loss, rice, biocathode

1. Introduction:

The environmentally-friendly conversion of biomass into energy is essential to produce both sustainable and renewable energy for the future. However, sugars and proteins in biomass (e.g. plant seeds or tubes) are presently part of the world's food supply. Other biomass, such as dead roots and shoot residues, are currently underutilized and left to decay naturally in fields where they support abundant microbial activities in the soil (Marschner et al., 2003). This neglected plant material has been recognized as an excellent source of biomass for bio-energy production. Among the present technologies able to transfer inedible biomass to energy, sediment microbial fuel cells (SMFCs) offer several unique advantages. SMFCs have a low environmental impact because they can transform organic matter into electrical energy without the transportation of biomass (Lovley, 2006).

This technology is classified as a type of photosynthetic microbial fuel cells (Rosenbaum et al., 2010). A typical SMFC with plants is constructed with an anode buried in reduced, flooded soil and a cathode placed at the air-water interface where it uses either oxygen reduction or other oxidizing chemicals. Electricity is then generated by the bacteria that oxidize the organic matter from soils or plant roots and release electrons (De Schamphelaire et al., 2008; Kaku et al., 2008).

In an SMFC with plants, wetland plants play a key role in supplying organic matter to the anode reaction (De Schamphelaire et al., 2008). As the most widely cultivated wetland plant, rice was used in initial studies on SMFCs with plants to supply organic matter for bacteria in anodes (De Schamphelaire et al., 2008; Kaku et al., 2008). Other plants, such as *Spartina anglica* and *Arundinella anomala*, were also able to supply organic matter for bio-energy production (Helder et al., 2010; Timmers et al., 2010). The SMFC with plants is considerably more complex than other MFC systems designed for wastewater treatment due to the added variable of plant growth. The factors influencing power generation by SMFCs are not yet well understood.

To adapt to anaerobic conditions in flooded fields, aerenchyma is developed in aquatic plants to allow for the downward transport of oxygen which can then facilitate a robust metabolism in wetland plant roots (Armstrong et al., 1991). The oxygen secreted from roots forms a micro-oxidizing environment in the rhizosphere. By using microelectrodes embedded in soil or sediment, early studies showed that the redox values reach up to 100 mV at the root tips (Flessa and Fischer, 1992). Recently, coupling with digital image analysis and rhizotron allowed for a rough depiction of the oxidized and reduced zones using 18 platinum electrodes (Schmidt et al., 2010). The micro-oxidizing zone was shown to extend 8 mm along the wetland plant roots (Frederiksen and Glud, 2006). This zone has been shown to influence greenhouse gas production (Segers, 1998; Van Bodegom et al., 2001) and the bioavailability of toxic elements (Chen et al., 2004; Liu et al., 2004). For SMFCs with plants, plant roots were often found to have penetrated through the buried anode (Kaku et al., 2008). As a result, the oxygen flux into the rhizosphere may worsen the anode performance and, as a consequence, the cell potential (Timmers et al., 2010).

Cathode reactions sometime become a limitation for MFC power generation due to activation, ohmic and mass transport losses (Rismani-Yazdi et al., 2008; Zhao et al., 2009). To reduce cathodic loss, electron mediators or oxidizing bacteria biocatalysts have been applied to MFCs and were found to yield much higher energy densities (De Schamphelaire et al., 2008). However, mediators require regular addition and may cause health problems, which is an unsuitable choice for sustainable power generation. Biocathodes with oxidizing bacteria are a good alternative because of their low costs and good operational sustainability (He and Angenent, 2006). In the wetland plant rhizosphere, iron oxidizing bacteria are abundant and found to be associated with iron plaques (Emerson et al., 1999; Weiss et al., 2011). However, the use of roots and iron oxidizing bacteria in the construction of biocathodes for SMFCs has never been done.

Here, a proof-of-concept experiment was conducted to test whether the root-contacting electrode buried in a rice paddy soil could accept electrons from rhizosphere oxygen. Estimates of the amount of oxygen released from rice roots were also made using the MFC cathode as an oxygen sensor. To do so, a novel SMFC with two cathodes was designed. One cathode was placed in the rhizosphere and another was fixed at the air-water interface. The voltages between the two cathodes and an anode were monitored during the plant growth season.

2. Materials and Methods

2.1 Plant pre-cultivation

Rice (*Oryza sativa* L.) cultivar Jiahua No.1 was selected. Seeds were sterilized by immersion in 10% H_2O_2 for 15 min, followed by germination in humid perlite. Before being transplanted into the SMFC apparatus, the seedlings were grown in a hydroponic culture in a climate-controlled room until the 4th leaf expanded (ca. 20 days). The room conditions were set at 25°C in daylight (14 hours, 180 μE m^{-2} s^{-1}) and 20°C in dark (10 hours).

2.2 SMFC set-up

An SMFC apparatus was employed that consisted of a 50ml centrifuge tube housed inside a lightproof plastic container (14cm × 10cm) (Fig. 1). The container was filled with fresh paddy soil collected from Jiaxing, Zhejiang province, China to a height of 10cm. A round, 3mm thick graphite mat (10cm diameter, 78cm^2 geometric area; Beijing Sanye Carbon Co., Ltd, Beijing, China) was placed 10mm from the bottom of the container. Before transplanting, the paddy soil and pre-placed graphite mat were flooded for one month to stabilize the anode without connection resistance.

A 50ml centrifuge tube with two graphite mats was placed into a root-sleeving electrode and half-inserted into the soil in the container. The conical bottom of the tube was cut off and holes (ca. 1 hole per 4 mm^2) were punctured into the tube wall using pins. The holes allowed for the passage of soil solution through the tube wall. One square graphite mat (3mm × 30mm × 97mm) served as the air cathode and was fixed on the top of the tube. Another graphite mat (3mm × 30mm × 84mm) was rolled and attached on the internal surface of the bottom of the tube. The graphite mat buried in the soils acted as the soil cathode in the cells.

Two rice seedlings were planted in the tubes. Rice roots were anticipated to expand along the tube wall and be in close contact with the soil cathodes. All of the electrodes were clean and used as received; they were conducted out with titanium wires.

2.3 SMFCs without an activated anode

Eight SMFC apparatuses were constructed. Two controls were used. One control consisted of an open circuit, and the other was a closed circuit with a connection of 1000 ohm resistance. Any plant grown in the control was quick removed when observed. For the treatments, 4 seedlings were planted in each container. Two of them were planted in the tube; the others were beside them. No fertilizer was added to the soils, plants were irrigated with deionized water. Among the six SMFCs with plants, two were in open circuits and the other 4 were connected with 1000 ohm resistance. All of the voltages were recorded using a data logger (USB-7660, ZTIC Co., Beijing, China).

2.4 SMFC with an activated anode

Six cells from experiments described in section 2.3 were incubated in the climate-controlled room for 56 days to allow for anode activation (Table S1). During the activation, no plant or organic matter was added to the cell, but native algae (mostly *Spirogyra*) were found in the surface water.

The cells were moved to a greenhouse after the activation. Among the six cells, three were planted with two rice seedlings into the root-sleeving tubes. The others and the controls consisted of two cells in a closed circuit or in an open circuit. Around 30g of dry paddy soils, mixed with urea ($0.2 g\cdot kg^{-1}$ as N), $CaHPO_4\cdot 2H_2O$ ($0.15 g\cdot kg^{-1}$ as P_2O_5), K_2SO_4 ($0.2 g\cdot kg^{-1}$ as K_2O), was added into the root-sleeving tube to support the plant growth. On day 1, root-sleeving tubes with seedlings were half inserted into the soil and subsequently topped with 4cm of deionized water (Fig. 1). For these flooded samples, the root and air cathodes were adapted to the new set of conditions. The soil cathodes were incubated in a mixture of soil, solution and air, while the air cathodes were fixed at the interface between the solution and air. On day 20, 10ml of nutrition solution (7.5mM N as NH_4NO_3, 4mM K as K_2SO_4 and 1.5mM P as NaH_2PO_4) was injected into the root-sleeving tube by a syringe. Three hours later, another 10ml of nutrition solution that had been deoxygenated by purging with N_2 for 30 min was injected. A variety of 10ml solutions were then injected including 1.5mM P solution (Day 22), deionized water (Day 26), 1.5mM P solution (Day 27), 4mM K solution (Day 28), and 7.5mM N solution (Day 29).

2.5 Net oxygen flux measurement

Net oxygen flux on root surfaces was detected using the scanning ion-selective electrode technique system (BIO-001A; Younger USA LLC.). The seedlings used for oxygen flux measurements were grown under the same conditions as described above. The plants were transferred into a measuring chamber filled with a hypoxic solution (Pang et al., 2006). One mature root was selected and fixed on the chamber to prevent floating. The positions of the microelectrode and camera were adjusted to insure the O_2-probe tip was adjacent to the root surface (< 10μm away). The O_2 microelectrode tip slowly vibrated (amplitude: 30 μm, 0.3-0.5 Hz) while away from the root surface using a computer-driven micromanipulator. Electrode movement did not disturb the solution.

Net oxygen flux was determined in a steady-state environment for 3 min at one position. Nitrogen gas was bubbled into the solution for 5 min between two measurements to insure hypoxia condition. When the root was replaced, the solution was also replaced. All experiments were accomplished by Xuyue Sci. and Tech. Co., Ltd.

3. Results and Discussion

3.1 Voltage fluctuation during plant growth

Towards better description, the abbreviations used in this study were explained in table 1. In the first trial, the highest $V_{1k\Omega}$ detected in the cells (102mV) was calculated using the air cathode in the control (Table S2), which was equivalent to 1.3mW·m^{-2}. The low electricity produced by the cells with 1000 ohm resistances might be due to a paucity of electrogenic bacteria on the anode surface. Rice plants were found to have an influence on the soil cathode. Significant positive soil $V_{1k\Omega}$s were recorded for more than 30 days when rice was planted in the root-sleeving tube (Table S2), which implies that there was continual oxygen excretion from the rice roots.

The activation of the anode for 56 days resulted in an increase and stabilization of the cell voltages. As shown in Figure 2, the air V_{open} of the cell without plants rapidly increased and oscillated between 500 to 708 mV, at the same time, the soil V_{open} decreased quickly and became negative in 22 hours. After reaching a minimum of -201 mV, the soil V_{open} exhibited a positive linear relationship with time. A similar tendency was also found when the circuits of the cells were connected with external resistance (Fig. 2). The air $V_{1k\Omega}$ varied from 125 mV to 248 mV, and the soil $V_{1k\Omega}$ linearly increased from -38 mV to - 24 mV from day 2 to 21 following an initial.

Due to the degradation of aquatic organisms and diffusion of atmospheric oxygen, top soil generally contains a higher concentration of organic matter and oxygen than soil at depth. The soil redox potential was often found to decrease with organic matter decomposition (Tanji et al., 2003; Gao et al., 2011), and it was recently reported that slight amounts of oxygen can increase the anodic metabolic activity which may cause a higher electron pressure at the cathode compared to the anode (Ajayi et al., 2010). Thus, both causes may result in the cell electrodes' polarity reversion.

The presence of plants in the root-sleeving tube had minimal influence on the air V_{open}, but greatly affected the variation patterns of the air $V_{1k\Omega}$, soil $V_{1k\Omega}$ and soil V_{open} (Fig. 3 and Fig. S1). The variation patterns could be divided into three stages. Initially, soil $V_{1k\Omega}$ and V_{open} dropped near to or below zero from days 1 to 3, respectively. At the same time, air $V_{1k\Omega}$ and V_{open} increased and varied around 90 mV and 700 mV, respectively. The air V_{open} then fluctuated above or below 800 mV. Stage two, from days 3 to 17, was characterized by the soil V_{open} and $V_{1k\Omega}$ increasing and stabilizing. In this stage, the soil V_{open} was slightly lower than the air V_{open}. Moreover, the soil $V_{1k\Omega}$ was even higher than the air $V_{1k\Omega}$ from days 11 to 17. The maximum voltages of an SMFC, 191mV of air $V_{1k\Omega}$ and 201 mV of soil $V_{1k\Omega}$, were observed during this period. Following the stabilization period, the soil V_{open} dramatically decreased from 780 mV to ca. 450 mV and the soil $V_{1k\Omega}$s gradually declined from day 17.

3.2 Estimation of oxygen flux from rice roots

In this study, the cathode of the SMFC was used as an oxygen sensor with continuous electrochemical signals being detected *in situ*. These signals were measured in the rhizosphere of the rice root for 32 days (Fig. 3B) and indicate that oxidized chemicals were reduced in the cathode reaction. Assuming the cathode half-reaction was $O_2 + 2H_2O + 4e^- = 4OH^-$, the oxygen depletion rate on the cathode was calculated based on the $V_{1k\Omega}$ curve (Fig. 3). The calculation was based on the equation: $N(O_2)/t = I/(nF)$, where I is the current, n is the number of the electrons consumed to reduce O_2, and F is Faraday's constant (96485 $C{\cdot}mol^{-1}$). The electrical current was calculated using ohm's law $I = V/R$, where V is voltage and R is the external resistance. Because no cathode reaction was observed in the control, the enhancement of the soil cathode's potential in the cells with plants must be due to the rice plants excreting oxygen. Thus, the oxygen depletion rate on the cathode could be used as a reliable estimate of the rate of rice root oxygen excretion.

In this study, the maximum daily oxygen depletion rate on the soil cathode of 20 $\mu mol{\cdot}plant^{-1}{\cdot}day^{-1}$ was recorded on day 15. Planar Optode is a two-dimensional oxygen mapping system with a luminescent indicator. This system has been used to investigate the oxygen dynamics in the rhizosphere of eelgrass *Zostera marina* (Frederiksen and Glud, 2006). An estimated total oxygen loss of 2.69 $\mu mol\ O_2\ plant^{-1}{\cdot}d^{-1}$ was yielded by assuming 12 hours in daylight and that oxygen was excreted from roots on the first two internodes. Although this estimate of oxygen loss from eelgrass is lower than the calculated oxygen flux value from the SMFC with rice plants, several facts imply that the calculated oxygen flux is underestimated in this study: 1) not all the oxidized material excreted from roots could reach the cathode surface; 2) the rice root only partially covered the cathode surface; 3) the rice root growth was restricted; and 4) the rice plants used in this study were juvenile. Thus, the efficiency of the rhizo-cathode was not optimal. The power density of the SMFC could be enhanced using a structure that separated the organic matter and oxidized material excreted out of the wetland plant roots.

3.3 Causes of soil $V_{1k\Omega}$ fluctuation

When the soil $V_{1k\Omega}$ declined to 30 mV, which was 15% of the highest soil $V_{1k\Omega}$, nutrition solution was injected into the adjacent soil cathode (Fig. 3B). The first injection included a 10 ml solution of N, P and K which induced a sudden voltage peak of 167 mV. In the same day, a 10 ml deoxygenated solution was injected and resulted in a voltage drop to -9 mV which quickly recovered. A wide peak of soil $V_{1k\Omega}$ was observed following the two injections. Sole element solution and deionized water were also added to investigate their effects. Many solutions changed the catholyte composition and led to a sharp or wide voltage peak, but none of them recovered the soil $V_{1k\Omega}$ to a level comparable to the air $V_{1k\Omega}$. Similar voltage changes were observed in another replicate (Fig. S1).

In the third stage, soil $V_{1k\Omega}$ and V_{open} were found to decrease after approximately one month. Three hypotheses could explain this phenomenon. First, the oxygen reducing in the rhizosphere could be due to a lack of adequate mineral uptake by the rice roots which were restricted in the root-sleeving tubes (Trolldenier, 1973; Liu et al., 2004).

Second, the oxygen excreted from the rice roots was not uniform along the roots (i.e. young rice roots were able to excrete more oxygen than the mature or aging rice roots) (Rubinigg et al., 2002; Kotula and Steudle, 2009). Third, over time microbial activity along the roots increased and microbes competed with the cathode for oxygen, effectively lowering the availability of oxygen on the root surface.

The first hypothesis was rejected by the fact that chemical fertilizer addition did not stop or delay the soil $V_{1k\Omega}$ decrease and no obvious symptoms of mineral deficiency were noted during the experiment (Fig. 3). Although the microbial communities in different stages in the rhizosphere soil and bulk soil were not directly investigated, the injection of nutrition solution and deionized water induced a voltage rise when the solution was oxygenated. This finding indicates that the voltage decrease in the third stage was mainly due to the decrease in oxygen on the cathode surface and not to changes in the microbial community. Interestingly, the peaks induced by the solution injections were short in duration even though the injected solutions were identical (see peak II and IV in Fig. 3B). One explanation is that the microbial community responds slowly to changes in the redox environment (Yuan et al., 2009). Unlike the sharp peaks which may be induced by oxygen, the peaks after NH_4NO_3 addition were wide and may be the result of cathode microorganisms slowly using nitrate as an electron acceptor (Clauwaert et al., 2007).

A detailed oxygen flux pattern along the root length was discovered by placing oxygen microelectrodes near rice roots during hypoxic and stagnant solution conditions. Both influx and efflux of oxygen were found on the surface of adventitious root (Fig. 4). The root cap and meristem region (0.15 mm behind root tip) consumed a considerable amount of oxygen at a rate of approximately 60 pmol·cm^{-2}·s^{-1}. Conversely, the elongation zone and maturation zone excreted oxygen, which reached its maximum at 20 mm from the tip (40 pmol·cm^{-2}·s^{-1}). Weak oxygen efflux was then detected around adventitious roots above 60 mm. When the cathode of the SMFC was used as an oxygen sensor, the maximum instant oxygen depletion rate on the soil cathode was 520 pmol·s^{-1}. This rate was calculated using the highest value of soil $V_{1k\Omega}$ recorded (201 mV). To compare the oxygen fluxes gained from the MFC experiments and microelectrodes, the geometric area of the soil cathode was assumed to be equivalent to the root area in contact with the soil cathode and that the oxygen depletion rate on the soil cathode was 20.6 pmol·cm^{-2}·s^{-1}. This value is comparable to the oxygen efflux rate detected by the O_2 microelectrode (< 40 pmol·cm^{-2}·s^{-1}) which supports the hypothesis that the cathode of an SMFC can be used as a sensor for oxidized material.

The oxygen flux pattern along rice roots supports the second hypothesis that young rice roots were able to excrete more oxygen than the mature or aging rice roots. Significant oxygen effluxes behind root tips along adventitious roots were also found in other rice genotypes when raised in a stagnant deoxygenated nutrient solution (Colmer, 2002). Only a few studies have investigated the redox potential or oxygen density in plant rhizosphere. Of these, marked increases in redox potential were found close to rice root tips (Flessa and Fischer, 1992), but oxidized areas were discovered to exist along the entirety of the active root (Schmidt et al., 2010). For seagrass *Zostera marina*, the oxygen concentration along its roots was highest in the region 2-4 mm behind the root tip (Frederiksen and Glud, 2006).

3.4 The implication of a biocathode in the plant rhizosphere

A thick soil layer was needed to prevent oxygen permeation to the anode in SMFCs. For example, He et al., (2009) designed a phototrophic SMFC with a cathode hung ca. 12cm above an anode and a 5cm soil layer between the air cathode and anode (Kaku et al., 2008). The distance between the air cathode and the anode in an SMFC often leads to changes in internal resistance, with a larger distance resulting in greater resistance (Rismani-Yazdi et al., 2008). The biocathode using plant roots excreted oxygen as an electron acceptor which allowed for a decrease in the distance between the two electrodes.

4. Conclusions

In summary, the oxygen excreted by rice roots has proved to be a viable alternative source of electron acceptors for SMFCs. A soil cathode has comparable efficiency to an air cathode, but has the advantage of a shorter distance between the cathode and anode because it can be placed directly in the soil. By counting the electrons transferred to soil cathode, the oxygen efflux from rice roots was able to be calculated. This study can aid in future research aimed at developing new kinds of SMFCs, as well as studies evaluating the effect of radius oxygen loss on ecological functions in wetlands.

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7. Figure Captions

Figure 1 Section plan of the experimental setup of the SMFC with air cathode and soil cathode. The anode was a round graphite mat, and two cathodes were tube-like shape by bending rectangle graphite mats. $V_{1k\Omega}$: the voltage between two electrodes with 1000 ohm resistance; V_{open}: the voltage between two electrodes without resistances.

Figure 2 the cell voltages fluctuation of sediment microbial fuel cell as a function of time. The data from day 8 to 10 were lost because of power-down.

Figure 3 the cell voltages fluctuation of SMFCs as a function of time. A: open circus voltage; B: voltage with 1000 ohm external resistance. Gray lines represent the voltages between anode and air cathode, black lines represent the voltages between anode and soil cathode. The data from days 8 to 10 were lost because of power-down. Peaks with Roman numbers means different solutions were injected into rhizosphere: I, mixed nutrition solution and deoxygenated solution; II, sole P; III, DI water; IV, sole P; V, sole K; VI, sole N.

Figure 4 net oxygen flux profiles along rice adventitious root on hypoxia condition. Positive value means influx into roots; negative value means efflux out of roots.

8. Tables and Figures

Table 1 Abbreviations used to describe the voltage differences between electrodes. V_{open}: open circus voltage; $V_{1k\Omega}$: closed circus voltage with external resistance of 1000 ohm; air V_{open} or $V_{1k\Omega}$: the V_{open} or $V_{1k\Omega}$ between air cathode and the anode; soil V_{open} or $V_{1k\Omega}$: the V_{open} or $V_{1k\Omega}$ between soil cathode and the anode.

	Description	Cathode location	External resistance
air V_{open}	Open circus voltage	water-air interface	no
air $V_{1k\Omega}$	Close circus voltage	water-air interface	1000 ohm
soil V_{open}	Open circus Voltage	soil or rhizosphere	no
soil $V_{1k\Omega}$	Close circus voltage	soil or rhizosphere	1000 ohm

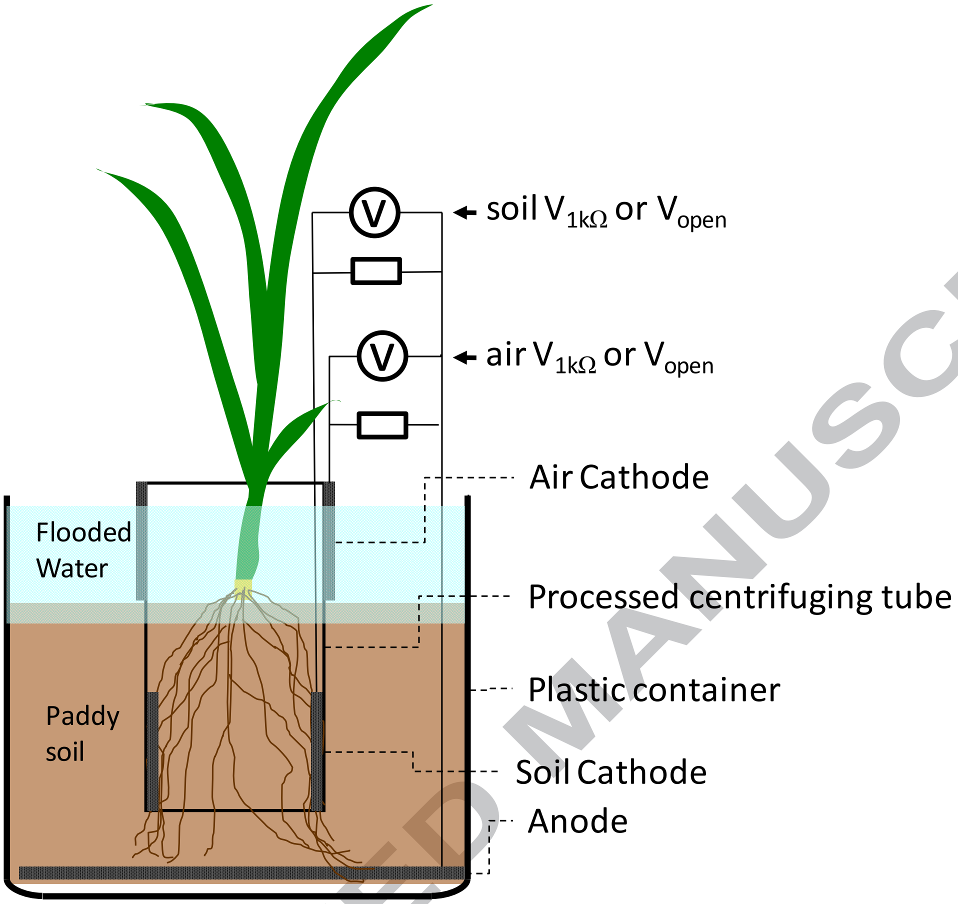
soil $V_{1k\Omega}$ or V_{open}
air $V_{1k\Omega}$ or V_{open}
Air Cathode
Flooded Water
Processed centrifuging tube
Plastic container
Paddy soil
Soil Cathode
Anode

Figure 1

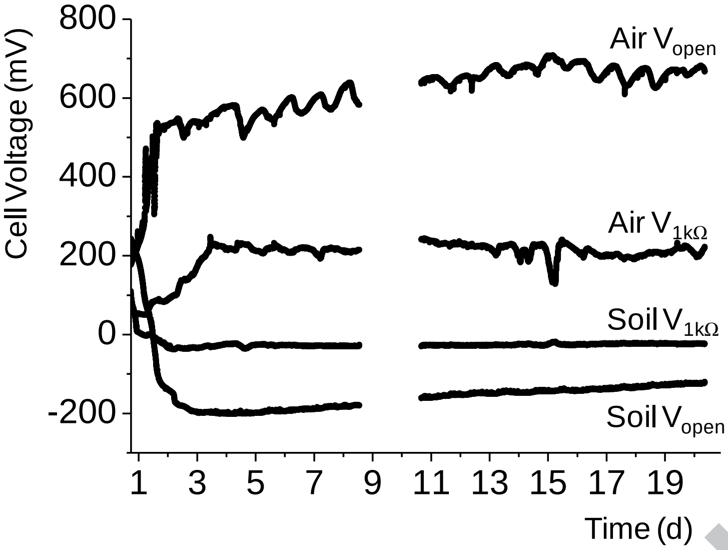
Cell Voltage (mV)
800
600
400
200
0
-200
1
3
5
7
9
11
13
15
17
19
Time (d)
Air V_{open}
Air $V_{1k\Omega}$
Soil $V_{1k\Omega}$
Soil V_{open}

Figure 2

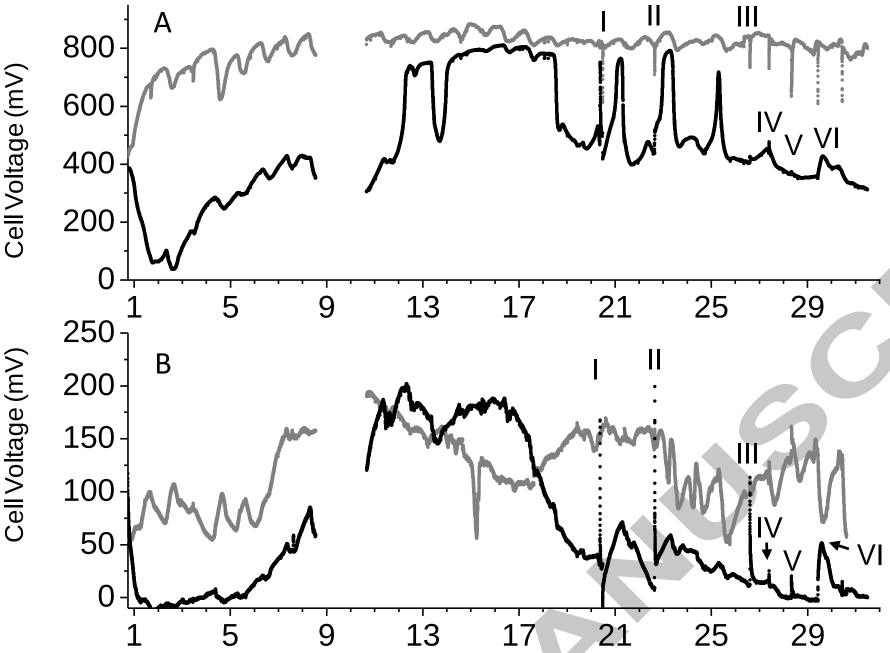
A
Cell Voltage (mV)
800
600
400
200
0
I
II
III
IV
V
VI
1
5
9
13
17
21
25
29
B
Cell Voltage (mV)
250
200
150
100
50
0
I
II
III
IV
V
VI
1
5
9
13
17
21
25
29

Figure 3

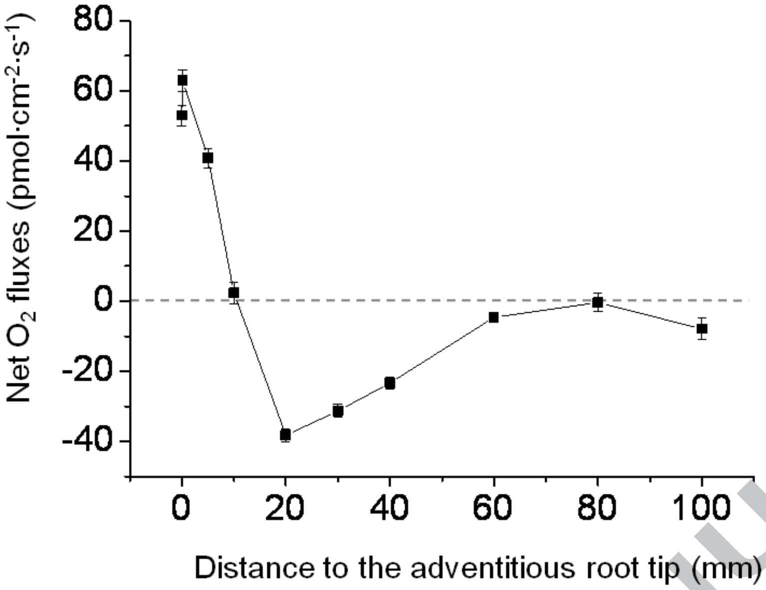
Net O_2 fluxes (pmol·cm^{-2}·s^{-1})
80
60
40
20
0
-20
-40
0
20
40
60
80
100
Distance to the adventitious root tip (mm)

Figure 4

Highlights

- The MFC cathode works well in soils where it can use oxygen excreted from wetland plant roots.
- A biocathode buried in the rice rhizosphere has comparable efficiency to an air cathode.
- Maximum oxygen efflux was 20 μmol·plant^{-1}·day^{-1} based on cell current calculations.