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RESEARCH ARTICLE

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Essential factors that affect bioelectricity generation by *Rhodopseudomonas palustris* strain PS3 in paddy soil microbial fuel cells

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Summary

A plant-associated phototrophic bacterium, *R. palustris* strain PS3, was inoculated into a soil-based MFC to generate electricity. We evaluated the performance of this soil-based microbial fuel cell (MFC) and elucidated the essential factors that contributed to power generation. PS3 showed the potential to enhance power generation, especially when the apparatus was operated in a sealed chamber with illumination. We deduced that the improved power performance was due to the enhanced electron transport through the living electrode that was grown as a PS3 biofilm via photoheterotrophic metabolism. In addition, we suggested that the interplay between phototrophic fixation of ambient CO_2 and anaerobic oxidation of ferrous iron in soil was also involved in the increased power output. We implemented CMOS (complementary metal-oxide-semiconductor) technology with the soil-based MFC to harvest energy in a more efficient and stable manner. The above system is expected to provide a potentially low-cost and low-energy system with a high power conversion efficiency for practical applications in the future.

KEYWORDS

biofilm, CMOS, carbon dioxide fixation, PGPR, Phototrophic bacteria, soil-based microbial fuel cell

1 | INTRODUCTION

Microbial fuel cells (MFCs) are bioelectrochemical devices that use bacteria as catalysts to oxidize organic and inorganic matter and generate current. Electrons produced by the bacteria from these substrates are transferred to the anode (negative terminal) and flow to the cathode (positive terminal), which are linked by a conductive material containing a resistor or operated under a load.[1-5]

A diverse range of microorganisms has been reported to act as electricigens. For example, *Geobacter* and *Shewanella* species are the most commonly used bacteria in a variety of MFC systems.[6,7] These electricigens have been reported to take advantage of a variety of membrane

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redox-active proteins (cytochromes) or conductive pili to transport the electrons from bacterial cells to electrodes.[8,9] It has also been reported that the biofilm production of electricigens is proven to increase the electron transfer rate.[4,5,10,11]

In addition to these two bacterial genera, non-purple sulfur photosynthetic bacteria, such as *Rhodopseudomonas* spp. was applied as electricigens frequently. For example, *R. palustris* strain DX-1 could produce electricity at relatively high power densities (approximately 2700 mW/m^2) in a liquid MFC system.[11] In addition, *R. palustris* strain RP2 was reported to possess nanowires that were capable of producing a maximum current density of 21 ± 3 mA/m^2.[12] These electrophotographic bacteria are widely distributed in various aquatic ecosystems as well as in sediments, moist soils, natural wetlands, sludge, alkaline water and paddy fields.[13-16] They can convert complex organic compounds, such as root exudates, pollutants, or aromatic compounds, into biomass or bioenergy.[11,12,17] Electrophotographic bacteria can sustain themselves in different metabolic states, including photoautotrophic, photoheterotrophic, chemoautotrophic and chemoheterotrophic states, and they play an essential role in nutrient cycles.[18] A plant-associated *R. palustris* strain PS3 was isolated from a paddy field, which has been proven to improve crop growth and nutrient utilization in soil.[19] According to the whole genome sequence data (NCBI accession number: CP019966), there were more than 70 cytochrome genes identified in PS3.[20] Furthermore, it has been proven that PS3 can synthesize biofilms either in a vegetative state or with plant roots.[20] As mentioned, cytochromes as well as biofilms of electricigens play essential roles in transportation of the electrons from bacterial cells to electrodes.[8-11,21] Accordingly, we propose that the *R. palustris* strain PS3 has the potential to serve as an electricigen in MFCs under special conditions.

MFCs can extract bioelectricity from a broad spectrum of soluble or dissolved substrates, such as complex organic wastes and renewable biomass.[22] Soil contains a mixture of organic matter, minerals, gases, liquids, metal, and organisms that have been considered nutrient-rich media for energy generation.[23] The essential electrochemical reactions that occur in soil-based MFC systems are exogenous or endogenous microbes that utilize organic matter and directly convert chemical energy to electrical energy.[24,25] The advantages of soil-based MFCs include their ease of access, low cost, clean energy generation, and renewability.[21] To date, there have been several studies integrating the MFC principle with soil for power applications.[26-28] For example, a self-powering wireless system using soil energy has been developed to monitor temperature and humidity in environments.[21,29] In addition, a soil-based MFC has also been reported to drive a single-hop wireless sensor network (WSN).[30]

In general, the output power of soil-based MFCs is in a mV range, which is insufficient to power off-the-shelf electronics directly.[29] To extract the maximum amount of energy from these systems, a power management circuit with adaptive maximal power extraction and management of power delivery for wide-range input power is indispensable[31]; for example, the use of discrete, complementary metal-oxide-semiconductor (CMOS) integrated circuits (ICs), and so on in these systems.[32] Recently, CMOS technology has been used for constructing ICs in MFCs.[33] They are low cost and provide a small form factor, low energy consumption, high power conversion efficiency, and quick operation among components.[30,34,35] In a previous study, we applied CMOS technology with a power management IC to develop a high-efficiency DC/DC converter for harvesting increased electrical power from a soil-based MFC equipped with carbon-zinc (C-Zn) electrodes.[36]

In this study, we inoculated the plant-associated phototrophic bacterium *R. palustris* strain PS3 into a soil-based MFC to generate electricity. We evaluated the performance of this soil-based MFC and elucidated the essential factors that contributed to electricity generation. Furthermore, we also integrated this cell with an improved CMOS power management system to harvest electricity from the soil more efficiently.

2 | MATERIALS AND METHODS

2.1 | Preparation of the microbial inoculant

R. palustris strain PS3 (DSM 29314) was used as a microbial inoculant for the soil-based MFC. This bacterium was cultivated in purple nonsulfur bacteria (PNSB) broth at 37°C.[8,19] Three milliliters of the precultured broth was inoculated into a 250-ml Erlenmeyer flask containing 50 mL of fresh PNSB broth and cultivated on a shaker (200 rpm) at 37°C for 22 hours. Subsequently, 50 mL of the above inoculum was transferred into fresh PNSB broth in a 1000-ml Erlenmeyer flask. After culturing for an additional 22 hours, the inoculum was used as a seed culture for batch fermentation that was performed in a fully automated benchtop fermenter (MS-F1, Major Science CO., LTD, Taiwan) with 3 L of fresh PNSB broth. The concentration of the fermented broth of PS3 was around 2×10^9 CFU/mL.

2.2 | Construction and operation of soil-based MFC

Soil sample was taken from rice paddy field in Taiwan, and some of its physicochemical properties, such as pH, electrical conductivity (EC), and organic matter (OM) are shown in Table 1. The soil-based MFC apparatus used in this study was made of acrylic plates with a volume of 10 cm × 10 cm × 12 cm (1200 cm^3), which was divided into four compartments (Figure 1A). As shown in Figure 1B, each compartment was filled with 300 g of paddy field soil (dry weight), and one zinc rod (a length of 20 cm and a diameter of 10 mm), which was surrounded by four carbon rods (a length of 15 cm and a diameter of 8 mm), was inserted into the soil (Figure 1B), as described in our previous study Yang *et al*.[37] Zinc and carbon serve as the anode and cathode, respectively. In contrast to those electrodes in the conventional single or two chamber MFCs, these two parts (anode, cathode) were not separated by the proton exchange membrane (PEM). The two electrodes were then connected to an external circuit with a resistance of 100 Ω. For the control experiment without PS3 inoculation, 300 mL of deionized water was gently poured into each apparatus. For the experiments with PS3 inoculant, 300 mL of the fermented broth (2×10^9 CFU/mL) was gently poured into each apparatus and sat for overnight until the inoculant was uniformly infiltrated into the soil, indicating that the concentration of PS3 cells in the soil was equivalent to 10^7 CFU/g soil. The resulting soil-based MFC was then operated at 25°C to 28°C in a temperature-controlled room and under different conditions, including sealed with a lid, open-lid (unsealed), illumination with T5 LED lamp from a distance of two meters above the apparatus (1.1 W/m^2) and dark conditions for 90 days. Voltage (V) and current (I) values were measured by a multimeter, and the power (P) calculation was made according to the following formula, P (watt) = V × I. All experiments were conducted in at least triplicate.

2.3 | Scanning electron microscopy analysis of the carbon rod electrodes

Both vegetative and colonized PS3 cells were observed by scanning electron microscopy (SEM). For vegetative cells, we took 3 mL of PS3 broth and centrifuged the solution at 5000 rpm for 12 minutes. The pellet was immersed in a 2.5% formaldehyde solution overnight to fix the sample, dehydrated stepwise in a graded series of water/ethanol solutions (25%, 50%, 75%, and 100% v/v EtOH; 0.5 hours for each treatment) and dried at the CO_2-critical point for 3 hours as described by Bozzolo *et al*.[38] In a preliminary test with plate count method, we noticed that PS3 cells were only detected on the surface of the C electrode but were not detected on the surface of the Zn electrode after operation of the soil-based MFC (data not shown). To observe the cells of electricigen colonized on the C electrode, we pulled the carbon rod electrodes from the soil-based MFC and cut them into 1.0-cm-long pieces; the pieces were immersed in a 2.5% formaldehyde solution overnight to fix the samples. The samples were then dehydrated stepwise in a graded series of water/ethanol solutions, as described above, and dried at the CO_2-critical point for 3 hours. The electrode samples were then mounted on copper specimens using a copper contact adhesive. Then, the samples were sputter-coated using a gold target and observed by SEM (JSM-5410, Jeol, Japan). The resulting SEM images were captured digitally.

2.4 | DNA extraction from paddy soil

DNA was extracted from paddy soil that was filled in the soil-based MFC by FastDNA SPIN Kit (MP Biomedical, Santa Anna, CA). The species specific primers were used to detect the electricigens (*R. palustris*., *Shenawella* spp., and *Geobacter* spp.) by polymerase chain reaction (PCR), and their sequences and conditions were shown in

TABLE 1 Physiochemical analysis of soil treatment with 0 and 30 days of inoculation

	Days of inoculation		
	0	30	
Soil properties	**(−) PS3/light**	**(−) PS3/light**	**(+) PS3/light**
Texture	Clay	Clay	Clay
Water content (%)	75%	76%	74%
pH	5.60 ± 0.30 c	6.28 ± 0.40 b	7.90 ± 0.35 a
EC (ms/cm)	0.10 ± 0.01 a	0.13 ± 0.02 a	0.15 ± 0.05 a
OM (%)	3.10 ± 0.20 a	2.80 ± 0.35 a	3.10 ± 0.10 a

Abbreviations: EC, electrical conductivity (ms/cm); OM, organic matter (Percentage, %).

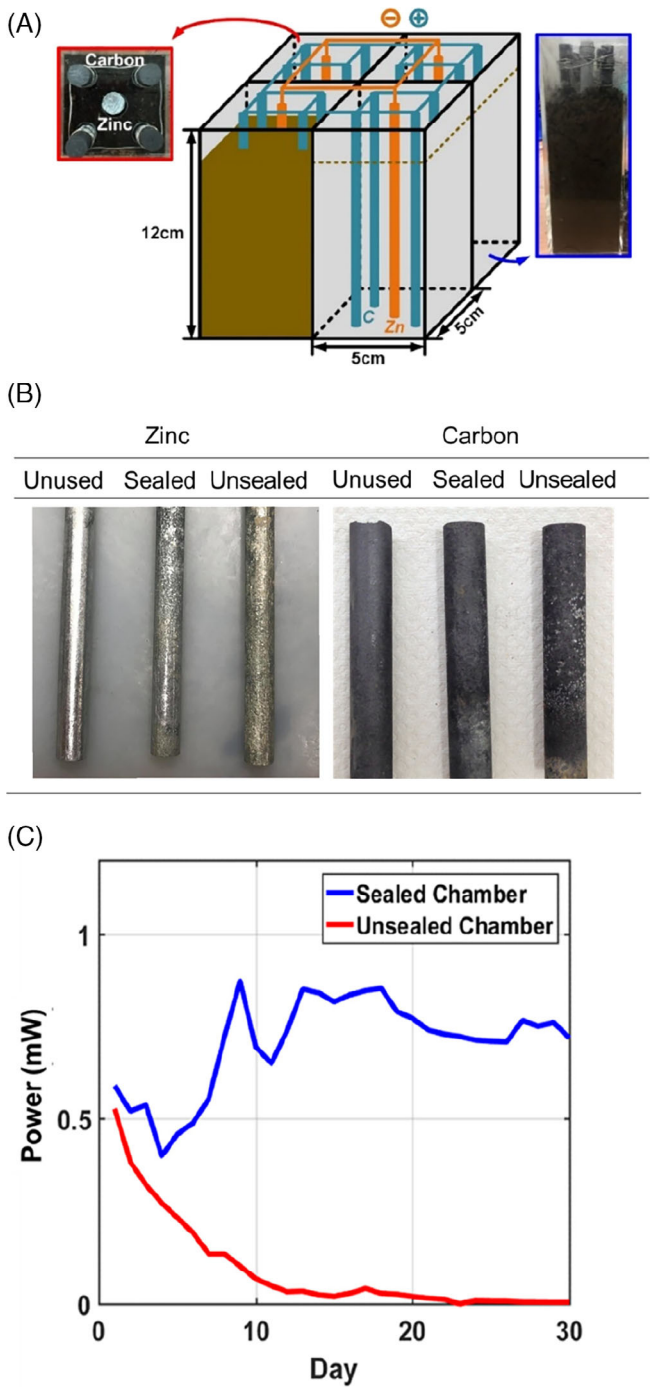

FIGURE 1 Architecture and operation of the soil-based MFC (without microbial inoculation). A, Diagram of the soil cell apparatus. B, Carbon and zinc electrodes in the sealed and unsealed (open lid) chambers after 10 days of operation. C, Measured power from the soil-based MFC operated under sealed and unsealed (open lid) conditions. The resistance values of the electrodes in the unsealed and sealed chamber were measured by an ohm meter with values of 350 Ω and 3.38 Ω, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

Table S1. PCR products were examined by gel electrophoresis and followed by DNA sequencing.

2.5 | Determination of Fe (II)/Fe (III) in soil-based MFC

Ferrous and ferric ions contained in the soil samples were determined by the colorimetric ferrozine-based assay.[39] Approximately 1.0 g of soil sample was added to a preweighed vial containing 5 mL of 0.5 M HCl for an hour. Later, 200ul of each soil suspension sample was aliquoted into a centrifuge tube that contained 10 mL of the Fe(II) buffer with 3.5 mM ferrozine and 50 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid). Ferrozine was reacted with ferrous iron in the soil suspension to form a complex that can be used to measure Fe (II). After being mixed for 10 minutes, the absorbance was determined at 562 nm (A562) using the UV-vis spectrophotometer, and the concentration of Fe (II) was calculated on the basis of a standard curve. For determining Fe(III), one gram of hydroxylamine hydrochloride was added to 100 mL of the above Fe (II) buffer as a Fe(III) reducing solution. Two hundred microliter of the above soil suspension was pipetted into the solution, and placed in dark for 4 hours to completely reduce all Fe (III) into Fe (II). Then, the total concentration of Fe (II) in the reaction sample was determined as described above, and the Fe (III) was calculated from the difference between these two values.

2.6 | Determination of the carbon dioxide effect on the performance of the soil-based MFC

A ventilation transmitter (JVT-D210, Jeter Electronics Co. Ltd., Taiwan) was used to examine the carbon dioxide amount in the soil-based MFC. Detectors were placed inside the sealed chambers, and the concentrations of carbon dioxide (CO_2) were recorded in real-time (Figure S1). To verify the effects of CO_2 on the power generation capabilities of the soil-based MFC with the PS3 inoculum, calcium hydroxide (slaked lime) was placed in an inner trench to remove CO_2 from the sealed chamber (Figure S1).

2.7 | Integration of an energy harvesting system for constantly extracting electrical power from the soil-based MFC

To maximize and stabilize the power extraction from the soil-based MFC, we applied a silicon chip that contained a CMOS DC/DC converter with a 2D-maximal power point tracking (2D-MPPT) controller according to our previous study.[36] To illustrate the performance of the

proposed chip, one set of soil-based MFCs implemented with the DC/DC converter and an LED array with "NCTU" printed letters was set up, as shown in Figure 9.

3 | RESULTS AND DISCUSSION

3.1 | Generation of electricity from a soil-based MFC equipped with C-Zn electrodes

A soil-based MFC used in this study consisted of four electric generating units (Figure 1A). Carbon (C) and zinc (Zn) were selected as electrodes for their high potential difference (~1 V) in redox reactions as described in our previous study, which would allow the electronic circuits to be easily driven.[29,36] Initially, the apparatus was operated without microbial inoculation under either open-lid (unsealed) or sealed chamber conditions, and the electricity generation efficacies were evaluated. By the way, the open-circuit voltage of the soil-based MFC was around 1.0 V under either sealed or unsealed (open lid) condition. Figure 1B shows the images of the respective C/Zn electrodes under the sealed and unsealed chamber conditions. Both the sealed and unsealed zinc electrodes showed corrosion on the surface, and the latter appeared more yellowish-brown than the former, indicating zinc oxidation during the intensive process. On the other hand, there was no dramatic difference in the carbon electrodes between the sealed and unsealed chamber treatments. As shown in Figure 1C, the average output power of the open-lid cell was approximately 0.3 mW over the first five days, which then rapidly declined to almost zero. On the other hand, that of the sealed cell remained at approximately 0.7 mWduring the first 30 days. This indicated that the power generation efficacy and voltage stability of the latter were remarkably better than those in the former. It has been known that the ionic conductivity of zinc electrodes is reduced with increasing corrosion.[40] As mentioned above, the zinc electrode was more corroded in the open-lid cell (Figure 1B). We further determined the electrical resistance of the zinc electrode in the soil-based MFC by multimeter after operation. The resistance in the unsealed chamber was 350 Ω, which was approximately 100 times larger than that in the sealed chamber (3.38 Ω). Accordingly, we deduced that the dramatic power loss was caused by an impediment in the electron transfer of the unsealed electrodes.

We also determined the soil water content in the sealed and unsealed cells during operation at room temperature (25-28°C). As shown in Figure 2, the soil water content remained almost at the same level in the sealed cell. On the other hand, it decreased dramatically in the unsealed cells, which led to dry-out in two days. We proposed that the quick decline in power in the unsealed cell (Figure 1C) was also due to the rapid loss of soil moisture. Accordingly, we adopted the sealed chamber condition for the following experiments to extend the operation time.

3.2 | Improvement of power performance through the inoculation of an electricigen

Geobacter spp., *Shewanella* spp., and *R. palustris*. are commonly studied microbes for electricity generation. To

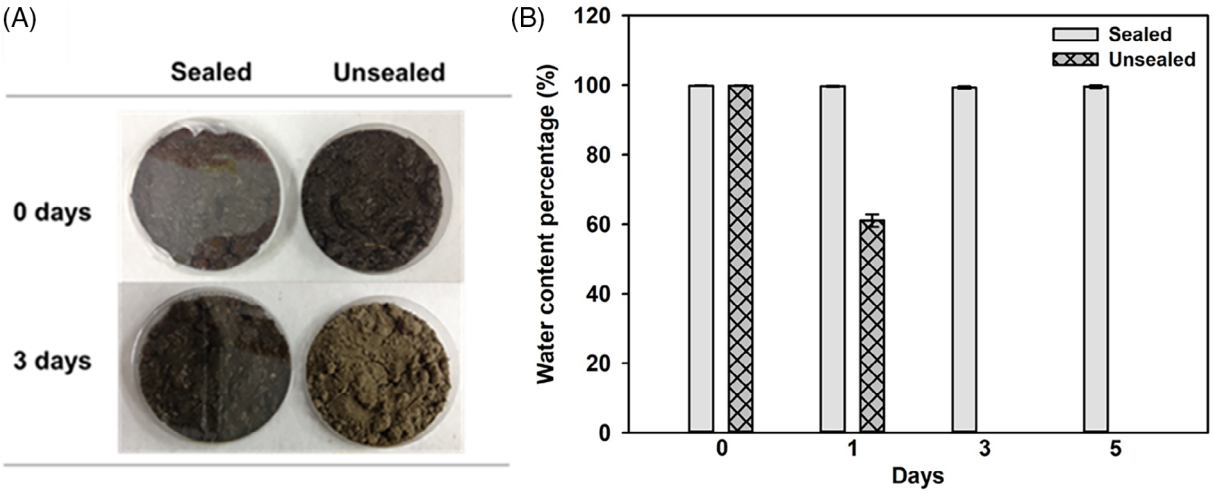

FIGURE 2 Soil water contents in both the sealed and unsealed soil-based MFCs. Soil was sampled from the respective soil-based MFC after 0 and 3 days of operation. A, Soil appearances derived from different apparatuses. B, Soil water content percentage (%). Data represents the mean (data point) and SEs (error bars) of three replicate assays [Colour figure can be viewed at wileyonlinelibrary.com]

confirm whether these microbes were already existed in the rice paddy soil that was filled in the soil-based MFC, we extracted DNA from the apparatus and performed PCR with the specific primers for detection. As shown in Figure S4, non of them were detected in the tested soil samples.

To verify whether *R. palustris* strain PS3 could act as an electricigen, we inoculated this bacterium into the soil-based MFC and determined its power performance. The power output (mW) was measured by a multimeter for the respective treatments (ie, soil-based MFCs that were operated with or without PS3 inoculant) during operation. As shown in Figure 3, the power outputs were low for both treatments (w/ wo PS3) over the first few days. We found that the power output of the soil-based MFC that was operated with PS3 inoculant remarkably increased after day 5. It achieved the highest value (2.5 mW) on day 15 and then subsequently decreased.

Afterwards, we extracted DNA from the surrounding soil of electrodes in the apparatus, and the existence of this bacterium was confirmed by PCR with *R. palustris*-specific primers (Figure S4). In contrast, the power output of the soil-based MFC that was operated without PS3 inoculant maintained at low level (approximately 0.5 mW). These results indicated that *R. palustris* strain PS3 had the potential to enhance power generation in a soil-based MFC.

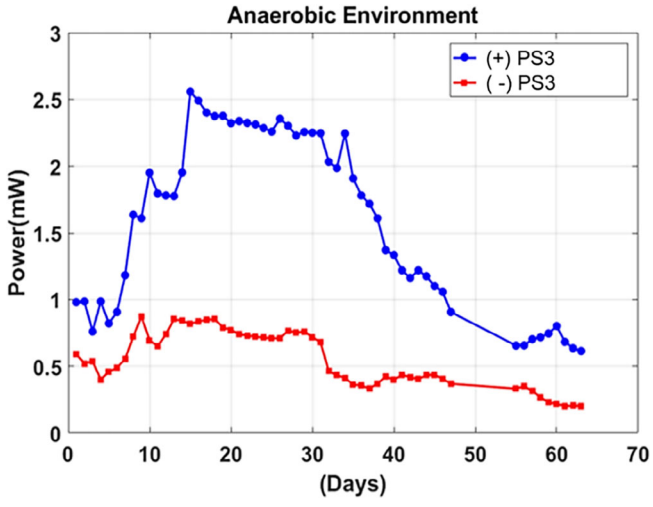

FIGURE 3 Power output of the sealed soil-based MFC inoculated with *R. palustris* strain PS3. The blue curve indicates the power output in the presence of PS3 inoculant in the cell, and the red curve indicates the power output without PS3 inoculation. The power output was calculated by taking the product of the voltage and current (P=I x V), which was represented in milliwatts (mW). This experiment was repeated three times [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Biofilm and filamentous structures formed by PS3

We determined the vegetative morphology of PS3 by scanning electron microscopy (SEM). As shown in Figure 4A,B, the rod-shaped PS3 cell (approximately 1 μm in diameter) contained a single polar flagellum. According to previous studies, PS3 was able to form biofilms on either biotic surfaces (plant roots) or abiotic surfaces (test tubes).[20] We observed the surfaces of the two electrodes by SEM after operation of the soil-based MFC and noticed that the PS3 cells were only detected on the surface of the C electrode (Figure 4C-F). In contrast, PS3 cells were unable to be detected on the surface of the Zn electrode by plate count method (data not shown). We speculated that the multilayered, rough and porous structure of the carbon electrode provided extended surfaces for the initial adhesion and colonization of PS3 (Figure 4C,D). As shown in Figure 4E,F, many PS3 cells as well as microbial clumps were observed on the surface. It has been regarded that electrode-associated microbial biofilms can facilitate the transport of electrons between electrodes and cells, as well as among cells fixed within the film for power generation of MFCs.[10,41] A few bacterial genera, such as *Geobacter*, *Shewanella*, *Synechocystis*, and *Rhodopseudomonas*, have been reported to transfer electrons through nanowires (pili and flagella) directly.[12,26,42,43] We observed that there were several extracellular filamentous structures along with the PS3 cells during the SEM examination (Figure 4B), which were proposed to function as nanowires. Taken together, we deduced that the power performance improvement of the soil-based MFC was due to the enhanced electron transport through the living electrode that was grown as a PS3 biofilm.

3.4 | Power generation of the soil-based MFC in response to light illumination

Rhodopseudomonas bacteria are known to transform light energy into chemical energy, which is then converted into electric energy.[44] In this study, we evaluated the effect of light illumination on the performance of bioelectricity generation for soil-based MFCs. The sealed apparatus inoculated with PS3 was operated under T5 LED lamp light illumination (1.1 W/m^2) for 90 days (Figure 5A). During the illuminated operating period, the highest power output rapidly increased to 3.2 mW and then declined slowly (Figure 5A, in the blue curve). Under dark conditions, only 2.0 mW was recorded as the highest power output, and it declined rapidly after 7 days (Figure 5A, in the red curve).

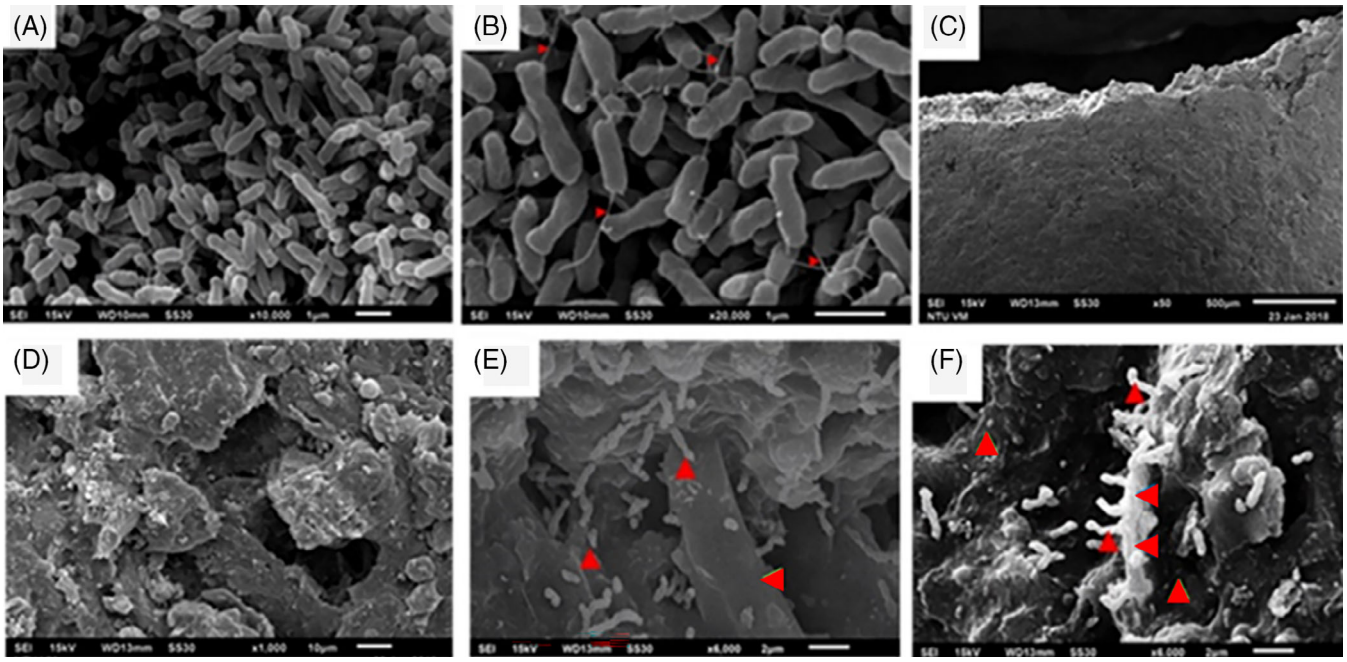

FIGURE 4 Scanning electron microscopy images of *R. palustris* strain PS3 on the carbon electrodes. A, Morphological characterization of the PS3 cells. B, Flagellum of PS3 (red arrowheads). C, D, Carbon electrode surface colonized with PS3. E, F, Biofilm formation on the carbon electrodes. The red arrows indicated there were PS3 cells on the rod and biofilm formation by bacterial cells [Colour figure can be viewed at wileyonlinelibrary.com]

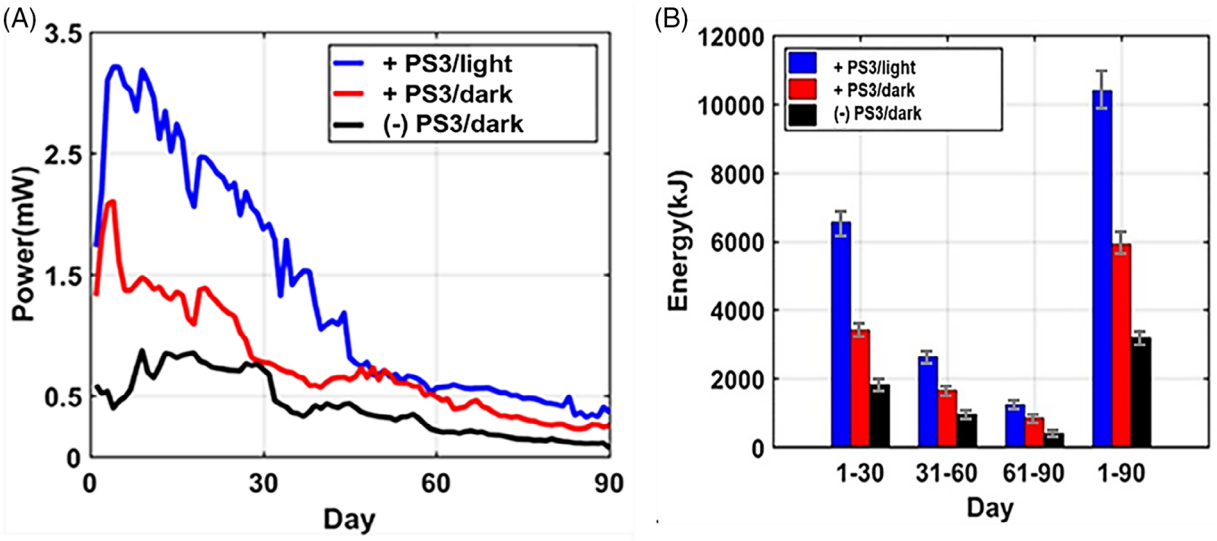

FIGURE 5 Effect of illumination on the power output and total energy production of the soil-based MFC. A, Power output of the respective soil-based MFCs under different treatments. The sealed apparatus inoculated with/without PS3 was operated in the dark and under illumination for 90 days. The power output was measured by a multimeter. B, Total electricity of the respective soil-based MFCs harvested by the DC/DC converter. + PS3/light indicates the sealed soil-based MFC apparatus inoculated with PS3 and operated under illumination. + PS3/dark indicates the sealed apparatus inoculated with PS3 and operated in the dark. (−) PS3/dark group indicates the sealed apparatus without the PS3 inoculation and operated in the dark. All experiments were performed three times [Colour figure can be viewed at wileyonlinelibrary.com]

We also determined the total energy harvested by the DC/DC converter of the cell during operation. The total energy is equal to the power output (P) multiplied by the operation time period (kilojoules, kJ). As shown in Figure 5B, it was approximately 10 000 kJ±1000 under illumination (90 days); moreover, almost all of the energy

was obtained in the first month of operation (1-30 days) and then decreased over time. On the other hand, it was approximately 6000 kJ±500 in the dark, which was 40% less than that of the former. This result indicated that the generation of bioelectricity could be remarkably enhanced by light illumination. Many studies have indicated that illumination can enhance the electrochemical activities of photosynthetic bacteria-based MFCs.[12,42,45] It has been reported that *R. palustris* can grow using various metabolic modes, including photoautotrophy, photoheterotrophy, organoheterotrophy, and chemoautotrophy.[18,46,47] Accordingly, we deduced that *R. palustris* PS3 grew via phototrophic metabolism under anoxic conditions and illumination, while the apparatus was operated. That is, PS3 might obtain energy from the illumination and took advantage of organic compounds in the soil as electron donors.

3.5 | Effect of carbon dioxide on the power output and total energy production

Soil respiration refers to the CO_2 released from the soil surface, which is produced by the biological activity of soil organisms, including plant roots, microbes, and soil animals.[48] We measured the concentration of CO_2 in the apparatus to evaluate the level of microbial activity in the soil-based MFC. In a preliminary experiment, we found

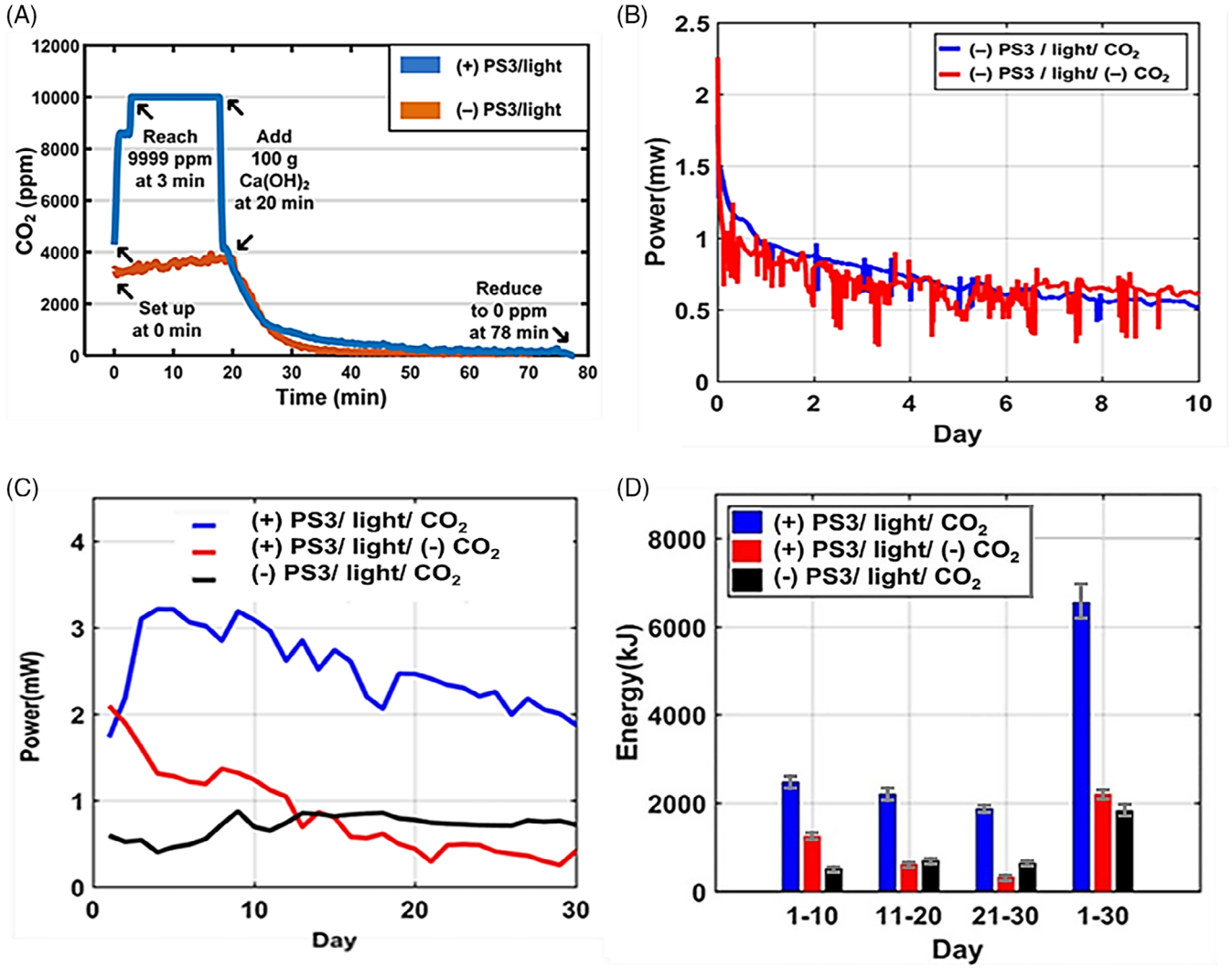

FIGURE 6 Effect of carbon dioxide (CO_2) on the power output and total energy production of the soil-based MFCs. A, Carbon dioxide measurement in the presence and absence of the PS3 inoculation. B, Power output from the sealed chamber without the PS3 inoculation in the presence and absence (ie, trapped by $Ca(OH)_2$) of CO_2 under illumination for 10 days of operation. C, D, Power output and energy from the sealed chamber with the PS3 inoculation in the presence and absence of CO_2 under illumination for 30 days of operation. Carbon dioxide was monitored by a CO_2 sensor. The data are represented in average values and all experiments were performed in triplicate [Colour figure can be viewed at wileyonlinelibrary.com]

that the CO_2 concentration in the sealed soil-based MFC without PS3 inoculation was significantly elevated and achieved 4000 ppm in 20 minutes (Figure 6A). Since oxygen (O_2) initially existed in the cell, we speculated this CO_2 mainly resulted from aerobic microbial decomposition of soil organic matter to obtain energy for the growth and functioning of the microbes inhabited in the apparatus. We noticed that the concentration of CO_2 quickly exceeded the detection limit (> 9999 ppm) within 3 minutes after inoculation of PS3 (Figure 6A).

To evaluate the effect of CO_2 on power output, we added approximately 100 g of $Ca(OH)_2$ powder (ie, slaked lime) along a small channel within the sealed chamber to trap CO_2 gas from the system (Figure S1). The powder did not contact the soil, and the concentration of CO_2 in both the (−) PS3/light and (+) PS3/light treatment decreased dramatically and was removed within 80 minutes (Figure 6A). As shown in Figure 6B, there was no dramatic difference between the power outputs in the presence or absence of CO_2 in the system where no PS3 was inoculated, although that of the latter (red curve) showed a more intense fluctuation than that of the former during operation. While PS3 was inoculated in the system, we found that the power output of the soil-based MFC was remarkably increased, and it achieved the highest value (3.2 mW)on day 3 (Figure 6C). On the other hand, when CO_2 was removed from the apparatus, the power output continued to decline (red curve), although the PS3 cells were still viable (Figure S2). The average power output of the (+) PS3/light/CO_2 group was 2.0 mW during the one-month operation; in contrast, that of the (+) PS3/light/(−) CO_2 group was only 0.5 mW. Furthermore, we also determined the total energy harvested by the DC/DC converter of the cell during operation. As shown in Figure 6D, it was more than 6000 kJ±500 in the (+) PS3/light/CO_2 group (30 days); however, it dramatically decreased by approximately one third (approximately 2000 kJ±100) when CO_2 was removed from the apparatus. Therefore, it suggests that CO_2 is indispensable for PS3 to generate higher electricity levels.

R. palustris is able to fix CO_2 into carbohydrates via photoautotrophic process under anaerobic and illumination conditions.[17] In addition, it has also been demonstrated that this phototrophic CO_2 fixation process is directly connected to the extracellular electron transfer of *R. palustris* for obtaining energy.[49] Accordingly, it suggests that the relative high electricity generation of the soil-based MFC as shown in Figure 6B was related to the phototrophic CO_2 metabolism of *R. palustris* PS3. That is to say CO_2 may act as either carbon source or as terminal electron acceptor for PS3 when grown in anoxic illuminated environments.

As mentioned above, the power output of the sealed soil-based MFC was immediately and dramatically reduced when CO_2 was removed from the apparatus (Figure 6C), however, the viability of the PS3 cells was not affected (Figure S2). Accordingly, we assumed that PS3 did not metabolize the ambient CO_2 as carbon source. Alternatively, this bacterium might use the carbon sources derived from paddy soil and nutrient broth for vegetative growth.

3.6 | Effects of nutritive substances in paddy soil and broth on the performance of soil-based MFC

There are many reports indicated that the efficacies of the conventional MFCs (single or two chambers) are related to the amounts of substrates, such as acetate, glucose, carboxymethyl cellulose (CMC) arabitol, and lignocellulosic waste in the devices.[22,50-53] In this study, paddy soil was used as main substrate for the soil-based MFC. Since soil electrical conductivity (EC) can serve as an easy indicator of nutrient availability,[54] we measured the EC levels for the paddy soil in the presence or absence of PS3 inoculation by a conductivity meter. Although the PS3 broth contained many nutrients, such as CH_3COONa, NH_4Cl, and yeast extract, we found that the EC level of the paddy soil with PS3 inoculation (0.15 ± 0.05 mS/cm) was not significantly varied from that without PS3 inoculation (0.10 ± 0.01 mS/cm) (Table 1). We noticed that the pH of the paddy soil in the absence of PS3 inoculation was increased from 5.60 ± 0.30 to 6.28 ± 0.40 during operation, and that of the PS3 inoculated soil was increased further to 7.90 ± 0.35. We assumed that the increase of acidic soil pH was due to the metabolism of nutrient substrates by the microbes inhabited in the paddy soil, including the *R. palustris* PS3 inoculated. Soil organic matter (OM) refers to all organic materials derived from plant, animal and microbial residues in the soil, which is an important source of nitrogen, phosphorus and sulfur[55]. The microbial biomass decomposes OM to release carbon dioxide and plant available nutrients. After 30 days of operation, we found that the OM content of the paddy soil in the absence of PS3 inoculation ($2.80 \pm 0.35\%$) was not varied from that with PS3 inoculation ($3.1 \pm 0.1\%$)(-Table 1). Accordingly, we deduced that inoculation of PS3 broth did not cause too much alteration in the composition of the major nutritive substances in the paddy soil.

On the other hand, we found that the power output and energy production were remarkably boosted up, while PS3 was inoculated in the soil-based MFC

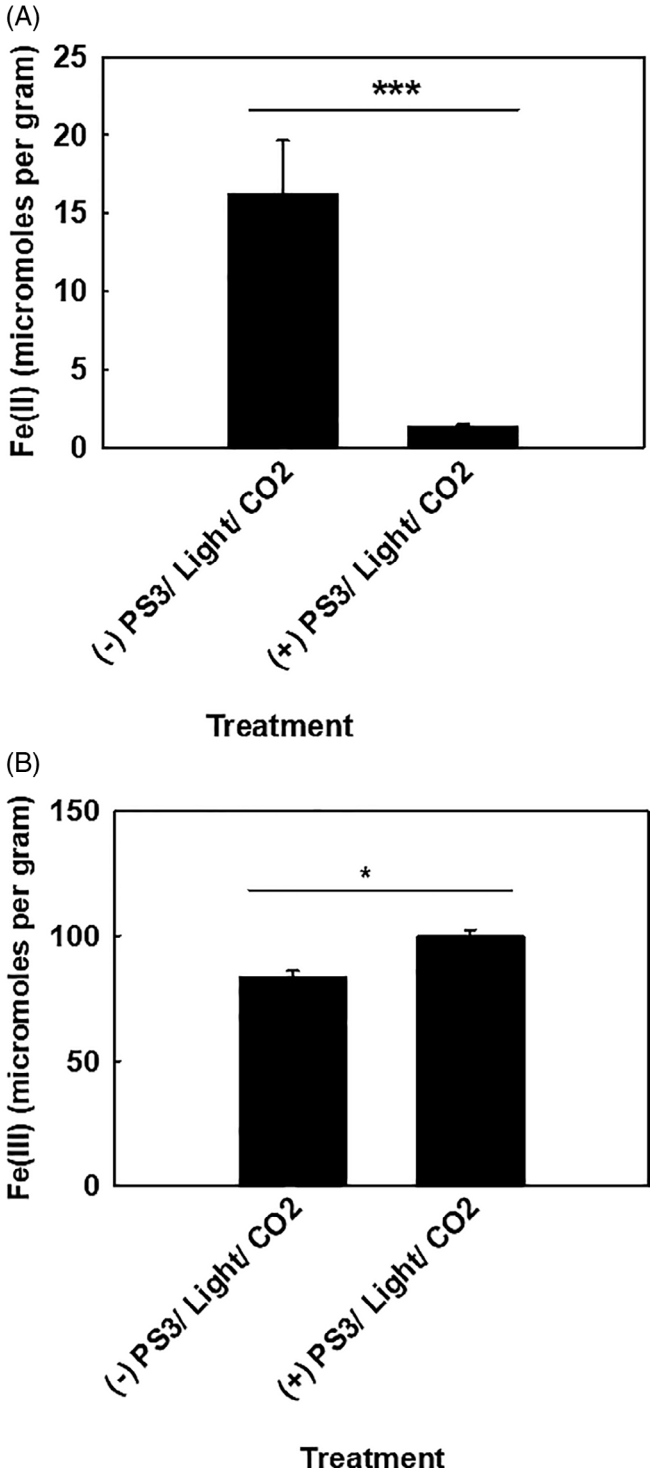

FIGURE 7 Determination of the concentrations of ferrous and ferric ions in the soil-based MFC by ferrozine assay. Respective soil sample was taken from the apparatus under different treatment after 30 days of operation. (−) PS3/Light/CO_2 represented a sealed chamber operated under light and without bacteria condition. (+) PS3/Light/CO_2 represented a sealed chamber operated under light and treated with PS3 bacteria. A, Fe (II) concentrations. B, Fe (III) concentrations. Data represents in mean ± SE of five replicate samples. Asterisk means significant difference, as tested by Student's *t*-test ($P \leq .05$)

(Figure 5). As mentioned above, it has been considered that the relative high electricity generation of the soil-based MFC was related to the phototrophic CO_2 metabolism of *R. palustris* PS3. We found that the concentration of CO_2 was quickly exceeded the detection limit (> 9999 ppm) after adding the PS3 broth, and the power output of the soil-based MFC (+PS3/light/CO_2) was remarkably increased (Figure 6). The rapid increase in CO_2 suggested that the soil respiration rate was elevated remarkably. We assumed that the addition of the PS3 broth with rich nutritive substances was initially utilized by all the microbes (including PS3) existed in the soil-based MFC to produce large amounts of CO_2. Since we did not measure the power output of the apparatus that was only added the nutrient medium without PS3, we were not able to discriminate the contribution of the enhanced power generation that was derived from the added nutritive substances. However, in comparison with the data derived from (+) PS3/dark (Figure 5, red curve and bars) and + PS3/light/(−)CO_2 (Figure 6C,D, red curve and bars, respectively) treatments, there were both electricigen (PS3) and nutritive substances (culture broth) existed in the apparatus, their performances were not as good as that of +PS3/light/CO_2. Furthermore, as mentioned above, the EC level of the paddy soil with PS3 inoculation was not significantly varied from that without PS3 inoculation after 30 days of operation (Table 1), indicating that not the medium added but the abundance of the nutritive substances in soil is essential for long time operation of the soil-based MFC.

Taken together, we deduced that although the nutritive substances of broth can aid the microbial activity of *R. palustris* PS3 initially, the performance of the soil-based MFC was mainly determined by the metabolic activity of *R. palustris* PS3 in fertile soil under favorable conditions (such as anaerobic and illumination).

3.7 | Power output of the soil-based MFC was related to anaerobic ferrous iron oxidation

Metal ions play important roles in the performances of MFCs by influencing the redox activities and metabolism of microorganisms.[56] Iron is a major constituent in soils, which exists in ferrous [Fe (II)] and ferric [Fe (III)] forms. We determined the concentrations of Fe (II) and Fe (III) in the soil-based MFC after 30 days of operation by ferrozine assay. As shown in Figure 7A, the concentration of Fe (II) was remarkably decreased in the soil-based MFC with PS3 inoculation. In contrast, the concentration of Fe (III) in this apparatus was higher than that without PS3 inoculation (Figure 7B). It suggests that the

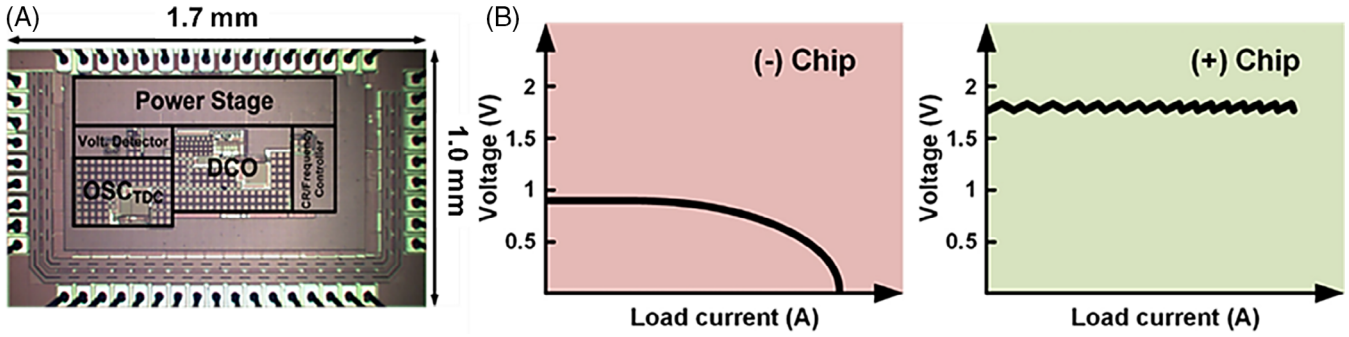

FIGURE 8 Chip micrograph of the DC-DC converter with CMOS technology and its performances in voltage stabilization. A, Micrograph of the silicon chip and key components. DCO, digitally controlled oscillator; OSC, on-chip RC oscillator; TDC, time-to-digital converter; and CR/frequency controller: MPPT Controller. B, Voltage comparison of the soil-based MFC with (+) the chip and without (−) the chip [Colour figure can be viewed at wileyonlinelibrary.com]

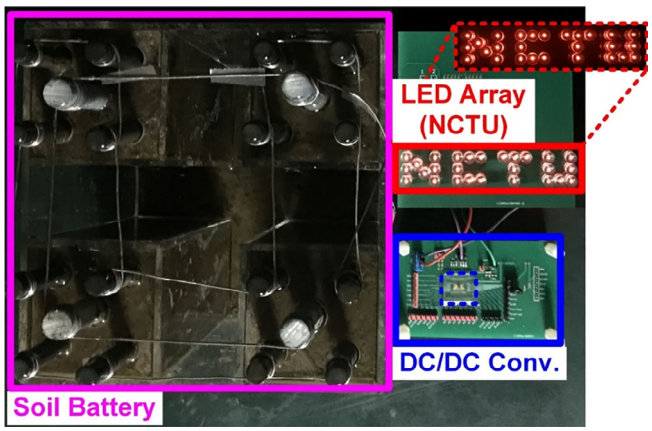

FIGURE 9 Experimental results of lighting LEDs using the proposed soil-energy harvesting chip design. A soil battery that contained the PS3 inoculant was connected with the silicon chip. The DC/DC converter on the silicon chip was responsible for the energy harvesting and transfer to load with the 2D-MPPT. The transferred energy made the "NCTU" LED array light up [Colour figure can be viewed at wileyonlinelibrary.com]

indigenous ferrous iron [Fe (II)] was oxidized into Fe (III) by PS3 in soil under anaerobic and illumination conditions.

As mentioned, the ambient CO_2 produced by the microbes inhabited in the soil-based MFC is crucial for PS3 to generate higher electricity levels (Figure 6). It has been demonstrated that *R. palustris* is able to couple the oxidation of Fe (II) to reductive CO_2 fixation (ie, CO_2 acts as electron acceptor) for electron transfer and energy transduction in anoxic illuminated environments.[57,58] Taken together, we deduced that the enhanced power output of the soil-based MFC was due to a relatively more efficient electron transfer of PS3, which was caused by the interplay between phototrophic fixation of ambient CO_2 and anaerobic oxidation of ferrous iron in soil. Further studies are required to verify this hypothesis.

3.8 | Soil energy harvesting with the proposed CMOS power management IC

To stabilize the power extraction, an efficient soil-energy harvesting system developed previously was introduced to the soil-based MFC in this study. CMOS technology was applied to implement the DC/DC converter on a silicon chip.[36] The components of this power management integrated circuit (IC) included a digitally controlled oscillator, an on-chip resistor-capacitor (RC) oscillator and a time-to-digital converter (Figure 8A and Figure S3). This chip was also installed with a proposed adaptive conversion-ratio charge pump and a maximal power point tracking (MPPT) controller.[36,59] While the load without the chip was directly connected to the soil-based MFC, the voltage value was approximately 0.8 V at a relatively low load current and then dramatically decreased at a high load current (Figure 8B). This indicated that the harvesting power decreased and became unstable with increasing load current. On the other hand, when the silicon chip was equipped with the load, the voltage value increased from 0.8 to 1.0 V to 1.7 to 1.8 V, and does not decrease even at a high load current (Figure 8B). This result indicated that the silicon chip could buffer the dynamic load currents to maintain high power output performance. Accordingly, our data demonstrated that the silicon chip with CMOS technology could harvest energy from the soil-based MFCs in a more efficient and stable manner. The soil-based MFC connected with the chip and the DC/DC converter successfully harvested electricity to illuminate the LED array (Figure 9). Thus, the above system could be used to enhance the production of electricity from PS3 and maintain stable performance.

We compared the performance of this soil-based MFC with those reported in the other studies, although it was not able to make equivalent comparisons due to these

devices using different components, substrates, operation conditions, or electricigens, etc.[11,60-64] As shown in Table S2, the average power density (16.5 $\mu W/cm^2$) of this apparatus was not particularly prominent, however, it presented potential for stable and remarkably durable for long-term MFC operation (90 days, Figure 5).

4 | CONCLUSIONS

In this study, a soil-based MFC inoculated with a photosynthetic *R. palustris* strain PS3 was implemented for electricity generation under anaerobic and illumination conditions, and the factors that influence power generation were elucidated. Compared with that without PS3 inoculation, the energy generation was enhanced by three times over a 90-day measurement period. PS3 showed the ability to generate electricity, and it had the potential to enhance power generation in a soil-based microbial fuel cell. We deduced that the enhanced power output of the soil-based MFC was due to a relatively more efficient electron transfer of PS3, which was caused by the interplay between phototrophic fixation of ambient CO_2 and oxidation of ferrous iron in soil. Furthermore, we introduced an electricity harvesting system, which contained a CMOS DC/DC converter with a 2D-maximal power point tracking (2D-MPPT) controller on a silicon chip and stabilized the power extraction of the soil-based MFC. Thus, the above could provide a potentially low-cost and low-energy system with a high power conversion efficiency for practical applications in the future.

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