Water evaporation–induced electricity with *Geobacter sulfurreducens* biofilms

Qichang Hu\(^1,2,\dagger\), Yongji Ma\(^4\), Guoping Ren\(^1\), Bintian Zhang\(^3\), Shungui Zhou\(^1\)*

Water evaporation–induced electricity generators (WEGs) have recently attracted extensive research attention as an emerging renewable energy–harvesting technology that harvests electricity directly from water evaporation. However, the low power output, limited available material, complicated fabrication process, and extremely high cost have restricted wide applications of this technology. Here, a facile and efficient WEG prototype based on *Geobacter sulfurreducens* biofilm was demonstrated. The device can generate continuous electric power with a maximum output power density of \( \sim 685.12 \mu W/cm^2 \), which is two orders of magnitude higher than that of previously reported analogous devices. The superior performance of the device is attributed to the intrinsic properties of the *G. sulfurreducens* biofilm, including its hydrophilicity, porous structure, conductivity, etc. This study not only presents the unprecedented evaporation potential effect of *G. sulfurreducens* biofilms but also paves the way for developing hydrovoltaic technology with biomaterials.

**INTRODUCTION**

Approximately 71% of Earth’s surface is covered by water, which not only is vital to life but also represents massive energy storage on Earth (1). Unfortunately, to date, most of the energy is lost to evaporation rather than captured for productive use (2). The utilization of water energy by human beings stems from early hydroelectric power, which mainly uses the kinetic energy of a large amount of flowing water and is limited by geographical location, hydrological conditions, and high infrastructure cost (2, 3). In recent years, a variety of energy conversion modes related to water flow, waves, drops, moisture, and water evaporation have been proposed on the basis of the “hydrovoltaic effect” (2, 4, 5), providing more ways to harvest water energy. Among these approaches, the water evaporation–induced electricity generator (WEG) produces electricity directly from the water evaporation process, which is attracting attention because of its highly spontaneous and continuous electricity generation (2, 6).

In earlier works (7, 8), water evaporation was used as a force to drive a certain form of motion and can indirectly generate electricity. For example, Sahin and co-workers (8) found that pulsed and low electricity can be generated from the bending and straightening motion of bacterial spores, which is driven indirectly by water evaporation. Guo and co-workers (2, 6) first reported in 2017 that an electricity generator based on porous carbon black can generate continuous electricity directly from water evaporation. Subsequently, some studies found that electricity generation driven directly by water evaporation takes place on specific nanostructured materials with large surfaces, abundant voids, hydrophilicity, and charged surfaces, such as carbon nanotubes (9), graphene (10), and graphene oxide (11). Considering the required properties of the material, only limited types of nanostructured materials are capable of generating electricity directly through WEG technology. Most nanostructured materials need posttreatment to obtain a hydrophilic surface, which is essential for electricity generation in the system because it is conducive to the interactions of material with evaporation-induced water flow (12, 13). Even with the limited materials for developing WEG technology, the output power density on devices is still low (2, 6). In general, although positive progress has been made in generating electricity by WEGs, the low power output, limited available materials, complicated fabrication process, and extremely high cost still restrict their wide application. Thus, methods for developing naturally suitable hydrovoltaic material systems and exploring the factors affecting the electricity generation process are urgently needed to further improve the performance of WEGs. Excitingly, biomaterials including protein nanowires and whole-cell *Geobacter sulfurreducens* with abundant hydrophilic groups have been used in the preparation of hygroelectricity generators (HEGs; a different type of hydrovoltaic technology) (14, 15), which exhibit excellent performance, and reveal that biomaterials are likely to have great potential in hydrovoltaic technology. To the best of our knowledge, however, whole-cell *G. sulfurreducens* biofilms have not been used in WEGs.

In the present work, we developed a prototype of WEG based on a *G. sulfurreducens* biofilm through a simple and economical method. The device can generate electric power with a maximum output power density of \( \sim 685.12 \mu W/cm^2 \), which achieves a record power density among the reported analogous WEG devices (10, 14–25). Next, we systematically investigated the correlations between electricity generation performance and internal factors of biofilm WEGs and proposed the working mechanism of these WEGs. In addition, the magnitude of electrical output can be amplified through parallel/series connections to light commercial light-emitting diodes (LEDs). This study provides insights into the further broadening and development of hydrovoltaic technology with biomaterials.

**RESULTS**

Fabrication and characterization of a biofilm WEG

A biofilm WEG device was fabricated by deposition of a *G. sulfurreducens* suspension onto the substrate. The active area of the device was defined...
by a molded well. As shown in Fig. 1A, a 300-μl \( G. \text{sulfurreducens} \) bacterial suspension was dripped into a rectangular well on a glass slide and dried naturally at room temperature. After removing the outside mold, a whole-cell biofilm was obtained. Then, the device was successfully fabricated by fixing two Cu electrodes with different distances on the film, as presented in the last image of Fig. 1A. Many uniform particles were evenly distributed in the biofilm in the optical image (Fig. 1B) and the scanning electron microscopy (SEM) image (fig. S1). In addition, carbon, oxygen, sulfur, and nitrogen were evenly distributed on the surface of the \( G. \text{sulfurreducens} \) biofilm, as shown in Fig. 1 (F to I), indicating the formation of a homogeneous biofilm. As seen from the cross-sectional SEM image, the thickness of the biofilm was approximately 10 μm (Fig. 1C), which was obtained by one dropping-coating-drying cycle.

We focused on testing the characteristics of the resulting biofilms. The contact angle of the surface was measured to be 59° ± 1° (Fig. 1D), indicating that the biofilm has good intrinsic hydrophilicity. The hydrophilicity of biofilms was considered to be mainly due to abundant hydrophilic functional groups in the film in previous research. This result was further confirmed by Fourier transform infrared (FTIR) and x-ray photoelectron spectroscopy (XPS) measurements. As shown in Fig. 1D, there are many vibration peaks from the hydrophilic functional groups, such as the C=O stretching vibration of amide (~1657 cm\(^{-1}\)), NH in-plane bending and CN stretching vibration of amide (~1532 cm\(^{-1}\)), symmetric stretching vibration of COOH (1409–1396 cm\(^{-1}\)), and OH deformation of carboxylate (~1239 cm\(^{-1}\)). According to the XPS results (fig. S2), the oxygen content mainly comes from COOH groups in the \( G. \text{sulfurreducens} \) biofilm, accounting for approximately 58.78% of the total oxygen. The nitrogen content mainly comes from −NH\(_2\) (accounting for approximately 93.12%) and NH\(_4^+\), which is consistent with the FTIR results (Fig. 1D). Previous work has found that the hydrophilicity of material plays an important role in the electricity generation process for WEGs. Guo and co-workers fabricated a WEG using a carbon black sheet, and this carbon black sheet was posttreated with annealing and plasma to obtain a hydrophilic surface. The hydrophilic surface helps the film react with water in the biofilm absorbed by the capillary effect. The oxygen-containing functional groups (COOH) in the \( G. \text{sulfurreducens} \) biofilms were ionized after contact with water, and the small cations (such as H\(^+\) and NH\(_4^+\)) were dissociated in aqueous solution (water). Correspondingly, the larger anions (COO\(^-\)) were difficult to move and remained in the film, so the surface of the \( G. \text{sulfurreducens} \) film was negatively charged (see Fig. 1E). A negative zeta potential of the biofilm surface means that water channels in the film (driven by water evaporation) preferentially transport cations and exclude anions. Thus, compared with conventional hydrovoltaic materials, biofilms with natural hydrophilic functional groups have natural advantages for fabricating high-performance WEGs.

**Fig. 1.** Construction and characterization of the \( G. \text{sulfurreducens} \) biofilm. (A) The fabrication process of the \( G. \text{sulfurreducens} \) biofilm and device. (B) Magnified view of the whole-cell biofilm under an optical microscopy. (C) SEM image of the cross-sectioned biofilm; the biofilm thickness is ~10 μm. (D) FTIR spectrum of the \( G. \text{sulfurreducens} \) biofilm. (F) Carbon, oxygen, sulfur, and nitrogen mapping of the \( G. \text{sulfurreducens} \) biofilm by x-ray energy dispersive spectroscopy. a.u., arbitrary units.
Water evaporation–induced electrical output performance of the device

After fabrication of the device, the output performance of the biofilm WEGs was tested systematically (see Materials and Methods). During the test, one side of the film was inserted into the water, while the bottom electrode was kept above the water level (shown as the last image in Fig. 1A). The schematic circuit diagram for testing the electrical output of the device is illustrated in the inset of Fig. 2A. The device generated an open-circuit voltage ($V_{oc}$) of ~0.53 V and a short-circuit current ($I_{sc}$) of ~2.28 $\mu$A at 90% relative humidity (RH), as observed from the current-voltage (I-V) curves (Fig. 2A). An approximately linear I-V curve between the two electrodes indicated quasi-ohmic behavior (Fig. 2A). The maximum power density is the value when the external resistance is equal to the internal resistance of the battery. The maximum power density value is controlled by the maximum rectangular area voltage within the I-V curve range (shaded area in Fig. 1A), which can be calculated by $P_{max} = \frac{V_m \times I_m}{S}$. The maximum output power density of the WEG approached ~400 $\mu$W/cm$^2$. The stability of the output performance of the WEG was determined by monitoring $V_{oc}$ and $I_{sc}$ continuously for 6 hours at 4-min intervals at ~90% RH (Fig. 2B).

To evaluate the interference of the galvanic effect, comparative experiments using various electrodes, including Au, Ag, Pt, Ti, and carbon nanotube, in WEGs to hinder the galvanic current have been performed. Experimental results (fig. S3) showed that the $I_{sc}$ of WEGs with various electrodes is comparable to that of the device with Cu electrode, indicating that the galvanic effect was almost zero during the process of power generation. Thus, the impact of the galvanic effect is negligible to $G. sulfurreducens$ biofilm WEGs within the test time frame. To optimize the performance of the device, the effect of different effective areas and film thicknesses was investigated. Figure S4 presents the performance of WEGs with different effective areas. $V_{oc}$, $I_{sc}$, and the output power density of the WEG increased with the effective area. The output power of the WEG increased with the thickness of the biofilm with a maximum $V_{oc}$ of approximately 10 $\mu$m and then decreased slightly after the thickness exceeded 15 $\mu$m (fig. S5). The $V_{oc}$ value increased with the distance between the two electrodes (fig. S6A), but the output power and $I_{sc}$ of the WEG decreased with the distance, which is attributed to the resistance increase (fig. S6B). Inversion of the electrode immersed in water caused the polarity of voltage output to be reversed, but the amplitude remained the same (fig. S7). The experiment was designed to invert the device without changing the circuit connection, which led to a reverse of the current flow. This result further confirmed that the source of water required for evaporation in the film was the absorption of water by the capillary effect. The statistical histograms of electrical output from 78 WEGs (Fig. 2C and fig. S8) showed that $G. sulfurreducens$ WEGs have good reproducibility and stability. To comprehensively demonstrate the biofilm WEGs as a power supply, the output voltage ($V_{load}$) and current

![Fig. 2. Electrical output measurement of the WEG.](image-url)
density ($I_{\text{load}}$) under varied load resistances were measured, and the results are summarized in fig. S9, indicating a $V_{\text{load}}$ and $I_{\text{load}}$ of $\sim$0.36 V and $\sim$0.82 μA, respectively, under 500-kilohm resistance in the external circuit. The output power density was calculated from the results of $V_{\text{load}}$ and $I_{\text{load}}$. Figure 2D shows the power density of the WEG under different load resistances of the external circuit, and the inset presents the circuit diagram. An output power density of 293.12 μW/cm$^2$ was obtained with an external resistance of 500 kilohms (Fig. 2D). The device generated power continuously under 500-kilohm resistance in the discharge circuit for 6 hours with only slight attenuation (Fig. 2E). It is important to emphasize that the $G. \text{sulfurreducens}$ biofilm WEG has the highest recorded output power density, which is approximately two orders of magnitude higher than that of previously reported analogous devices thus far (Fig. 2F) (10, 16–19). A systematic performance comparison of reported WEGs and HEGs based on different materials is shown in tables S1 and S2 (10, 15–25), respectively. We ascribe the superior electricity generation to biofilms, which have unique features.

**Analysis of effect factors of WEG’s electrical output**

To further improve the WEG’s electrical output, we focused on investigating the factors that affect the electricity generation process. First, the water evaporation rate increases with increasing temperature. By adjusting the temperature, the relationship between the evaporation rate and temperature was investigated, as shown in fig. S10A. Then, the effect of temperature on the WEG’s electricity generation performance was studied. The results showed that the power generation performance of the device increased with increasing temperature (fig. S10B). On the basis of the results from fig. S10 (A and B), the electrical output performance of the WEG with different water evaporation rates in biofilms could be derived (Fig. 3A). The results showed that the electricity generation performance of the WEG increased with increasing evaporation rate. Moreover, it was found that $V_{\text{oc}}$ increased with increasing wind flow velocity in the laboratory environment (fig. S11). Nitrogen (N$_2$) was used to artificially create different wind speeds, thus eliminating the interference of active oxygenation. When the N$_2$ wind speed was 0, 1, and 2 m/s, the device generated a $V_{\text{oc}}$ of 0.31, 0.42, and 0.51 V, respectively. We propose that the wind speed promotes the water evaporation rate, which is beneficial to improving power generation. A similar experiment was designed to confirm the effect of the evaporation rate on the electrical output of the device. The output power of the device decreased with increasing RH (fig. S12). This result further confirms the positive effect of the evaporation rate on the output power because the evaporation rate decreases with increasing humidity. Obviously, the evaporation rate is a very important factor for the performance of WEGs.

Second, to determine the effect of ionization on electricity generation, the electrical output performance of WEGs in solvents with different polarities was investigated (see Fig. 3B and fig. S13, A and B). The results demonstrated that the power density of WEGs operating in solvent followed the order of deionized (DI) water > ethylene glycol > methanol > ethanol, which corresponds to the order of the polarity magnitude of the solvents (16). In other words, the electricity generation capacity of WEGs increased with the polarity of the solvent. The more polar the solvent used by the generator, the higher the ionization level of the hydrophilic functional groups in the biofilm, and the more cations (H$^+$ or NH$_4^+$) that could be dissociated in the solvent. As mentioned earlier, anionic groups (such as COO$^-$) are fixed on the film surface, which is demonstrated by the higher negative potential of the film (fig. S14). At this time, more cations move toward the top electrode under the same suction force driven by evaporating water flow, resulting in a streaming current and creating a higher potential at the top electrode. According to the above experimental results, it is reasonable to believe that ionization is positively correlated with the electrical output performance of these WEGs.

Moreover, the effect of conductivity on electricity generation was investigated. The conductivity of the solution was regulated by controlling the concentration of NaCl solution, and the relationship between the NaCl concentration and the conductivity of the solution is shown in fig. S15A. The electrical output performance of devices corresponding to solvents with different NaCl concentrations is shown in fig. S15B. Combining the results of fig. S15 (A and B), it is deduced that the conductivity of the solvent affects the electrical output performance of the device (see Fig. 3C). With increasing NaCl concentration in the solution, the conductivity of the solution increased, leading to a significant reduction in the internal resistance of WEGs (fig. S16), which in turn effectively increased the power density. This result indicated that the conductivity is positively correlated with device performance, which is consistent with a previous study (28). The $G. \text{sulfurreducens}$ biofilm used in this work has already been confirmed to have naturally good conductivity, which greatly reduces the internal resistance of the device and thus improves the WEG’s electricity generation performance. On the basis of these results, it is conceivable that seawater with high ionic concentrations in the ocean is suitable for WEGs, which potentially expands the wide range of applications for WEGs.

Furthermore, the effect of average pore diameter of the $G. \text{sulfurreducens}$ biofilm on the WEG electricity generation performance was investigated. Biofilms with different morphologies were obtained by applying different vertical pressures on the surface. As shown in fig. S17 (A to D), the morphologies of the $G. \text{sulfurreducens}$ films changed from a large-aperture stacking structure to compact stacking with increasing vertical pressure. The Brunauer-Emmett-Teller (BET) results showed that a smaller pore structure appeared in the film with increasing vertical pressure (fig. S18). In addition, the pore diameter of the $G. \text{sulfurreducens}$ film decreased with vertical pressure (fig. S19A). Figure S19B shows the effect of the vertical pressure on the device performance, indicating that the electrical output performance improves with increasing vertical pressure. On the basis of fig. S19 (A and B), it can be deduced that the output performance decreased with increasing average pore diameter in the $G. \text{sulfurreducens}$ biofilm (Fig. 3D). Reducing the average pore diameter increases capillary force and facilitates water entry into the biofilm (12, 13). The water evaporation rate further increases with increasing water flow in the film, thereby improving the performance of the WEG. On the basis of the above results, we optimized the device to obtain the best performance of the device. The $G. \text{sulfurreducens}$ biofilm–based WEG was observed to generate continuous electricity with an output power density of $\sim$685.12 μW/cm$^2$ with a resistance of 500 kilohms for 24 hours under the conditions of $\sim$95% RH, 30°C, and a wind speed of 2 m/s (see fig. S20).

In addition, since moisture always exists along with water, it is necessary to perform experiments to confirm that the power generation of devices is mainly induced by water evaporation rather than humidity. The device structure of the WEG in this work is completely different from that of HEGs. For HEGs, the original driving
The force of electricity generation is the different degrees of group dissociation caused by the moisture gradient from the surface to the interior of the film (29, 30). The current flows vertically from the surface to the interior of the film. However, the current of the WEG flows from the bottom to the top electrode along the surface. There are two humidity gradients in this work: one is the vertical humidity gradient between the top and bottom electrodes on the biofilm above the water level (parallel to the direction of the current), and the other is the horizontal humidity gradient between the surface and interior of the biofilm surface (perpendicular to the direction of the current). Therefore, to confirm whether power generation is caused by water evaporation, it is necessary to eliminate the interference associated with HEGs. Therefore, a layer of oil was dropped on the water surface to isolate the contact between the water and air and to ensure that there was no vertical humidity gradient on the film surface above the water level. The results showed that the WEG could still generate electricity after isolating water and air, with little voltage drop (Fig. 3E). To further eliminate the interference of the horizontal humidity gradient in the vertical direction between the film surface and the interior, the surface of the G. sulfurreducens film was covered with tape, and the power generation performance of the WEG exhibited little change (Fig. 3F). The specific sealing position was between the two Cu electrodes, but the top of the film is not completely sealed. When one end of the device was inserted into water, part of the biofilm on the top electrode was still exposed to air, which allowed for further water evaporating. Water evaporation drove the water flow absorbed by capillary action uphill through the porous biofilm, thus realizing continuous electricity generation. By sealing the area in between the two electrodes, humidity effect was eliminated, but the $V_{oc}$ remains the same, which indicates that it is not a humidity-driven process but an evaporation-driven process arising from capillary action (14). Therefore, the above experimental results demonstrated that the power generation of the device is mainly caused by water evaporation.

Electricity generation mechanism of biofilm WEG devices

Analysis of the above results suggest that the water evaporation–induced electricity generation mechanism is a relatively complex process and related to at least four factors: the water evaporation rate, ionization level, conductivity, and pore structure. Partial least squares path modeling (PLS-PM) analysis was conducted to probe complex relationships among four factors. The simulation results showed that the output power density was directly affected by the four factors (Fig. 4A), where the water evaporation rate, ionization level, and conductivity were positive factors on the output power, while the average pore diameter was a negative factor. The simulation results of the main positive factors (evaporation rate and ionization level) influenced by multiple factors are shown in fig. S21 (A and B). The evaporation rate was positively correlated with the temperature and air flow rate and negatively correlated with the RH. The ionization level increased with increasing solvent polarity and film thickness. The large pore diameter was a negative factor for output power, while the smaller pore diameter could increase capillarity and promote the water evaporation rate, thereby improving the output power of the WEG. Moreover, it was found that the conductivity was the weak factor regarding output power under the effects of other factors, consistent with our previous work (14). Furthermore, the standardized mean effects indicated that the
water evaporation rate, ionization level, and average pore diameter all had distinctly direct effects on output power, while pore diameter and electric conductivity also had weak indirect effects on output. (Fig. 4B).

According to the aforementioned results, we proposed a feasible mechanism for electricity generation in biofilm WEG devices, as illustrated in Fig. 4C. The biofilm is composed of *G. sulfurreducens* with porous structures, and these porous structures provide a bottom-top microchannel that facilitates capillary absorption of water. Once DI water contacts the porous structure of biofilms, the abundant hydrophilic groups of the biofilms easily react with water and dissociate to form a negative potential in the film, and H⁺ and NH₄⁺ are ionized from these functional groups in a water environment. Then, the capillary force and evaporation drove the water flow uphill through the porous biofilm. The capillary flow water also carried free H⁺ and NH₄⁺ to uphill through the porous biofilm. This process produces a streaming current and creates a higher potential at the top electrode in the vertical direction (parallel to the direction of the current). Last, the water involved in this process is eventually consumed through evaporation. The combination of capillarity and water evaporation allows the WEG to generate electricity continuously, and *G. sulfurreducens* biofilms provide an excellent platform for this process.

### Potential application of the biofilm WEG device

The electrical output performance of the WEG devices could be easily scaled up in series or in parallel (shown in Fig. 5, A to C). Figure 5B shows that the total voltage value increased linearly with the number of biofilm WEGs in series. The inset depicts the series circuit diagram. Similarly, the total current value also quasi-linearly increased with the number of biofilm WEGs in parallel, as shown in Fig. 5C.

When five devices were connected in series, the voltage/current of the integrated battery reached 2.38 V and 36.2 μA, respectively (Fig. 4D). This battery could charge a capacitor and then be used to drive a commercial LED. The charged capacitor was used to light a single LED (Fig. 5E) and the “FAFU” (Fujian Agriculture and Forestry University) pattern on the LED pattern (Fig. 5F and movie S1). In addition, commercial capacitors could be charged through a single WEG device (Fig. 5G). The results showed that all the capacitors could be charged through the WEG to a stable voltage of approximately 0.4 V. A capacitor of 1 μF was charged by the *G. sulfurreducens* WEGs in
parallel to 0.43 V in 5 min. The above results indicated the excellent practical application potential for *G. sulfurreducens* biofilm WEGs.

**DISCUSSION**

Although WEGs have been greatly developed in recent years, their output power is still low. Developing previously unexplored suitable hydrovoltaic material systems represents a shortcut to improving the performance of devices. In this work, a WEG using a *G. sulfurreducens* biofilm was prepared by a simple and economical method. The device can continuously generate electric power with a maximum output power density of ~685.12 µW/cm², which is higher than that of previously reported analogous hydrovoltaic devices. Why do *G. sulfurreducens* biofilm–based WEGs have a much higher output power density than traditional nanostructure material–based WEGs? The reason remains unclear. We considered possible explanations based on the effect factor analysis of WEG performance. One explanation may be that the film naturally exhibits hydrophilic characteristics without any treatment, helping to maintain hydrophilicity during the power generation process. In contrast, conventional materials require posttreatment to obtain hydrophilic properties, which is more likely to revert to surface hydrophobic properties during electricity generation. Therefore, a WEG device based on biofilm is better for stably and continuously generating electricity. In addition, our previous experimental results (Figs. 3B and 5A) demonstrated that a high ionization level is conducive to improving electricity generation performance. In this work, biofilms have abundant oxygen–containing functional groups, which can interact well with the water absorbed into the film by capillary action to dissociate H⁺ and NH₄⁺ ions. This process leads to an increase in the ionization level, which helps to improve the WEG performance. Moreover, *G. sulfurreducens* biofilms have good electrical conductivity (14), which is conducive to reducing the internal resistance of the device and increasing the output power density. This fact is consistent with the mentioned result obtained earlier here. In addition, the unique structure of the biomaterial may affect the carrier transport of the device (15). The surface of the *G. sulfurreducens* biofilm is uniform, with a microscopic porous structure, and the individual bacterial cells themselves are smooth ellipsoids, which are conducive to forming smooth ion transport channels in the film so that ions are free and move unhindered driven by evaporative water. Therefore, the superior output performance of the biofilm WEG is attributed to the intrinsic advantages of *G. sulfurreducens* biofilms, including their natural hydrophilic characteristics, water absorption capacity, and good electrical conductivity, which are conducive to absorbing water and charge transport. This has greatly improved the understanding of the operation mechanism of WEGs.

The novelty of *G. sulfurreducens* biofilm WEG lies not only in its excellent output performance but also in the advantages of whole-cell microorganism materials. The *G. sulfurreducens* biofilm WEG differs from the protein nanowire HEGs in previous work in terms of material, device structure, and work mechanism. The protein nanowire used in the HEGs fabrication is a component extracted from whole-cell *G. sulfurreducens* film (only 5% weight), with...
complex and time-consuming procedures, and the yield is very low, while the use of the *G. sulfurreducens* biofilm WEG technology greatly simplifies the process by eliminating the complicated nanowire extraction. Furthermore, *G. sulfurreducens* biofilms are environmentally friendly, intrinsically hydrophilic and conductive, and highly reproductive through self-metabolic processes. These advantages ensure possible wide-range applications of biofilm WEGs in the future. Research on the operation mechanism of biofilm WEGs is still in the preliminary stage. Further investigation of the operation mechanism and performance optimization (such as reducing the internal resistance), as well as the preparation of large-area devices to improve the electrical output, are the focus of future research.

In summary, we have reported an unprecedented evaporating-potential effect of *G. sulfurreducens* biofilms. The first prototype of WEG based on *G. sulfurreducens* biofilm was successfully fabricated. The device can continuously generate electricity with an output power density of ~685.12 μW/cm², which is two orders of magnitude higher than that of previously reported WEG devices. Moreover, the electrical output of the WEG device can be easily scaled up to power small electronic devices or for energy storage. The super- or output of the device is attributed to the intrinsic properties of the *G. sulfurreducens* biofilms. We believe that our findings indicate a pioneered way to develop hydrovoltaic technology with biomaterials.

**MATERIALS AND METHODS**

*Collection of the G. sulfurreducens bacterial precursors*

*G. sulfurreducens* is an anaerobic microorganism that requires sodium acetate (15 mM) as an electron donor and fumarate (40 mM) as an electron acceptor to grow normally. In addition, we provided other nutrients, as shown in table S3, for the growth of *G. sulfurreducens* cells. The medium was deoxygenated for at least 1 hour under an N₂/CO₂ (80/20%) atmosphere and then covered with Teflon-coated rubber on a sterile worktable. The *G. sulfurreducens* cells were cultured in a constant temperature incubator at 30°C until the OD₆₀₀ (optical density at 600 nm) value reached the exponential phase (OD₆₀₀~0.6). Subsequently, the *G. sulfurreducens* cells were collected.

*Fabrication of the biofilm WEGs*

Ordinary glass or polytetrafluoroethylene was used as the substrate for the whole-cell WEGs. A rectangle of various areas (0.5 to 4 cm²) was fixed on the substrate with 1-mm-wide tape. A certain amount of *G. sulfurreducens* solution was dropped into the fixed rectangle and then air-dried naturally. The water in the *G. sulfurreducens* suspension was evaporated, and a dry *G. sulfurreducens*-based biofilm was obtained. This process resulted in most of *G. sulfurreducens* cells being inactivated (fig. S22). Generally, a 50 μl/cm² *G. sulfurreducens* solution (10 mg/ml) formed a *G. sulfurreducens* whole-cell biofilm of approximately 10 μm. Two copper sheets (1 × 10 mm) were fixed at different positions on the *G. sulfurreducens* biofilm as different electrodes.

*Characterization of biofilm WEGs*

The morphology of the *G. sulfurreducens* biofilm was characterized by optical microscopy (Eclipse E200 MV, Nikon, Japan) and SEM (SU8020, Hitachi, Japan). Functional group analysis was characterized by XPS (ESCALAB 250Xi, Thermo Fisher Scientific, USA) and FTIR ( Nicolet iS50, Thermo Fisher Scientific, USA) spectroscopy. A Krüss drop shape analysis (DSA) 10 Mk2 system (Germany) was used to test the contact angle of the *G. sulfurreducens* biofilm with water. The element type and content of the *G. sulfurreducens* biofilms composition were tested by energy dispersive x-ray spectroscopy (EDAX, USA). The average pore diameter of the *G. sulfurreducens* film was tested by an automatic surface area and porosity analyzer (ASAP 2460, Mack Instruments Inc., USA). The zeta potential of the *G. sulfurreducens* solution was tested by a Zetasizer Nano ZS (Malvern, UK). A conductivity meter (DDS-307A, China) was used to analyze the ionic conductivity of the *G. sulfurreducens* solution. A LIVE/DEAD BacLight Bacterial Viability Kit (Invitrogen, CA) was used to stain the prepared microbial biofilm, which is a common method for identifying dead or living cells (31). Then, the biofilm was examined with a confocal laser-scanning microscope (LSM880 NLO, ZEISS) to obtain the three-dimensional image.

*Electrical measurements*

The Vₑₒ and Iₑ of the *G. sulfurreducens* biofilm WEG were tested and recorded through an electrical measurement system (Zennium pro, Zahner, Germany), in which the current and the voltage of the circuit parameter of output tests were set to zero. The Vₑₒ and Iₑ of the discharge circuit with variations in load resistance (0.1, 200, 0.5, 1, 10, and 50 megohms) were measured by electrical measurement systems. Electrochemical impedance spectra of HEGs were measured and analyzed in the frequency range of 0.01~10⁶ Hz using an electrochemical workstation (PalmSens4, The Netherlands). Various supercapacitors (1, 10, 100, and 1000 μF) were charged using WEGs under ~75% RH by an independent WEG device unit. FAFU LED plate and the LED (YY-SVQCMS5712UC, Geesled, China) were lit by charged capacitors.

*Statistical analysis*

The interaction between various factors and WEG power density was explored through PLS-PM. The model included the following variables: evaporation rate (RH, air flow, and temperature), average pore diameter, conductivity, and level of ionization (polarity of solvent and film thickness). Path coefficients were calculated after 999 bootstraps, with significance levels indicated by *P < 0.05, **P < 0.01, and ***P < 0.001.

**SUPPLEMENTARY MATERIALS**

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