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Self-sustaining, solar-driven bioelectricity generation in micro-sized microbial fuel cell using co-culture of heterotrophic and photosynthetic bacteria

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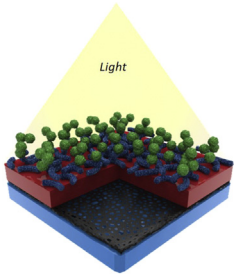
HIGHLIGHTS

- We generated a self-sustaining electricity from bacterial syntrophic interaction.
- Without additional fuel, the device generated current for more than 13 days.
- The mixed bacterial culture produced more current than the photoautotrophs only.

GRAPHICAL ABSTRACT



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ABSTRACT

Among many energy harvesting techniques with great potential, microbial fuel cell (MFC) technology is arguably the most underdeveloped. Even so, excitement is building, as microorganisms can harvest electrical power from any biodegradable organic source (e.g. wastewater) that is readily available in resource-limited settings. Nevertheless, the requirement for endless introduction of organic matter imposes a limiting factor to this technology, demanding an active feeding system and additional power. Here, we demonstrated self-sustaining bioelectricity generation from a microliter-scale microbial fuel cell (MFC) by using the syntrophic interaction between heterotrophic exoelectrogenic bacteria and phototrophs. The MFC continuously generated light-responsive electricity from the heterotrophic bacterial metabolic respiration with the organic substrates produced by photosynthetic bacteria. Without additional organic fuel, the mixed culture in a 90-μL-chamber MFC generated self-sustained current for more than 13 days, while the heterotrophic culture produced current that decreased dramatically within a few hours. The current from the mixed culture was about 70 times greater than that of the device with only photosynthetic bacteria. The miniaturization provided a short start-up time, a well-controlled environment, and small internal resistance. Those advantages will become the general design platform for micropower generation.

1. Introduction

Developing self-sustainable micropower sources are of critical importance for independent, sustainable, maintenance-free, and

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continuous operation of a wide array of wireless applications deployed in remote and resource-limited field locations [1,2]. Among many energy harvesting techniques for self-sustainable micropower generation, such as ambient vibrations, heat or light [1], miniaturized microbial fuel cells (MFCs) are receiving a great deal of attention for those applications because of their self-organizing and self-maintaining properties [3–8]. MFCs are powered by the respiration of heterotrophic exoelectrogens that harness energy from a wide range of soluble or complex organic wastes and renewable biomasses, which are readily available even in challenging conditions [9,10]. Despite their vast potential, miniaturized MFCs have not been integrated with practical power applications because the devices require a power-driven active feeding system (e.g. magnetic, piezoelectric, and electrochemical actuation techniques) to continuously introduce organic fuel [11], and, even worse, the fuel may not be available, especially in dry, desert climates. On the other hand, photosynthetic MFCs (PMFCs) can provide self-sustainable power generation by using the photosynthetic and respiratory activities of photosynthetic microorganisms [6,12–15]. The PMFCs continuously produce electricity from solar energy without additional organic substrates because light energy absorbed by the photosynthetic reaction splits water and generates oxygen, protons, and electrons [16]. However, PMFCs are very constrained as a self-sustainable energy technology because of their persistent power limits [6,17,18]. Their power densities are typically several orders of magnitude lower than that of even the smallest power MFCs [6].

Recently, the “Plug and Play” photosynthetic concept has been proposed by the Jones' group at Arizona State University to provide MFCs with power self-sufficiency and increase the power performance of the PMFCs [19]. The light and dark reactions can operate independently, coupling microbial respiratory metabolism to electricity generation using photosynthetic co-cultures. Also, several studies generated a wealth of new scientific and technological results that clearly demonstrated synergistic cooperation between photosynthetic microorganisms and heterotrophic bacteria [20–22]. Their studies showed increased power generation from mixed microbial communities. However, much of this work is in its nascent stages; the evolution of this technology will require additional exploration through a practical application of established techniques and comprehensive systematic integration.

In this work, we created a micro-sized MFC that establishes the groundwork for advances in sustainable energy. The device maximized syntrophic interactions between photosynthetic and heterotrophic bacteria in well-controlled micro-chambers. Biofilm extracellular polymeric substance produced by the heterotrophic microbes facilitated adhesion of phototrophic microbes enhancing their biofilm formation and electron coupling with higher electricity generation through their synergistic interaction (Fig. 1). Moreover, organic compounds and oxygen for heterotrophic microbial respiration was internally regenerated by photosynthetic microbes whose reactants, such as carbon dioxide and water, were the products of the heterotrophic bacteria, and consequently exhibited self-sustainable capabilities. The heterotrophic bacterial biofilm (*Shewanella oneidensis* MR-1), which formed first at the bottom of the anode, oxidized organic substrates, and efficiently transferred electrons to the anode while photosynthetic bacterial biofilm (*Synechocystis* sp. PCC 6803) formed over the heterotrophic bacteria and provided the in-situ organic substrates (Fig. 1). Without the external input of organic fuels, the MFC utilizing the mixed culture generated self-sustained current more than 70 times greater than that of the device using only photosynthetic bacteria. In addition, MFC miniaturization inherently produced favorable conditions for increasing power density by reducing internal resistance and improving mass transport. Because small-scale biological fuel cells are more energy dense than larger units, one possible way to scale up MFCs may be through connecting multiple small-scale MFCs in a stack configuration. As far as we know, this is the first research work that has made efforts to integrate heterotrophic and photosynthetic bacteria into a microliter-scale MFC for high power density and self-sustainability.

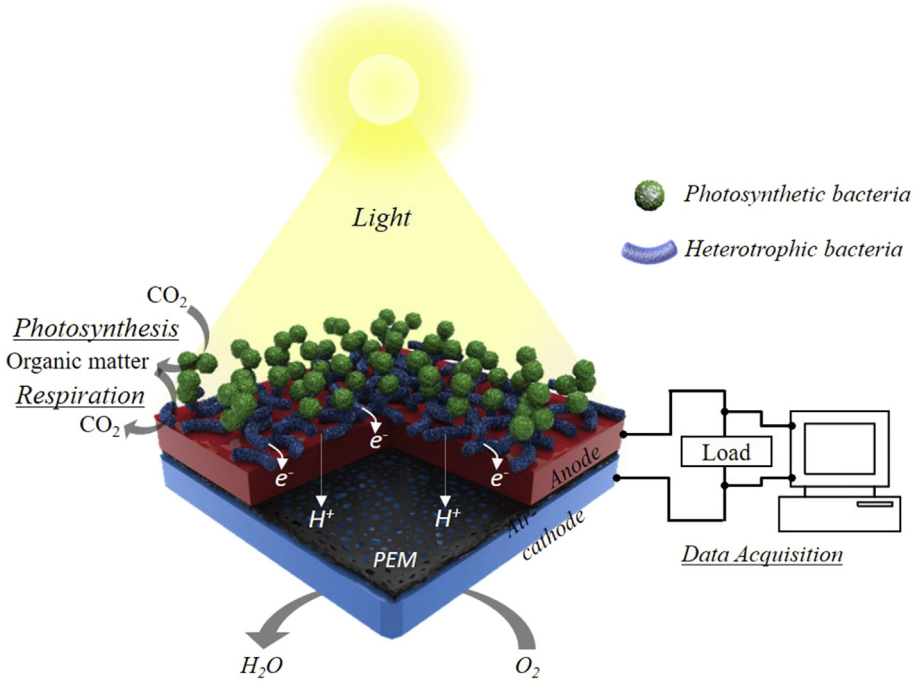

Fig. 1. Conceptual illustration of the hybrid bio-solar cell based on the synergistic cooperation between photosynthetic and heterotrophic bacteria.

2. Experimental section

2.1. Device fabrication for direct visualization of bacterial biofilm

The anode chamber was defined in a microfluidic channel, part of which was sandwiched with the cathode chamber by a proton exchange membrane (PEM) (Fig. 2). The device had a 140 μL-sized anode chamber and a 70 μL-sized cathode chambers separated by a PEM. Each layer except for PEM (Nafion 117) was first micro-patterned by using laser micromachining (Universal Laser System VLS 3.5). The electrodes (0.07 cm^2) were prepared by depositing 10 nm gold on polymethyl methacrylate (PMMA) substrates with chrome as the adhesion layer. The anode was transparent for visualizing live cells in situ. The layers were manually stacked in sequence to form the channels and expose the electrodes to solutions introduced in the channels while carefully aligning the tubing holes. All the layers were thermally bonded at 100 °C for 1 h. Copper tape (3M™ copper conductive tape) was attached to the contact pads with silver conductive paint (PELCO® Colloidal Silver). Fig. 2 shows a fully assembled device with tubes (CODAN, 0.35 mL volume).

2.2. Device fabrication for power generation

The hybrid MFC was assembled as shown in Fig. 3, where the main body of the device comprised of four polymethly methacrylate (PMMA) substrates and the sandwiched electrode assembly. Five functional layers were cut by the laser cutting machine: (i) a top PMMA layer, (ii) a PMMA microfluidic chamber layer, (iii) a rubber gasket, (vi) an anode (carbon cloth)/PEM (Nafion 117)/air-cathode sandwiched electrode assembly, and (v) a bottom PMMA layer. We used one of the most common anode materials for bacterial-based fuel cells, a carbon-based material, which possesses a large surface area and functional organic groups favoring cell vitality [4]. Bacterial biofilms on the carbon anode appeared dense and compact. All layers were carefully aligned and assembled with 10 small screws. The device used the air-cathode to allow freely available oxygen to act as an electron acceptor by the installation of the catalyst side of the air-cathode to face toward the chamber while the opposite side was exposed to air. The air-cathode was fabricated from 30% wet-proofed carbon cloth with four layers of polytetrafluoroethylene (PTFE) coating [23,24,25,26]. The other side of the cathode was coated with Pt/C catalysts (0.5 mg/cm^2 Pt loading). The electrodes were pierced with a 0.5 mm thick Ti wire as a current collector. The MFC had two holes for fluidic inlet/outlet. Tubes (CODAN, 0.35 mL volume) were plugged into the holes with adhesive to form a fluidic channel. The 1.6 mm-thick PMMA microfluidic chamber layer (ii) and 0.508 mm-thick rubber gasket layer (iii) were precisely laser-machined to define a 90 μL chamber over the electrodes. The size of a completely assembled device was

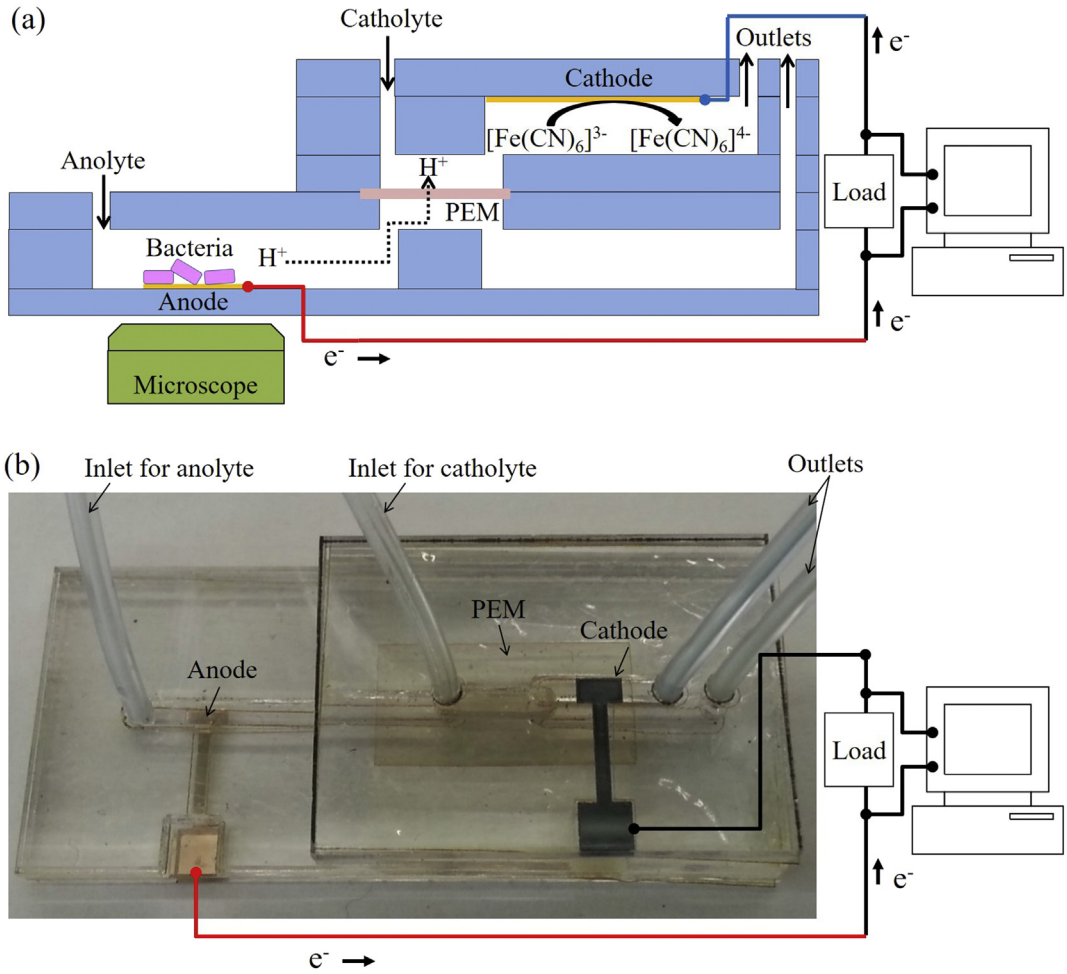

Fig. 2. A microscale MFC with a protruded anode chamber for direct visualization of bacterial biofilm with measurements of bacterial electron transfer. (a) schematic and (b) photo-image of the device.

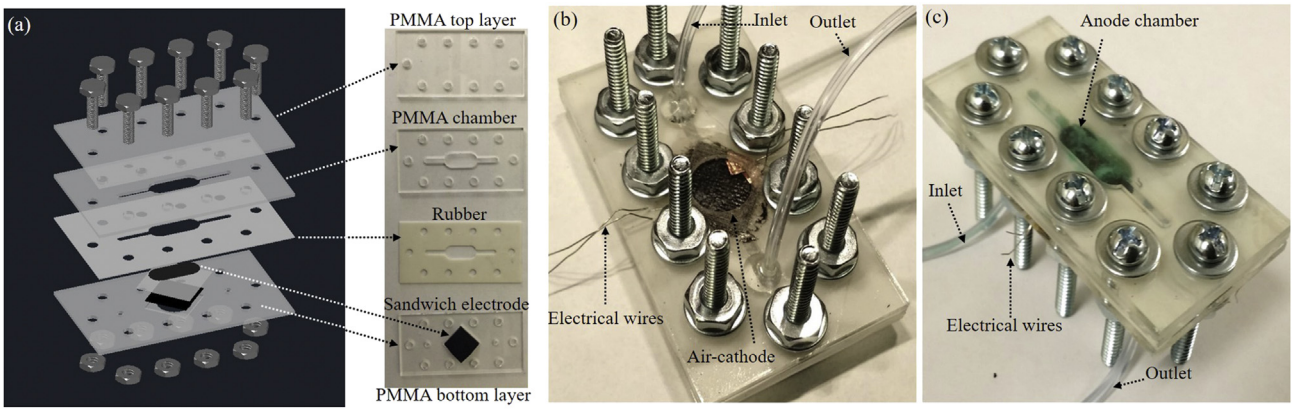

Fig. 3. (a) Schematic diagram of the individual layers for the device and photo-images of the fully assembled biological solar cell: (b) the bottom view and (c) the top view.

6 cm × 3.5 cm. The assembled device was sterilized with 70% ethanol and ultraviolet light for 24 h.

2.3. Inoculum and measurement setup

Synechocystis sp. PCC 6803 (phototrophs) were grown from −80 °C glycerol stock cultures by inoculating 15 mL of BG-11 medium with gentle shaking under a 24 h light cycle (12 h light/dark). The BG-11 contained 1.5 g $NaNO_3$, 40 mg K_2HPO_4, 75 mg $MgSO_4$, 36 mg $CaCl_2$, 1 mg of EDTA, and 6 mg of citric acid and of ferric ammonium citrate per 1 L of distilled water. Fluorescent lamps provided the continuous aeration and illumination for 2 weeks. Growth was monitored by measurement of the optical density at 600 nm (OD_{600}) and the culture we used reached an OD_{600} of 1.4. *Shewanella oneidensis* MR-1 (heterotrophs) were grown from −80 °C glycerol stock cultures by inoculating 20 mL of L-broth medium with gentle shaking in air for 24 h at 35 °C. The L-broth media consisted of 10.0 g tryptone, 5.0 g yeast extract and 5.0 g NaCl per liter. The culture we used reached an OD_{600} of 2.0. Both cultures were then centrifuged at 5,000 rpm for 5min to remove the supernatant. The bacterial cells were re-suspended in a new medium and used as an anolyte for the device.

2.4. Measurement setup

We measured the voltages generated between the anodes and the cathodes with a data acquisition system (National instrument, USB-6212), and recorded the readings every 1 min via a customized LabView interface. An external resistor connected between the anode and the cathode closed the circuit. The current through this resistor was calculated using Ohm's law.

3. Results and discussion

3.1. Biofilm formation

Before we integrated bacterial co-cultures into the proposed micro-sized MFC, we first examined individual and synergistic

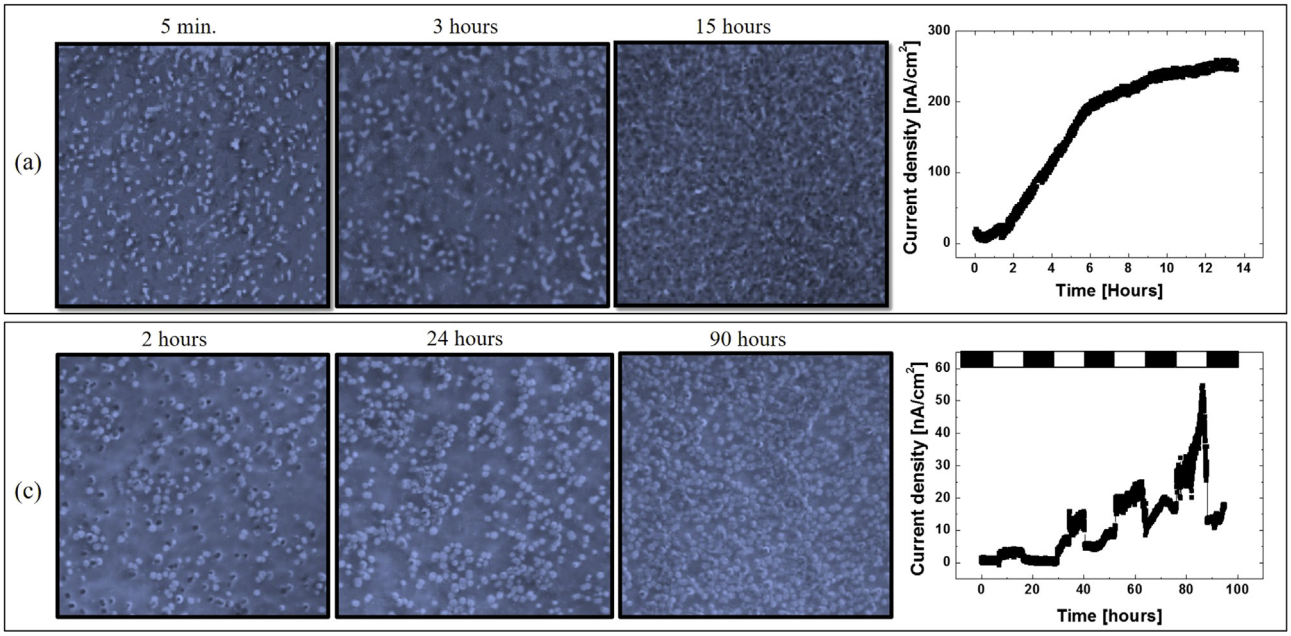

Fig. 4. Current produced from (a) *Shewanella oneidensis* MR-1, and (b) *Synechocystis* sp. PCC 6803 at different stages of biofilm development. The white bars indicate the illuminated period and the shadow indicates the dark period.

microbial roles in a well-designed microsystem. As shown in Fig. 2, the microfabricated transparent microsystem was designed based on a typical MFC configuration but featured a protruded anode chamber. The device was directly placed under an optical microscope to observe live bacterial behavior in situ with real-time and simultaneous measurements of bacterial electron transfer. The real-time analysis of the relationship between bacterial electricity-generating capabilities and their biofilm development is scientifically important to investigate fundamental factors that maximize microbial energy production and its sustainability. However, in the literature, reported work on the characterization of the microbial biofilm at a new level of detail and efficiency is either unavailable or quite limited [23]. Our novel microscale analytical tool facilitates studies of microbial behavior in a smaller group of cells with excellent control over the microenvironment and allows for better-characterized correlations between MFC performance and biofilm behavior. The anode chamber was defined in a microfluidic channel, part of which was sandwiched with the cathode chamber by a proton exchange membrane (PEM). Microorganisms introduced in the microfluidic channel accumulated and acclimated on the anode and their biofilm gradually formed. The microorganisms oxidized organic fuels and completed respiration by transferring electrons to the anode. Electrons that were transferred to the anode flowed to the cathode through the external resistor. The protons, after traveling through the PEM, and the electrons, after reaching the cathode, were reduced with ferricyanide, $[Fe(CN)_6]^{3-}$ (1) (Fig. 2).

$$[Fe(CN)_6]^{3-} + e^- \rightarrow [Fe(CN)_6]^{4-} \quad (1)$$

First, we inoculated one device with individual heterotrophic bacteria (*S. oneidensis*) and a second with photosynthetic bacteria (*Synechocystis* sp. PCC 6803). We recorded the currents generated from their respiration and photosynthesis while observing their biofilm formation under an optical microscope. We continuously supplied organic fuel to the heterotrophic bacteria while the device with photosynthetic bacteria operated self-sustainably. Gradual increases in current were observed from both bacterial samples under the operational condition of 12hr light and 12hr dark consecutive cycles and consistent temperature at 30 ± 2 °C (Fig. 4). A positive light response of the photosynthetic bacteria was detected while the heterotrophic bacteria showed no light response. The change in current output did not correlate to the minor fluctuations in temperature (the control without bacteria showed no light or temperature response). In our configuration, protons generated from the anode might not have effectively transferred through the PEM along the long channel, triggering a pH gradient between the anode and cathode that severely degraded bioanode performance [8]. This limitation resulted in the low current densities, 265 μA/cm^2 (*S. oneidensis*) and 50 nA/cm^2 (*Synechocystis* sp. PCC 6803), respectively, which are significantly lower than previous micro-sized MFCs [6]. Further study needs to be done to improve our device configuration. However, optical measurement through microscopy allowed for real-time monitoring of bacterial proliferation and accumulation on the anode. As shown in Fig. 4, photosynthetic biofilm development on the anode surface was much slower; current generation was also significantly lower than that of the heterotrophic bacteria (*Shewanella* sp.). In addition, compared to heterotrophic bacteria, the biofilm hardly formed multiple layers. Although the photosynthetic bacteria demonstrated the self-sustainable capability of the device, these results indicated that improving the performance of photosynthetic bacterial biofilm may require a different strategy from those used for conventional MFCs.

As a potential solution, we constructed a model co-culture biofilm comprised of two microbes, namely *Shewanella oneidensis* MR-1 (heterotrophic) and *Synechocystis* sp. PCC 6803 (photosynthetic). After the heterotrophic bacterial biofilm was formed, the photosynthetic bacterial inoculum was introduced (Fig. 5). Fig. 5 shows the rapid (<12 h) and densely-packed biofilm formation by the photosynthetic bacteria to cover the heterotrophic bacterial

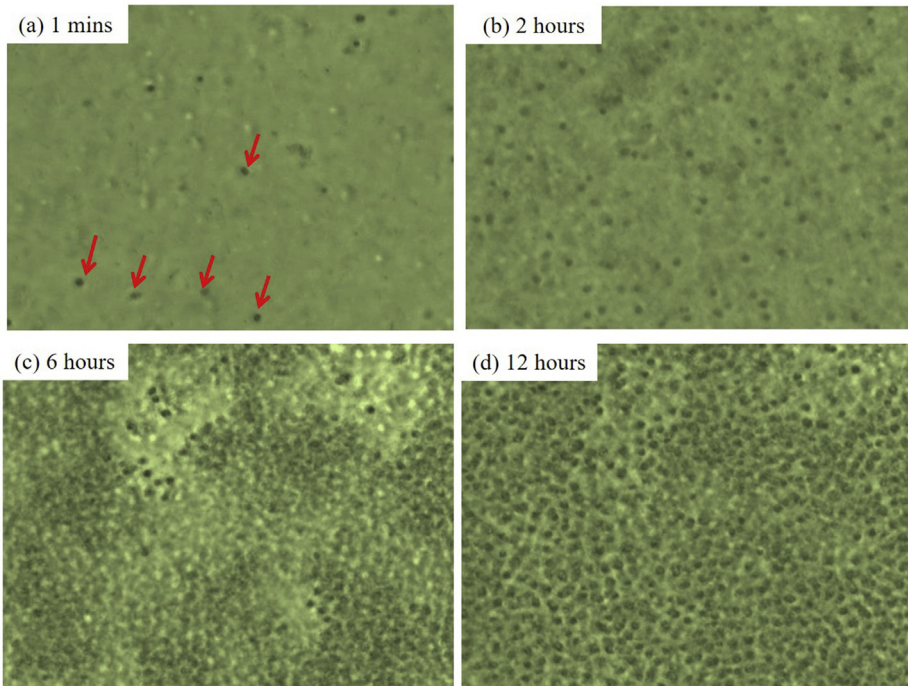

Fig. 5. Photosynthetic bacterial biofilm formed on heterotrophic bacterial biofilm. The extracellular polymeric substance of the heterotrophic microbes facilitates the adhesion of phototrophic ones rapidly forming their biofilm (Red arrows: photosynthetic bacterial cells). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

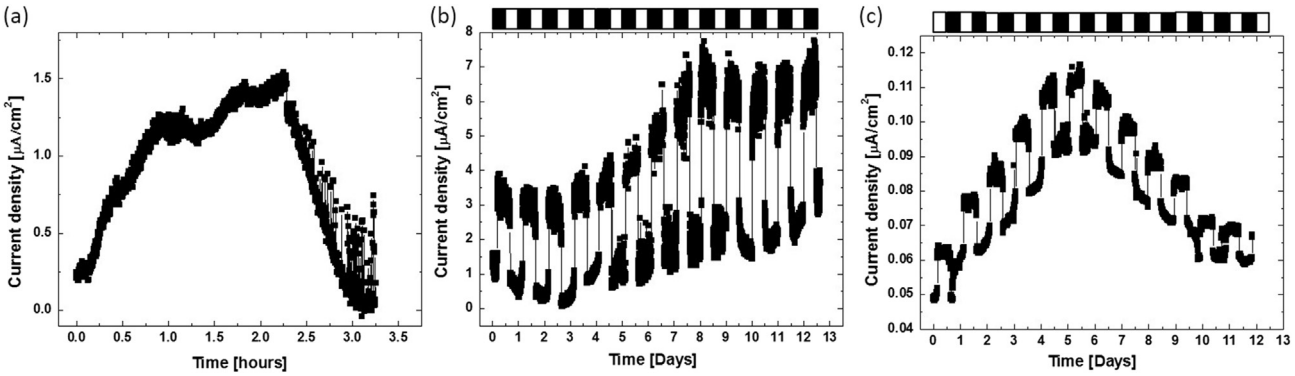

Fig. 6. Current densities (at 700 kΩ resistor) measured from the (a) heterotrophic bacteria (*Shewanella oneidensis* MR-1) only, and (b) co-cultures of heterotrophic bacteria (*Shewanella oneidensis* MR-1) and photosynthetic bacteria (*Synechocystis* sp. PCC 6803) and (c) photosynthetic bacteria only. The white bars indicate the illuminated period and the shadow indicates the dark period.

biofilm, indicating that cultivating photosynthetic bacterial biofilms can be maximized by using co-cultures with heterotrophic bacteria. Although further study needs to be done, we claim that the biofilm extracellular polymeric substance of the heterotrophic microbes facilitates adhesion of phototrophic microbes rapidly forming their biofilm (c.f. Fig. 4b).

3.2. Current generation from co-cultures

After we confirmed the cooperative biofilm formation between photosynthetic microorganisms and heterotrophic bacteria, we created a hybrid micro-sized MFC by using their syntrophic interactions. The photographs and schematics of the fully-assembled micro-sized MFC are illustrated in Fig. 3. The device featured (i) a small-scale microchamber (90 μL) to reduce the start-up time by increasing the probability of cell attachment and biofilm formation [7], (ii) a carbon-based anodic material to promote bacterial adhesion [4,6] and (iii) a sandwich configuration of the anode, PEM, and air-cathode to minimize the inter-electrode distance and significantly reduce the internal resistance [3–5]. Initially the *Shewanella* strain was introduced and a biofilm formed, after which the *Synechocystis* inoculum was introduced. Once we ensured the green biofilm formation (Fig. 3c), we stopped supplying the anolyte and clogged the tubes with clamps to prevent additional flow and to operate the device self-sustainably. Two other MFCs were prepared as a control; one with *Shewanella* sp. (heterotrophic bacteria) and the other with *Synechocystis* sp. (photosynthetic bacteria). All three devices received no additional organic fuels. The self-sustainability was substantially improved via co-cultures with photosynthetic bacteria while the heterotrophic pure culture produced a dramatically decreasing current output within a few hours (Fig. 6a). Without any additional introduction of organic fuels, the mixed culture in the 90 μL chamber-sized MFC generated a self-sustained current for more than 13 days (Fig. 6b). The efficiency of photosynthetic electron transfer also was improved via co-cultures with heterotrophic bacteria. The electron transfer rate among heterotrophic bacteria is much more efficient than that of photosynthetic bacteria, which leads to significantly increased power generation (Fig. 6c). Although the understanding of the individual and synergistic roles for key microbial populations is still missing, some studies have shown increased power generation from mixed microbial communities [21,22]. The MFC utilizing the mixed culture generated light-responsive current from organic matter released by the photosynthetic bacteria. It generated current more than 70 times greater than that of the MFC using only photosynthetic bacteria (Fig. 6b and c). Based on these results, the hybrid MFC is shown to be light-powered without needing an organic substrate as an energy source to maintain self-sustainable power generation. The experimental results were reproducible with about 11% variation, as measured across 3 devices per condition. The negative light response from the mixed culture is consistent with the mechanism where photosynthetic bacteria produces organic fuels that feed heterotrophic bacteria [12,14,24], and the respiratory electron-transfer chain of the heterotrophic bacteria is the source of electrons deposited on an anode. A current decrease during illumination presumably was due to the negative effect of photosynthetically evolved oxygen, which diverted electrons away from the anode [8,12]. The dark-induced rise of current generation made the day/night differences more distinctive, having a current magnitude of 300% greater during the night. This increase suggests that the electron transfer of extracellular respiration is maximized when the oxygen concentration is minimized during the dark phases. On the other hand, a positive light response was observed from the device only with the photosynthetic bacterial; approximately 25% higher current was generated during the illumination than during the dark phases. However, a gradual decrease in current generation was noticed after 6 days of operation. This might be the depletion of an essential element (e.g. Nitrogen) for long-term biological metabolism while synergistic cooperation can drive the nitrogen cycle between heterotrophic and photosynthetic microorganisms [12]. Further studies are required to monitor the nitrogen cycle. However, research into miniature MFC contributes essential knowledge about the cooperative biofilm formation and the syntrophic interactions between photosynthetic co-cultures that occur in a smaller group of microorganisms, all with excellent control over the microenvironment. The combination makes miniature MFCs a versatile platform for fundamental bio-solar energy studies. Integrating individual small-scale MFCs in a panel may enable substantial performance upgrades and bring power densities up to levels comparable to conventional batteries or chemical fuel cells [27]. It may also offer the potential ancillary benefit of establishing a general design platform for microfabrication that would facilitate further research.

4. Conclusion

We created a micro-sized MFC that continuously generated an entirely self-sustainable current by using syntrophic interaction between heterotrophic biocatalysts and photosynthetic bacteria. We could generate much higher and more self-sufficient current density (8 μA/cm^2, and up to 13days) than that of a device using only photosynthetic bacteria or only heterotrophic bacteria.

Moreover, by using an innovative device architecture in a miniaturized chamber, the bacterial adhesion was improved and the internal device resistance was reduced, leading to higher current and power generation. This work could result in barrier-transcending advancements in miniature solar-driven MFCs that could facilitate higher performance with self-sustainability, releasing MFC technology from research settings and installing it in practical, real-world applications.

Acknowledgments

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