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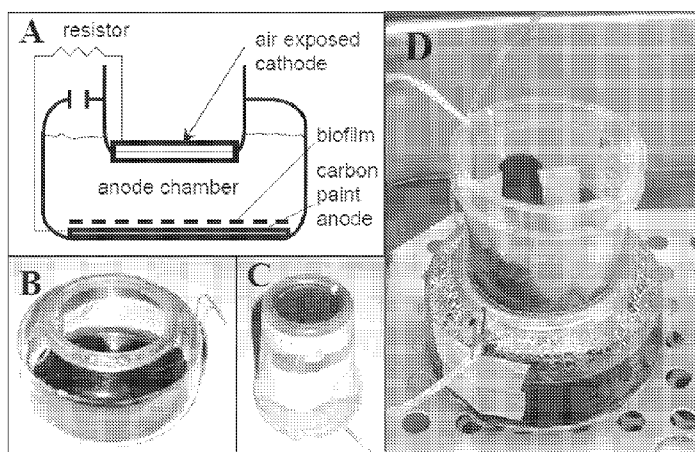
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(54) Title: GENERATING ELECTRICAL POWER BY COUPLING AEROBIC MICROBIAL PHOTOSYNTHESIS TO AN ELECTRON-HARVESTING SYSTEM



(57) Abstract: The present invention relates to an aerobic single-chamber photosynthetic microbial fuel cell (PMFC) that does not depend on organic substrate as an energy source but instead powered only by the energy of light. Its operation is CO₂-free and does not require buffers or exogenous electron transfer shuttles to provide the electrical energy.

Figure 1



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GENERATING ELECTRICAL POWER BY COUPLING AEROBIC MICROBIAL PHOTOSYNTHESIS TO AN ELECTRON-HARVESTING SYSTEM

CROSS-REFERENCE TO RELATED APPLICATION

[001] The present application claims priority to U.S. Provisional Patent Application No. 61/164,960 filed on March 31, 2009 the contents of which are hereby incorporated by reference herein for all purposes.

BACKGROUND OF THE INVENTION

Technical Field

[002] The present invention relates to the generation of electrical power, and more particularly, to the use photosynthetic microbial fuel cell that does not require organic substrates as energy sources but instead directs generation of electrical power by the coupling of microbial photosynthesis to an electron-harvesting system.

Related Art

[003] In recent years, biological approaches for generating energy in a self-sustainable manner have been predominantly focused on two large areas: (i) microbial fuel cells (MFCs) (1), and (ii) biological production of hydrogen (2-5). Hydrogen could be produced via splitting water through biophotolysis or via electrogenic oxidation of organic compounds, whereas MFCs harvest energy from oxidation of organic compounds including those present in waste waters. In MFCs, electrons are derived from respiratory electron-transfer chain, where a broad variety of organic substrates including cellulose can be utilized as a source of energy (6-9). Few aerobic MFCs have been developed in most recent studies (10,11), as the majority of MFCs operate under anaerobic conditions.

[004] In the previous studies, photosystems I and II from thermophilic cyanobacteria were utilized for producing light-powered or light-sensitive electronic devices (12,13). When isolated and immobilized on gold nanoelectrodes, cyanobacterial PSI or PSII were found to produce light-dependent electrical current. However, because of high rate of

photodamage seen in the devices consisting of immobilized enzymes, they are unlikely to be utilized for energy production in self-sustainable manner.

[005] Previous photosynthetic or solar-powered MFCs involved hydrogen production by a photosynthetic microorganisms/algae (3,4) or coupling of photosynthetic bioreactors with anaerobic oxidation of organic substrates produced through photosynthesis (14). Rosenbaum and coauthors introduced photosynthetic MFCs (PMFC)s that involved anode-catalyzed oxidation of hydrogen synthesized by green alga *Chlamydomonas* or the purple bacteria *Rhodobacter* (15,16). Permanent voltage has to be applied to the electrodes to oxidize hydrogen in these PMFCs. In recent study, Cho and coauthors described PMFC containing *Rhodobacter sphaeroides* that produced hydrogen under illumination. Hydrogen was then oxidized by the platinum-coated anode generating electrical current (17). This PMFC functioned only under anaerobic conditions and required the presence of carbon and nitrogen sources. Sensitivity of hydrogenases to oxygen limits the range of potential applications for MFCs that rely on hydrogen. Strick and coauthors showed that sustainable electricity production could be achieved by coupling algal photobioreactors with conventional MFC (14).

[006] In 1980-90's, a team from Japan's Institute of Physical and Chemical Research published a series of studies that described aerobic PMFCs that operated entirely using cyanobacterial cultures (18-20). This work provided the first illustration that positive light response (i.e. immediate increase in electrical current output upon illumination) could be achieved in PMFC. Positive light response is consistent with the idea that electrons could be supplied directly by photosynthetic electron-transfer chain, and not only derived from respiratory transfer chain or through oxidation of hydrogen. These studies, however, were performed using two-chamber PMFC with potassium ferricyanide in cathode chamber, and phosphate buffer and electron transfer mediator 2-hydroxy-1,4-naphthoquinone (HNQ) in the anode chamber. The PMFCs that required potassium ferricyanide, exogenous electron shuttle, and buffer imposed serious limitations for exploiting PMFCs for sustainable conversion of light energy into electricity.

[007] Thus, it would be advantageous to provide for a PMFC system that overcomes the shortcomings of the above-discussed prior art and provides for generating power in a self-sustainable manner using the energy of sunlight.

SUMMARY OF THE INVENTION

[008] In the current studies, an alternative approach has been presented that involves producing electrical power by photosynthetic cultures in aerobic solar-powered microbial fuel cells.

[009] Specifically, the present invention provides for electrons for electricity generation derived directly from photosynthetic electron-transfer chain, i.e. originate from splitting of water molecules induced by photons. The present system is powered directly by electromagnetic energy included in sun-light or light sources mimicking frequencies and wavelength of sunlight and does not depend on consumption of organic substrates as an energy source. Furthermore, the operation of PMFC is CO₂-neutral, because water is the substrate and the final product of electrochemical reaction.

[0010] In one aspect, the present invention relates to a system for generating electricity, the system comprising:

- a vessel wherein the vessel has an anode positioned on the bottom of the vessel and a cathode position near the top of the vessel and communicatively connected to the anode;
- a solution in the vessel, wherein the solution is aqueous;
- a photosynthetic aerobic microbial culture positioned in the vessel, wherein the culture includes microbes having photosynthetic ability and the ability to provide electrons from the electron-transfer chain; and
- an electromagnetic energy source that emits day-light energy.

[0011] The photosynthetic aerobic microbes used in the present invention must have photosynthetic ability and provide electrons from the electron-transfer chain. The photosynthetic aerobic microbes may include cyanobacteria, green and purple sulfur bacteria and algae. Preferably, cyanobacteria is used and can include but is not limited to strains including *Prochlorococcus*, *Synechococcus*, *Nodularia spumigena*, *Lyngbya aestuarii* and *Lyngbya majuscula*, *Trichodesmium erythraeum* and *Crocospaera watsonii*. In the present invention the marine *Synechococcus* strains have been found to be very effective.

[0012] In another aspect, the present invention relates to a system for generating electricity, the system comprising:

a vessel wherein the vessel has a anode positioned on the bottom of the vessel and a cathode position near the top of the vessel and communicatively connected to the anode, wherein the anode is carbon coated and further coated with an electrically conductive polymer;

a solution in the vessel, wherein the solution is aqueous and does not include exogenous electron mediators;

a photosynthetic aerobic microbial culture positioned in the vessel and adjacent to the anode, wherein photosynthetic aerobic microbial cultures is a fresh water biofilm or planktonic cyanobacteria; and

an electromagnetic energy source that provides for day-light energy.

[0013] An electrically conductive polymer may include, but is not limited to, such polymers as poly(acetylene)s, poly(pyrrole)s, poly(thiophene)s, polyanilines, polythiophenes, poly(p-phenylene sulfide), poly(p-phenylene vinylene)s (PPV), polyindole, polypyrene, polycarbazole, polyazulene, polyazepine, poly(fluorene)s, and polynaphthalene.

[0014] In another aspect, the present invention provides for a method of generating electricity, the method comprising;

providing an aqueous solution comprising a photosynthetic aerobic microbial culture, wherein the aqueous solution is in contact with an anode and cathode, wherein the anode is coated with a carbon surface and wherein the photosynthetic aerobic microbial culture is positioned on the anode; and

providing a source of day-light or natural sunlight to the vessel in amount sufficient to induce an electron transfer chain in the photosynthetic aerobic microbial culture.

[0015] Preferably, the carbon surface is further coated with an electrically conducted polymer such as polyaniline or polypyrrole and wherein the anode is separated from the cathode in an amount of from 1 to 100 cm and more preferably from about 1 to 5 cm and clearly dependent on the size of the container vessel.

[0016] In yet another aspect, the present invention relates to an aerobic solar-power microbial fuel cell that provides free electrons originating from the splitting of water induced by photons.

[0017] Importantly the present invention provides for microbial fuel cells wherein the electrical energy is derived directly from the source of electromagnetic energy, that being natural sunlight or a source mimicking sunlight and not dependent on the consumption of organic substrates.

[0018] These and other aspects of the present invention will be apparent from the detailed description of the invention provided hereinafter.

BRIEF DESCRIPTION OF THE FIGURES

[0019] Figure 1 shows a single-chamber PMFC. (A) Schematic diagram of PMFC. (B) Anode chamber with a bottom surface painted with electrically conductive carbon black. (C) Cathode. (D) PMFC with pond biofilm under operation.

[0020] Figure 2 shows the positive light response observed during PMFC operation. (A) Performance of PCC 6803-based PMFCs with non-treated (pink) and polyA-coated anode (red) between 2d and 9th day of operation. (B) Performance of biofilm-based PMFCs with non-treated (blue) and polyA-coated anode (green) between 14th and 18th days of operation. Performance of PCC 6803-based PMFCs is provided for comparison. (C) Performance of biofilm-based PMFCs with non-treated (blue) and polyA-coated (green) and polyP-coated anodes (brown) between 1st and 6th days of operation. Voltage from control PMFC with polyP-coated anodes that lacked biofilm is shown by black line. Arrows indicate gradual change in the shape of dark-phase voltage decay. PMFCs were operated under 1 K Ω external resistance. 12h-dark phases are indicated by black bars.

[0021] Figure 3 shows the dynamics of pH (○) and DO concentration (▲) during 12h/12h light/dark cycles monitored in anodic chambers with PCC 6803 (A) or mature biofilm (B). Three consecutive 12h/12h cycles are shown. In addition to periodic oscillations, long-

term upward trends in pH and DO are noticeable. 12h-dark phases are indicated by black bars.

[0022] Figure 4 shows fluorescence microscopy imaging of pond biofilm. Phase-contrast (left panels) and intrinsic fluorescence images (right panels) of biofilm taken with X20 objective (A) and X60 objective (B,C). Fluorescence images were collected from the two channels using EN-GFP-HQLP filter cube for red fluorescence and FITC C31001 filter cube for green fluorescence, and combined with the WCIF Image-J software. Eukaryotic algae include two major types: individual cells with spherical morphology (indicated by blue arrows) and sets of four cells that resemble green algae *Scenedesmus* (indicated by red arrows). Filamentous and non-filamentous (shown by green arrows) cyanobacteria were found in biofilm. Scale bars = μm .

[0023] Figure 5 shows voltage (A, C) and power density (B, D) as a function of current density (normalized by the cathode surface area) obtained by varying the external circuit resistance for the following PMFCs: PCC 6803, non-treated anode (■); PCC 6803, polyA-treated anode (●); pond biofilm, non-treated anode (▲); pond biofilm, polyA-treated anode (▼); pond biofilm, polyP-treated anode (◆). In (C) and (D), PMFCs were operated in the presence of 1 mM HNQ. Current densities were measured at 20th day of operation in the absence of HNQ and at 23d day in the presence of HNQ.

[0024] Figure 6 shows cyclic voltammetry curves generated at scan rate of 50 mV/s for the following PMFCs: no culture, non-treated anode – black line; PCC 6803, non-treated anode – red lines; PCC 6803, polyA-treated anode – pink lines; pond biofilm, non-treated anode – blue lines; pond biofilm, polyA-treated anode – green lines; pond biofilm, polyP-treated anode – brown lines. As judged from the voltammogram profiles (vs Ag/AgCl), the current output was found to increase in the following rank order (from the lowest to the highest): culture-free, non-treated anode < PCC-6803, non-treated anode < biofilm, non-treated anode < PCC-6803, polyA anode < biofilm, polyA anode < biofilm, polyP anode.

[0025] Figure 7 shows (A) Nyquist plot for anode electrodes recorded for the following biofilm-based PMFCs: with non-treated anode (Δ), with poly-A treated anode (∇), and with polyP-treated anode ($\diamond$). Solid lines represent the result of fitting to an equivalent

circuit. The impedance spectroscopy was performed 44 days after the pond culture was inoculated into anode chambers and at the 18th day of PMFC operation. (B) Equivalent circuit used for the analysis of impedance data.

[0026] Figure 8 shows the formation of pond biofilm on the anode surface composed of non-treated carbon paint (A), polyA-coated carbon paint (B), and polyP-coated carbon paint (C). Images were taken 3 weeks after transferring of biofilm suspension to the anode chambers and 1 day before PMFC operation. Two duplicates are shown for each anode type.

[0027] Figure 9 shows the positive light response observed for PMFCs with biofilm formed on carbon cloth anode coated with polyA (green) or with polyP (brown). PMFC operation started 6 days after transferring biofilm suspension to anode chambers. Plot shows PMFC performance between 1st and 6th day of operation under 1 K Ω external resistance. 12 h-dark phases are indicated by black bars.

[0028] Figure 10 shows fluorescence microscopy imaging shows complex architecture of biofilm where filamentous species intercalated between conglomerates of non-filamentous algae and cyanobacteria. Phase-contrast (left panels) and fluorescence images (right panels) of biofilm taken with X20 objective (A) and X60 objective (B). Fluorescence images were collected from the two channels using EN-GFP-HQLP filter cube for red fluorescence and FITC C31001 filter cube for green fluorescence, and combined with the WCIF Image-J software. Scale bars = μm .

[0029] Figure 11 shows the performance of biofilm-based PMFC with polyP-coated carbon paint anode recorded for 3 days prior to replacing of BG-11 media and for 6 days after the aged media was replaced with fresh BG-11. PMFCs were operated under 1 K Ω external resistance. The time point for replacing the media is indicated by vertical arrow. Small arrows indicate change in the shape of dark-phase voltage decay. Interestingly, medium replacement altered the kinetics of the dark-phase voltage decline. Instead of slow decay typical for old medium, the cell voltage showed rapid decline in the fresh medium. This result was consistent with previous observations that current production

during the dark-phase was likely to be powered by oxidation of photosynthetically produced organic compounds.

[0030] Figure 12 shows the cell voltage recorded for PMFC with biofilm formed on carbon paint anode coated with polyA (green), polyP (brown), or non-treated (blue). Biofilm suspension was transferred to anode chambers and incubated for 3 weeks in BG-11 medium for biofilm formation. Prior to PMFC operation, BG-11 medium was replaced with 50 mM phosphate buffer in BG-11. No light response was observed for six days of operation. After phosphate buffer in BG-11 was replaced with fresh BG-11 (as indicated by arrow), the positive light response was restored. PMFCs were operated under 1 K Ω external resistance. 12 h-dark phases are indicated by black bars.

[0031] Figure 13 shows A. Photosystem II and photosystem I of cyanobacteria. B. Q-cycle. When production of NADH is inhibited, electrons are transferred from ferredoxin to cyt-b6 creating excessive amounts of electrons on plastoquinone

[0032] Figure 14 shows that cyanobacterium *Synechocystis* FCC 6803 produced electrically conductive nanowires under conditions of CO₂ limitation and excess of light.

[0033] Figure 15 shows the reduction potential of microbial respiratory chain components.

DETAILED DESCRIPTION OF THE INVENTION

[0034] The present invention describes new technology for generating electrical power in photosynthetic microbial fuel cells via coupling of microbial photosynthesis to an electron-harvesting system.

[0035] Cyanobacteria are the evolutionary ancestors to chloroplasts. Cyanobacterial electron-transfer chain that conducts photosynthesis is similar to that of chloroplasts and consists of Photosystem II and Photosystem I (Figure 13 A). Photosystem II uses light energy to split water and release electrons. From photosystem II, electrons pass to plastoquinone (PQ), then to cytochromes (Cyt), and then to plastocyanin (PC). Another light reaction at photosystem I activates electrons for transfer to ferredoxin, and finally to NADP⁺. The electrons are used to reduce NADP⁺ to NADPH, while the protons released

from splitting of water become part of the energy source for ATP synthesis. The electron transfer from water to NADP + via photosystem II and photosystem I is non-cyclic (referred to as Z-scheme of electron transfer).

[0036] Because a bacterial cell needs to make more ATP than NADH, some of the electrons from ferredoxin are fed back to cyt-b6, short-circuiting the Z-scheme. Such cyclic transfer of electrons from photosystem I to ferredoxin, than to cyt-b6, and then back to photosystem I is called Q-cycle (Figure 13 B). The Q-cycle is specifically active under conditions of CO₂ limitation, when CO₂ fixation is slow, NADH is accumulated in excessive amounts and its further production is shut down.

[0037] Under bright light and CO₂ limitation, both Z-scheme and Q-cycle are active producing excessive amounts of electrons, which can not be all utilized due to low level of CO₂ fixation. Because two pathways of electron transfer, trough Z-scheme and Q-cycle, merges at cyt-b6, electrons are accumulated on plastoquinone (PQ) and need to be discarded. Production of electrical power in photosynthetic MFC exploits natural needs of cyanobacteria to discard electrons that are produced through splitting of water in Photosystem II under conditions of CO₂ limitation and excess of light.

[0038] In natural environment, many bacteria including cyanobacteria form nanowires (also known as pili). Nanowires facilitate transfer of excess electrons to other metabolic groups within complex bacterial communities. It is believed that nanowires provide a way of communication between members of bacterial communities supporting interspecies energy exchange. The nanowires also allow the bacteria to get rid of unwanted electrons generated during metabolism, transporting them to distant “electron dumps.” (Figure 14).

[0039] Cyanobacterium *Synechocystis* PCC 6803 was shown to produce electrically conductive nanowires under conditions of CO₂ limitation and excess of light (31). When cyanobacterium is unable to utilize electrons due to low level of CO₂ fixation, nanowires are believed to provide a route for donating excessive electrons into environment. In photosynthetic MFCs, cyanobacterial nanowires may provide a mechanism for discarding electrons to the anode surface.

[0040] The voltage that could be recovered in a MFC is dependent on where electrons exit the chain of respiratory enzymes (Figure 15). In classical MFCs, electrons exit the respiratory chain at the level of cytochromes c. In this case, bacteria could derive energy from the reduction potential between NADH and cytochromes c, whereas the MFC could be used to recover energy from the potential between cytochromes c and oxygen. In a photosynthetic MFC that utilizes cyanobacteria, electrons are expected to exit the Photosystem II at the level of plastoquinone (PQ). Electrons exiting at the level of plastoquinone should have sufficient reduction potential to be used in fuel cell for generating electrical voltage.

[0041] The present invention provides for a single chamber PMFC and tested its performance using two photosynthetic cultures, planktonic cyanobacteria *Synechocystis* PCC 6803 and fresh-water biofilm that comprising algae and filamentous cyanobacteria or consisting of same. Both cultures exhibited weak positive light response when used in PMFCs with an anode made of non-treated carbon paint. When carbon paint anode was coated with electrically conductive polymers polyaniline (polyA) or polypyrrole (polyP), biofilm-based PMFCs showed much stronger light response with a rapid increase in cell voltage upon illumination. PMFCs persistently showed higher cell voltage during the light-phase than the dark-phase despite an increase in dissolved oxygen (DO) and pH during the light-phase. Addition of exogenous electronic shuttle to PMFCs improved performance of PCC 6803-based PMFC to a much higher extent than biofilm-based PMFC arguing that biofilm PMFCs did not rely on electron shuttles as much as planktonic PMFCs. Although PMFC power outputs were substantially lower than those reported for anaerobic MFC, the PMFC operation was CO₂-neutral and did not require organic substrates, buffer or exogenous electron shuttles.

METHODS AND MATERIALS

[0042] PMFC design. PMFC consisted of a single 125 ml chamber (anode chamber) made of NALGENE Disposable Filter Units (Cat. N Nalgene, Rochester, NY), and an air-exposed cathode (Figure 1). The anode electrode was either a piece of carbon cloth (B1A no wet proof, E-Tek, Somerset, NJ, 5x5 cm, A_{an} = 25 cm²) or a bottom surface of anode chamber (A_{an} = 50 cm²) coated with electrically conductive carbon paint (Item No. 05006-AB, SPI Supplies Division, Structure Probe Inc, West Chester, PA). The bottom of

chamber was painted with four layers of carbon paint. For each layer, approximately 0.5 ml of carbon paint was equally distributed with a brush, allowed to air-dry for 10 min, then dried at 70°C in a pre-heated furnace for 5 min, and cooled down at room temperature. To prepare polyaniline treated anodes, the fourth layer was coated using a 10 mg polyaniline emeraldine salt (20 wt. % on carbon black, Cat. N. 530565 Sigma-Aldrich, St. Louis, MO) mixed with 0.5 ml carbon paint instead of carbon paint alone. To prepare polypyrrole treated anodes, the fourth layer was coated using a 10 mg polypyrrole, undoped (20 wt. % on carbon black, Cat. N. 577565 Sigma-Aldrich) mixed with 0.5 ml carbon paint instead of carbon paint alone. Standard drying conditions were used for the polyaniline- or polypyrrole-carbon paint layer. For coating of carbon cloth anode, polyaniline emeraldine salt (0.5 wt. % dispersion in mixed solvents, Cat. N. 649996 Sigma-Aldrich, St. Louis, MO) was diluted 4-fold with isopropyl alcohol, then carbon cloth was incubated in the polyaniline solution for 30 sec following being dried at 230 °C in a pre-heated furnace for 2 min.

[0043] The cathode ($A_{ca}=9.6 \text{ cm}^2$) consisted of (1) platinum catalyst layer (0.5 mg of Platinum/ cm^2 , E-Tek), (2) carbon cloth (B1B 30% wet proofed, E-Tek), (3) carbon base layer, and (4) four PTFE diffusion layers, and was prepared according to a previously published procedure (21). Two plastic caps with a hole in the bottom surface were used as holder for cathode that was permanently sealed using marine epoxy (Figure 1). Titanium wire was attached to anode and cathode. The distance between anode and cathode is about 2 cm.

[0044] Photosynthetic cultures. Synechocystic strain PCC 6803 and pond sediment were used as model photosynthetic cultures. Synechocystic PCC 6803 was grown at room temperature in BG-11 medium (1.5 g NaNO_3 , 0.04 g K_2HPO_4 , 0.075 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.036 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.006 g citric acid, 0.006 g ferric ammonium citrate, 0.001 g EDTA, trace metal mix A5: 2.86 g H_3BO_3 , 1.81 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.222 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.39 g $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 0.079 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.0494 g $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ per 1L, initial pH 7.1) under constant bubbling with air. After two weeks of cultivation, the cultures were diluted with fresh BG11 to $A=0.6$ o.e. as measured at 760 nm and transferred to anode chamber. PMFCs were assembled and their performance were evaluated under stationary cultivating conditions (no air bubbling).

[0045] Sediment collected from local pond in Columbia, MD was cultivated in the lab at room temperature in BG-11 medium in 250 ml plastic bottle under aerobic conditions (but no air bubbling) under 12/12 h light/dark cycles. After initial adaptation for approximately two months, notable biofilm appeared on the bottom surface. The surface was scratched with plastic pipet, then biofilm was gently vortexed to homogeneous cell suspension and transferred to the anode chambers; fresh BG-11 was added. Prior to PMFC operation, anode chamber was kept for 3-4 weeks to allow biofilm formation on anode surface.

[0046] During culture growth and PMFC operation, both Synechocystic PCC 6803 and pond cultures were placed in front of a day-light source (light intensity from about 100 to about 500 lux, color temperature 6500K) that operated under 12h/12 h light/dark cycles.

[0047] Characterization of PMFC performance. PMFCs were operated at a fixed external circuit resistance of $1\text{K}\Omega$, at constant temperature of $22 \pm 0.2^\circ\text{C}$ and under 12h/12h light/dark cycles. For each PMFC, the voltage was measured at 2-min intervals using a computer-controlled digital data acquisition system (PCI-6280, National Instruments, Austin, TX) and LabVIEW software (National Instruments) for data recording and analysis. For recording the polarization curve, PMFCs were stabilized at an open circuit potential, then the voltage was measured at variable external resistances (from $100\text{K}\Omega$ to 10Ω) allowing to stabilize for 15 min at each resistance. The current was calculated for each resistance using Ohms. An apparent internal resistance (R_{int}) was calculated from the linear region of the polarization curves according to equation: $R_{\text{int}} = -\Delta E / \Delta I$ as described by Logan (22).

[0048] The power curves were calculated from the polarization curves as previously described (23). The power output for planktonic PMFCs presumably depends on the reactor volume and on the anode surface area, whereas for the biofilm PMFCs is primarily dependent on the anode surface area. Therefore, to compare the performance of planktonic PMFCs with biofilm-based PMFC, the current density was calculated based on the cathode surface area (9.6 cm^2).

[0049] Electrochemical Analysis. Electrochemical measures were performed with a potentiostat (Reference 600, Gamry Instrument Inc., PA). Cyclic voltammetry (CV) was conducted at scan rates of 5 mV/s or 50 mV/s in the potential range from -0.7 to 0.7 V. Electrochemical impedance spectroscopy (EIS) measurements were carried in a frequency range from 100 kHz to 1 mHz with an AC signal of 10 mV amplitude at open circuit potential. The equivalent circuit (EC) was used to analyze the anodic impedance spectroscopy. The EC model consisted of an ohmic resistance (R_s), followed by a Randle's type circuit of an electrochemical charge transfer resistance (R_{ct}) and a Warburg's diffusion element (W) in parallel with a double layer constant phase element as shown in Figure 7. The sum, $R_s + R_{ct}$ is considered as the total internal resistance. Instead of a capacitor element, a constant phase element was employed during simulation because of a serious dispersion effect that relates to a rough electrode surface. In all electrochemical measurements, the working electrode was the anode and the counter electrode was the cathode. An Ag/AgCl reference electrode was used as a standard constant potential. All electrochemical analyses were conducted at 22 °C.

[0050] pH and Dissolved Oxygen measurements. Dissolved oxygen (DO) concentration (mg/L) and pH of PMFC anode chamber were measured every 10 minutes with an Orion four star-plus benchtop meter (Thermo Orion, Beverly, MA) using a Ross Ultra refillable glass pH electrode and a DO probe model 081010MD (Thermo Orion). DO and pH data were automatically recorded in real time using the Star Navigator Plus software (Thermo Orion).

[0051] Fluorescence Microscopy. Pond biofilms were grown on glass cover slips in the anode chamber or were transferred directly from the PMFC anode surface to new cover slips via sterile pipette tip. Samples were imaged on an inverted microscope (Nikon Eclipse TE200-U, Nikon Inc., Melville, NY) as previously described (24). Briefly, microscope was equipped with an X-Cite illumination system (EXFO Photonics Solutions Inc., Quebec, CA) connected by fiber-optics, and using a 1.3 aperture Plan fluor x150 numerical aperture and x10, x20 and x60 objectives. Digital images were taken with a cooled 12-bit Coolsnap HQ CCD camera (Photometrics, Surrey, CA) using EN-GFP-HQLP filter cube (excitation at 470 ± 20 nm, long-pass emission > 500 nm, Nikon Inc.), FITC C31001 filter cube (excitation at 480 ± 15 nm, emission at 535 ± 20 nm, Chroma

Technology Corp., Rockingham, VT) or Eth-Bro C31008 filter cube (excitation at 540 ± 12.5 nm, emission at 605 ± 27.5 nm, Chroma Technology Corp.) Images were processed with the WCIF Image-J software (National Institutes of Health, Bethesda, MD).

[0052] PMFC design. PMFC consisted of 125 ml plastic anode chamber and an air-exposed cathode containing platinum catalyst layer (Fig 1 A, B, C). The bottom of anode chamber was coated with four layers of electrically conductive carbon paint that served as an anode. To improve the yield of electron harvesting, the last layer of carbon paint was mixed with electrically conductive polymers, polyA or polyP when indicated. The cathode was submerged ~ 0.5 cm beneath the surface of media (Fig. 1 A, C). A layer of air above media surface connected with atmospheric air.

[0053] Positive light response. As photosynthetic culture, planktonic fresh-water cyanobacterium *Synechocystis* strain PCC 6803 was used. PMFCs were loaded with PCC 6803 in BG-11 media (no buffer or organic substrate), kept for several hours at open circuit until steady-state voltage was established, and then maintained at $1\text{ K}\Omega$ external resistance under 12h/12 h light/dark cycles at constant temperature 22°C for the entire operation. At $1\text{ K}\Omega$ resistance, PMFCs with PCC 6803 showed weak positive light response: the cell voltage increased slowly during the illumination phase and dropped gradually during the dark phase (Figure 2A). Coating of anode with polyA improved cell voltage only to a small extent (Figure 2A). While positive light response was observed persistently for several weeks, overall decline in cell voltage was observed within the first few days of operation. PMFCs loaded with BG11 media in the absence of PCC 6803 showed no response to light (data not shown).

[0054] Considering that PCC 6803 is planktonic (does not form biofilm) and that PMFCs were operated in the absence of exogenous electron transfer mediators, the weak light response could be attributed to a low yield of electron harvesting by the anode. To examine whether photosynthetic biofilm perform better than planktonic cyanobacteria, a fresh-water sediment collected from a local pond was tested. After initial adaptation to BG-11 media (no buffer or organic substrate) for approximately two months, photosynthetic biofilm formed on the surface of cultivation flasks. Cell suspension

harvested from biofilm was transferred to anode chambers and incubated for three additional weeks prior to PMFC operation to reform biofilm.

[0055] In the absence of polyA coating, the pond biofilm showed a weak light response similar to that of cyanobacterial culture PCC 6803, whereas the biofilm grown on polyA-coated anode displayed substantially higher amplitude of the light response as shown in Figure 2B. During each 24h light-dark cycle, the cell voltage in polyA-coated PMFC increased sharply immediately after the beginning of illumination reaching a plateau within 20-30 min and dropped rapidly during the dark stage of each cycle (Figure 2B).

[0056] Measurements of dissolved oxygen (DO) and pH revealed 24 h cycles that appeared as a result of light-dependent oxygen evolution in both planktonic and biofilm cultures (Figure 3). In contrast to sharp jump of cell voltage observed in direct response to illumination, both DO and pH displayed much more gradual increase during 12h illumination-phase. These results argue that the positive light response in biofilm-based PMFC was not due to change in DO concentration or pH. In fact, an increase in DO or pH creates unfavorable conditions for PMFC operation and is expected to reduce cell voltage.

[0057] To examine whether the positive light response is limited only to the polyA-treated anodes, additional experiments were performed using PMFCs with anodes coated with polyP. Comparing to non-coated anodes, both the polyA- and polyP-coated anodes showed significantly higher light response as shown in Figure 2C. PolyP PMFC performed even better than the polyA PMFC. Control PMFC that was not inoculated with biofilm suspension and only contained BG-11 showed no response to illumination (Figure 2C).

[0058] It is not clear, whether the difference in PMFC performances should be attributed exclusively to the positive effects of polymers on biofilm formation or better efficiency in electron collection by modified anodes. Visual examination of anode surface prior to PMFC operation revealed that pond biofilm formed substantially better on anodes coated with polyA or polyP than on non-treated anodes (Figure 8). However, plausible effects of electrically conductive polymers on efficiency of electron transfer from biofilm to anode should not be excluded considering that both polymers are positively charged.

[0059] To make sure that the positive light response is not limited to carbon paint-based anodes, performance of PMFCs was tested with carbon cloth that was attached to the bottom of anode chamber (to mimic the architecture of PMFC). When used as anodes in biofilm-based PMFCs, carbon cloth coated with polyA or polyP displayed positive light response similar to that of carbon paint-based anodes as shown in Figure 9.

[0060] Fluorescence Microscopy Imaging of Pond Biofilm. Fluorescence microscopy imaging revealed several major groups that compose pond biofilm including filamentous cyanobacteria, non-filamentous cyanobacteria and eukaryotic algae (Figure 4). Based on morphological evaluation, filamentous cyanobacteria most closely resembled the *Leptolyngbya* or *Phormidium* strains that are generally known to form freshwater microbial mats. The smaller but conspicuously segmented filaments resembled the *Pseudoanabaena* strain. The eukaryotic non-filamentous cells possessed spines or spicules, and appeared as linked sets of four cells (Figure 4C). This phenotype is characteristic of the freshwater green algae *Scenedesmus*. Members of this genus typically grow in nutrient rich waters and are also culturable in BG-11 medium (Jai et al., 2008). Another type of non-spiculated eukaryote was noted as individual, generally spherical cells (Figures 4A,B). Close examination by fluorescence microscopy revealed complex multi-layered architecture of biofilm where filamentous species intercalated between conglomerates of non-filamentous algae and cyanobacteria (Figure 10). Because intrinsic fluorescence was used for the imaging (no staining), species that lacked photosynthetic or fluorescent pigments (heterotrophs) could not be seen using this techniques. .

[0061] PMFC performance. To compare performance of PMFCs, polarization curves were collected during the light-phase at 20th day of PMFC operation using a series of circuit external resistances (Figure 5A). As judged from the slope that reflects the rate of voltage decline as a function of current production, all PMFCs could be classified into two categories: (1) cells with fast voltage drop (PCC 6803 with non-treated anode; pond biofilm with non-treated anode; PCC 6803 with polyA-treated anode) and (2) cells with relatively slow voltage decline (pond biofilm with polyA-treated anode; pond biofilm with polyP-treated anode). Interestingly, all PMFCs with weak light responses fell into the first category showing an apparent internal resistance $R_{int}=12.1\pm0.4\text{ K}\Omega$ as calculated from the

slopes (Figure 5A), whereas PMFCs with strong light responses belong to the second category with $R_{\text{int}} = 3.9 \pm 0.01 \text{ K}\Omega$.

[0062] Analysis of power density curves revealed the following rank order for the power outputs (from the lowest to the highest values): PCC 6803 with non-treated anode, 0.2 mW/m^2 ; pond biofilm with non-treated anode, 0.35 mW/m^2 ; PCC 6803 with polyA-treated anode, 0.63 mW/m^2 ; pond biofilm with polyA-treated anode, 0.95 mW/m^2 ; pond biofilm with polyP-treated anode 1.3 mW/m^2 (Figure 5B). As expected, biofilm PMFCs produced higher power outputs than planktonic PMFCs, and the polymer-coated anodes performed better than the non-treated anodes in PMFCs with both cultures.

[0063] To test the extent to which exogenous electron mediators improve performance of PMFCs, polarization curves and the power density were analyzed for PMFCs operated in the presence of 1 mM HNQ. As expected, HNQ increase substantially the power output of PCC 6803-based PMFCs: from 0.2 mW/m^2 to 0.59 mW/m^2 (195% increase) for PMFCs with non-treated anode, and from 0.63 mW/m^2 to 1.47 mW/m^2 (133% increase) for PMFCs with polyA-coated anode (Figures 5B, D). The increase in the power output was much smaller for the biofilm-based PMFCs: from 0.35 mW/m^2 to 0.45 mW/m^2 (28% increase) for PMFCs with non-treated anode, and from 0.95 mW/m^2 to 1.56 mW/m^2 (64% increase) for PMFCs with polyA-coated anode (Figures 5B,D). This experiment demonstrated that (i) planktonic PMFCs rely on electron shuttles to a much higher extent than biofilm PMFCs; and (ii) planktonic PMFCs could generate as high power output as biofilm PMFCs if electron mediators are provided. Furthermore, as judged from the polarization curves, planktonic- and biofilm-based PMFCs maintained voltage as a function of the current production equally well when operated in the presence of HNQ (Figure 5C).

[0064] To further examine the electrochemical activity of PMFCs cyclic voltammetry (CV) and impedance spectroscopy was employed. Regardless of a scanning speed rate (50 or 5 mV/s), cyclic voltammetry did not reveal any oxidation/reduction peaks suggesting a lack of endogenously produced electron mediators in both planktonic- or biofilm-based PMFCs (data not shown). As judged from the voltammogram profiles (vs Ag/AgCl) recorded as a function of applied anodic potential, the current output was found to increase

in the following rank order (from the lowest to the highest current): culture-free fuel cell, non-treated anode < PCC 6803, non-treated anode < pond biofilm, non-treated anode < PCC 6803, polyA-treated anode < pond biofilm, polyA-treated anode < pond biofilm, polyP-treated anode (Figure 6). CV analysis showed that (i) PMFC with pond biofilm had higher electrochemical activity than PMFC with PCC 6803, and that (ii) anodes coated with electrically conductive polymers exhibited better electron-transfer properties than the non-coated anode. The positive effects of polymer-coating can be attributed to an improvement of intrinsic conductivity of anode and/or active involvement of polymers in transfer of electrons from cellular membrane. Because of their long chains, polyA and polyP can physically interact with or intercalate into cell membrane enabling direct discharge of electrons to anode surface.

[0065] Impedance spectroscopy analysis of biofilm-based PMFCs revealed remarkable differences in the total resistance (R_{total}) between three PMFCs (Table 1, Figure 7). These differences were attributed entirely to the differences in the anodic charge transfer resistance (R_{ct}) that reflects the transfer of electrons from biofilm to carbon paint anode. As judged from R_{ct} values, coating of anode with polyP and, to a lesser extent, with polyA improved the electron-transfer processes. Relatively high values of R_{ct} can be due to intrinsically low conductivity of carbon paint. Comparison of R_{ct} values for biofilm-grown PMFC with those for control PMFCs that lacked biofilm demonstrated that biofilm markedly improved the electron-transfer properties of the anodes in all three cells (Table 1).

[0066] Table 1. Impedance spectroscopy analysis of biofilm-based PMFCs and PMFCs without biofilm.

culture	anode type	R_{total} , Ω	R_s , Ω	R_{ct} , Ω
*pond-biofilm	non-treated	383	63	320
in BG11 medium	polyA-coated	298	58	240
	polyp-coated	212	62	150
**BG11	non-treated	464	72	392
medium	polyA-coated	327	66	261

polyP-coated	343	73	270
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*the impedance spectroscopy was performed 44 days after the pond culture was inoculated into anode chambers and at the 18th day of PMFC operation.

** newly assembled, non-inoculated PMFCs containing BG11 medium

[0067] The ohmic or electrolyte resistance (R_s) was the same for all three biofilm-containing PMFCs ($R_s = 60 \pm 3 \Omega$) indicating that they had identical configuration. The relatively high ohmic resistance reflected intrinsically low ionic strength of BG-11 medium. When BG-11 medium was supplemented with 50 mM phosphate buffer, R_s dropped from $60 \pm 3 \Omega$ to $20 \pm 1 \Omega$ in all three PMFCs (data not shown). The result of impedance spectroscopy was consistent with the cyclic voltammetry tests and confirmed that polymer coating provided beneficial effects for anode performance.

[0068] Long-term performance of PMFCs. Biofilm-based PMFCs showed positive light responses for as long as PMFCs were kept under operation (up to one month). The amplitude of the light response, however, declined over the first month of operation by 60-80%, with the most substantial drop of up to 50% being observed in the first week (Figure 2C). Remarkably, the shape of cell voltage drop recorded during the dark-phases changed gradually over the first several days of operation (Figure 2C). Instead of fast exponential decay observed at the first few days of operation, the dark-phase voltage developed substantially slower kinetics of decay that presumably reflects accumulation of organic compounds by the photosynthetic biofilm.

[0069] Decline in the amplitude of positive light response could be due to gradual accumulation of DO in anode chamber, an increase in pH that causes insufficient supply of protons to the cathode reaction, deprivation of BG-11 medium, or some other reasons. Replacement of BG-11 media did not restore the amplitude of the cell voltage to the initial level suggesting that the voltage decline was not due to deprivation of the medium (Figure 11). Interestingly, replacement of the medium altered the kinetics of the dark-phase voltage decay. Instead of slow decay, which was typical for the mature biofilm and old medium, the cell voltage showed fast drop in the fresh medium. This result was consistent with previous observation that the current production during the dark-phase was likely to be powered by photosynthetically produced organic compounds that were accumulated by biofilm during the light-phase.

[0070] The concentration of dissolved oxygen and pH value were found to gradually increase over long-term (Figure 3). In fact, in biofilm-based PMFCs, pH typically reached values of 8.5-9.0 just after several days of operation. In attempt to control pH, performance of PMFCs was tested where BG-11 medium was supplemented with phosphate buffer. Surprisingly, no light response could be detected for PMFCs containing phosphate buffer (Figure 12). After phosphate buffer in BG-11 medium was replaced by BG-11 medium alone, positive light responses were restored confirming that the buffer did not damage irreversibly the vitality of biofilm nor did it alter the electrochemical properties of PMFC (Figure 12). It is not clear why the phosphate buffer completely abolished the positive light response.

[0071] PMFCs with planktonic cyanobacterium *Synechocystis* PCC-6803. To test whether positive light response can be observed using planktonic photosynthetic culture instead of biofilm-forming culture, we used strictly planktonic fresh-water cyanobacterium *Synechocystis* PCC-6803. PCC-6803 is one of the best studied cyanobacterial species with respect to genetics, physiology and biochemistry. In recent studies, PCC-6803 was shown to produce electrically conductive nanowires (31) and, therefore, appeared to be a well-suited candidate for light-powered electricity production in PMFCs. PMFCs were loaded with *Synechocystis* PCC-6803 in mBG-11 media (no buffer or organic substrate), kept for several hours at open circuit until steady-state voltage was established, and then maintained at 1 K Ω external resistance. During operation at 12h/12h light/dark cycles, PMFCs with PCC-6803 showed weak positive light responses: the cell voltage increased slowly during the light-phases and dropped gradually during the dark-phases (Figure 2B). Coating the anode with polyA improved cell voltage only to a small extent (Figure 2B). Considering that PCC-6803 is strictly planktonic (does not form biofilm) and that PMFCs were operated in the absence of exogenous electron mediators, the weak light response could be attributed to a low yield of electron harvesting by the anode.

Discussion

[0072] The current studies provided direct illustration that electrical power can be generated in aerobic sun-powered single-chamber PMFCs. The rapid positive light response is consistent with the mechanism postulating that the photosynthetic electron

transfer chain is the source of the electrons harvested on the anode surface. This mechanism is fundamentally different from the one exploited in previously designed anaerobic MFCs, sediment MFCs or anaerobic PMFCs, in which the electrons collected on anode are derived from bacterial respiratory electron transfer chain (25). In anaerobic MFCs, electrons exit bacterial respiratory chain via cytochromes c (26,27). It is currently unknown how electrons in PMFC are transported from the photosynthetic transfer chain, which is localized in thylacoid membranes, to the cell membrane. It is possible that electrons exit photosynthetic chain using cytochrome c6. Cytochrome c6 is a water-soluble protein that is used by photosystem II to transfers electrons from cytochrome f to photosystems I. Like other components of photosystem II or I in cyanobacteria, cytochrome c6 is predominantly localized in thylakoids. However, it has also been found in substantial amounts in locations distant from the photosynthetic membranes including the periplasmic space (28,29).

[0073] In recent studies, PMFCs inoculated with photosynthetic cultures were reported to display negative light response (i.e. increase in cell voltage during a dark-phase) (30). The negative light response is consistent with the conventional mechanism of MFC operation in which respiratory electron transfer chain provides electrons. In the current study it was found that the intensity of dark-phase voltage gradually increases over several days of PMFC operation. This may indicate accumulation of organic biomass within photosynthetic biofilm and involvement of respiratory electron transfer chain in generating power during the dark-phase. Therefore, the current configuration of PMFC appear to be suitable for harvesting electrons from both photosynthetic and respiratory electron transfer chains during light- and dark-phases, respectively.

[0074] The present invention provides a useful framework for the future design and operation of PMFCs with positive light response. Exploiting biofilm-forming photosynthetic cultures instead of planktonic cultures was shown to improve substantially the yield of electron harvesting and remove the needs for exogenous electron shuttles. Indeed, addition of the exogenous electron shuttle HNQ did not provide significant benefits to the biofilm-based PMFCs as it did to the PCC 6803-based PMFCs with respect to the power output. These results argue in favor of direct electron transfer from microbial/algae consortia of biofilm to the anode surface.

[0075] Coating of anode surface with polyA or polyP was found to improve substantially the positive light response. Both polymers accelerated biofilm growth (Figure 8) and improved electrochemical properties of the anodes (Figures. 6,7 and Table 1). The observations discussed herein of the polymer effects on biofilm formation was consistent with previous findings that positively charged natural surfaces enhance adhesion of bacteria due to negatively-charged bacterial cell surface. In studies of mixed culture MFCs, the power production was improved with treatment of the carbon surface with ammonia that increased the positive charge the surface (32). As judged from CV and impedance spectrometry in the current work, polyA and polyP were also found to improve the electrochemical property of anode, presumably, via increasing the yield or efficiency of electron harvesting. Furthermore, consistent with previous studies with anaerobic MFC (33), impedance spectroscopy analysis revealed that the anodic charge transfer resistance was reduced upon initial biofilm formation (Table 1) indicating direct involvement of biofilm in electrochemical reaction of electron transfer.

[0076] As judged from fluorescence microscopy imaging, biofilm was composed of eukaryotic algae and cyanobacterial species including filamentous bacteria. It is unknown whether heterotrophs were constituent part of the biofilm. Photosynthetic biofilms or mats are completely self-sustainable communities that can grow on sediments in a variety of environments (34). These important characteristics provide unique opportunities for designing fully self-sustainable, solar-powered, aerobic PMFC bioreactors. A typical property of mats is their multilayered laminated structure in which different functional groups of microorganisms occupy different vertical layers. Cyanobacteria are the most successful mat-building organisms and form the top, aerobic layer of mats (34). A layer of oxidized iron may separate the cyanobacterial oxygenic layer from anoxygenic layer composed of purple sulfur and green sulfur bacteria. In marine mats, anaerobic sulfate-reducing bacteria can be found throughout the mat below the top layer of cyanobacteria. Sulfate-reducing bacteria play a major role in recycling waste and decomposing organic materials produced by cyanobacteria. The joint metabolic activity of microorganisms in mats results in steep gradients of light, oxygen, carbon dioxide, pH, and sulfide; these gradients shift markedly during a 24-h cycle. Photosynthetic activity in mats causes fast depletion of CO₂ throughout the day time (34). It is intriguing to speculate that the layer of oxidized iron is used by cyanobacteria to dump excess electrons during the CO₂-depletion cycle and/or to share excess electrons within the microbial mat community.

Consistent with this hypothesis, cyanobacteria were shown to produce nanowires under excess of light and CO₂-limitation (31).

[0077] The dense biomass of photosynthetic organisms in the mats results in high rates of photosynthesis. On a surface area basis, the photosynthetic productivity compares to that of rain forests, which are considered the most productive ecosystems on Earth. Of the 1.5×10^{22} kJ of energy reaching the earth each day from the sun, 1% is absorbed by photosynthetic organisms and converted into biomass. Cyanobacteria account for 20-30% of all world-wide conversion of sun energy into biomass (35). They can be found anywhere near the surface of the earth, from Antarctica to hot springs. The major requirements for growth are light as an energy source, water as an electron donor, and CO₂ and N₂ as a carbon and a nitrogen sources.

[0078] The power density produced in the newly designed PMFC although substantially lower than power outputs reported for anaerobic MFC in recent years (1,36,37), was compatible to the first generations of MFCs (1) or the sediment MFC installed in fresh water rice paddy field (38). The main advantage of an aerobic PMFC versus anaerobic MFC is that it is easy to set up and maintain under normal atmospheric conditions. The presence of oxygen, however, appears to present the most significant challenge for achieving high power outputs, as it causes protons to be reduced to water before the electrons can reach anode. The current set-up showed high concentration of oxygen in both planktonic and biofilm cultures because no precautions were employed to deprive dissolved oxygen (Figure 3). However, several strategies can be used to rectify this problem, including purging oxygen with CO₂ or co-culturing with aerobic chemoheterotrophs that consumes oxygen for cellular respiration. In previous studies, Kayano and coauthors employed aerobic *Bacillus subtilis* for removing oxygen from photochemical fuel cell reactor and increasing hydrogen production (4). In natural photosynthetic mats, the concentration of dissolved oxygen drops rapidly through the depth of mat reaching undetectable level at the depth of 2 mm during the day and 1 mm at night (34). Employing three-dimensional porous material instead of carbon paint should promote formation of highly dense photosynthetic biofilm with higher yield of light energy harvesting per anode footprint area.

[0079] Low conductivity of BG-11 medium and high pH values were among other factors that contributed to the low PMFC power output. As such, the present invention includes using marine photosynthetic cultures that grow in highly conductive saline media instead of fresh-water cultures will help to overcome high PMFC internal resistance. The pH profile mimicked quite accurately both the 24-hour dynamics and the long-term gradual increase in dissolved oxygen concentration, as the change in pH is known to be a direct result of photosynthetic activity. Basic pH makes the proton transfer between biofilm and cathode highly unfavorable.

[0080] PMFC with positive light response has several advantageous over anaerobic MFCs and, therefore, may serve different practical applications. First of all, PMFC is 100% light-powered and does not require organic substrate as an energy source. Second, the net production of CO₂ in PMFC is zero. Water is the “substrate” (i.e. the source of electrons) and the end-product of PMFC operation. Third, PMFC does not require a buffer or exogenous electron shuttles for its operation. These conditions are essential for establishing a new, PMFC-based technology for generating energy in a self-sustainable, CO₂-neutral manner using renewable energy sources.

[0081] References

[0082] The contents of all references cited herein are hereby incorporated by reference herein for all purposes.

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CLAIMS

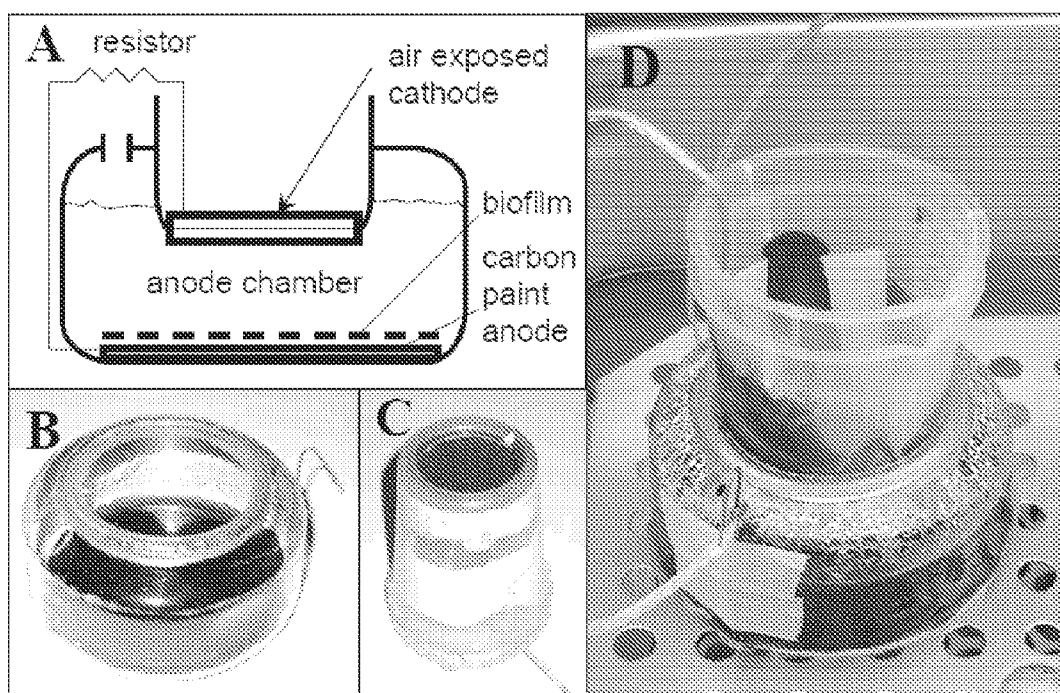
That which is claimed is:

1. A system for generating electricity, the system comprising a vessel wherein the vessel has a anode positioned on the bottom of the vessel and a cathode position near the top of the vessel and communicatively connected to the anode;
a solution in the vessel, wherein the solution is aqueous;
a photosynthetic aerobic microbial culture positioned in the vessel, wherein the culture includes microbes having photosynthetic ability and the ability to provide electrons from the electron-transfer chain; and
an electromagnetic energy source that emits day-light energy.
2. The system of claim 1 wherein the anode is coated with a conductive carbon layer.
3. The system of claim 2, wherein the anode is further coated with an electrically conducted polymer.
4. The system of claim 3, wherein the electrically conducted polymer is polyaniline or polypyrrole
5. The system of claim 1, wherein the vessel consists of a single compartment filled with fresh water or salt water.
6. The system of claim 1, wherein the photosynthetic aerobic microbial cultures is a fresh water biofilm or planktonic cyanobacteria.
7. The system of claim 1, wherein the anode and cathode are separated by a distance of 1 to 5 cm.
8. The system of claim 6, wherein the planktonic cyanobacteria is *Synechocystis* PCC 6803.

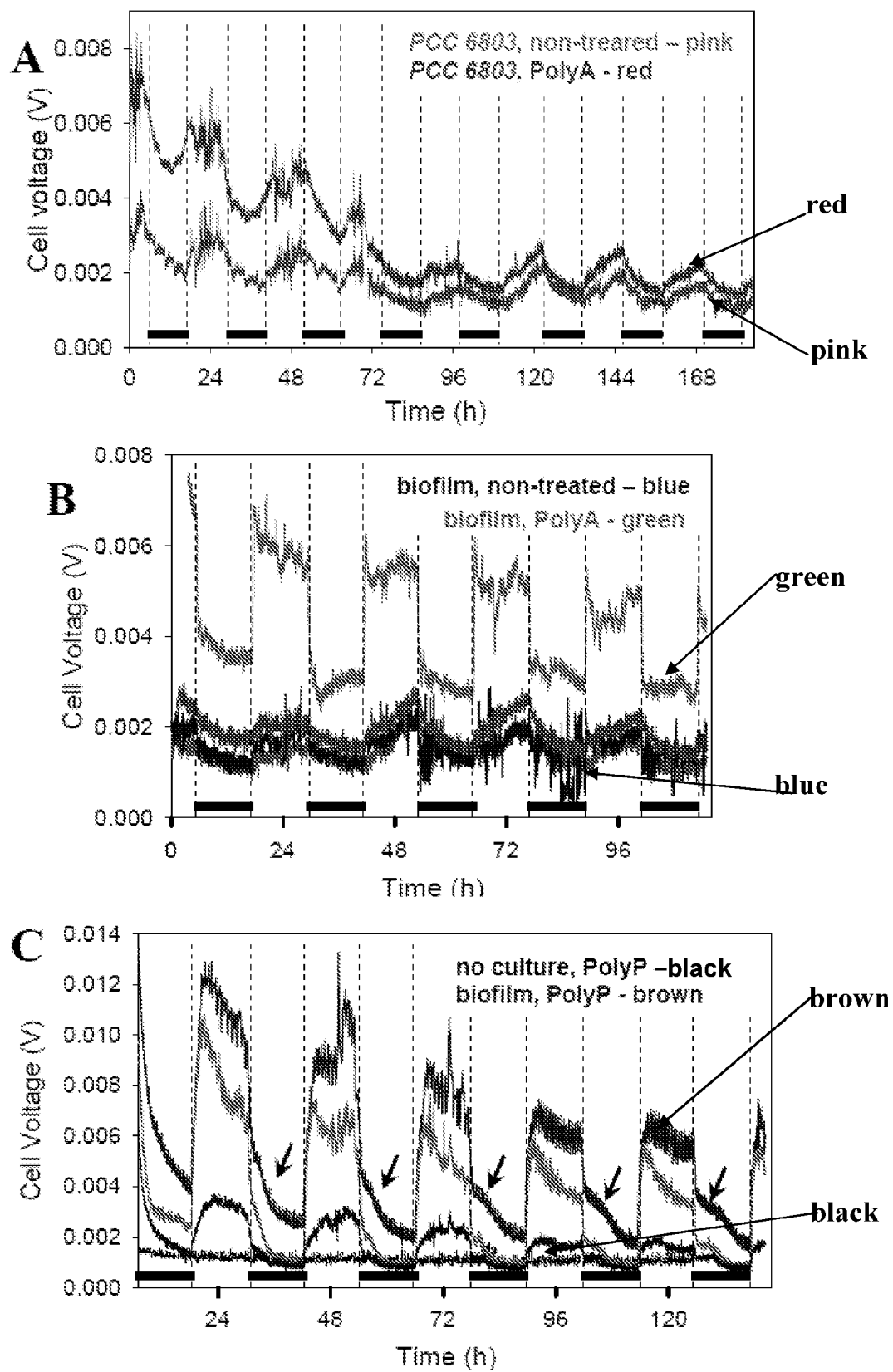
9. The system of claim 1 where in the source of day-light energy is applied for about 10 to 14 hours.
10. The system of claim 1, wherein the aqueous solution does not include exogeneous electron mediators or buffers.
11. The system of claim 1, wherein the microbes are positioned on the anode.
12. The system of claim 1, wherein the electromagnetic energy source is sunlight.
13. A method of generating electricity, the method comprising:
providing an aqueous solution comprising a photosynthetic aerobic microbial culture, wherein the culture includes microbes having the ability to provide electrons from an electron-transfer chain, wherein the aqueous solution is in contact with an anode and cathode, wherein the anode is coated with a carbon surface and wherein the photosynthetic aerobic microbial culture is positioned on the anode; and
providing an electromagnetic energy source that emits day-light energy to the vessel in amount sufficient to induce an electron transfer chain in the photosynthetic aerobic microbial culture.
14. The method of claim 13, wherein the anode is further coated with an electrically conductive polymer.
15. The method of claim 14, wherein the electrically conducted polymer is polyaniline or polypyrrole
16. The method of claim 13, wherein the photosynthetic aerobic microbial cultures is a fresh water biofilm or planktonic cyanobacteria.
17. The method of claim 13, wherein the anode and cathode are separated by a distance of 1 to 5 cm.

18. The method of claim 16, wherein the planktonic cyanobacteria is *Synechocystis* PCC 6803.
19. The method of claim 13, where in the sunlight is applied for about 10 to 14 hours.
20. An aerobic solar-power microbial fuel cell wherein the electrical energy is derived directly from energy of sunlight and not dependent on the consumption of organic substrates.

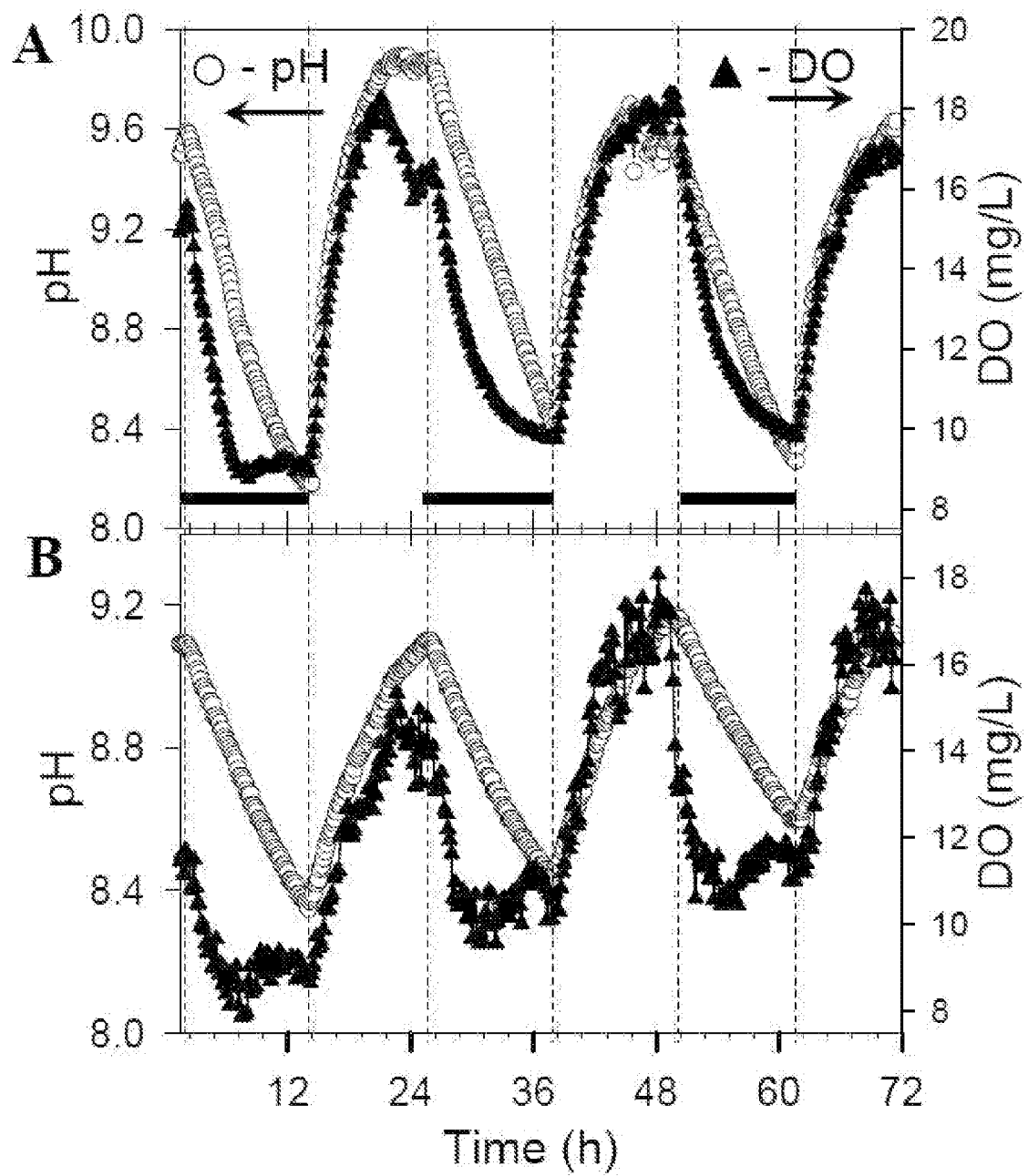
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**Figure 1**

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**Figure 2**

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**Figure 3**

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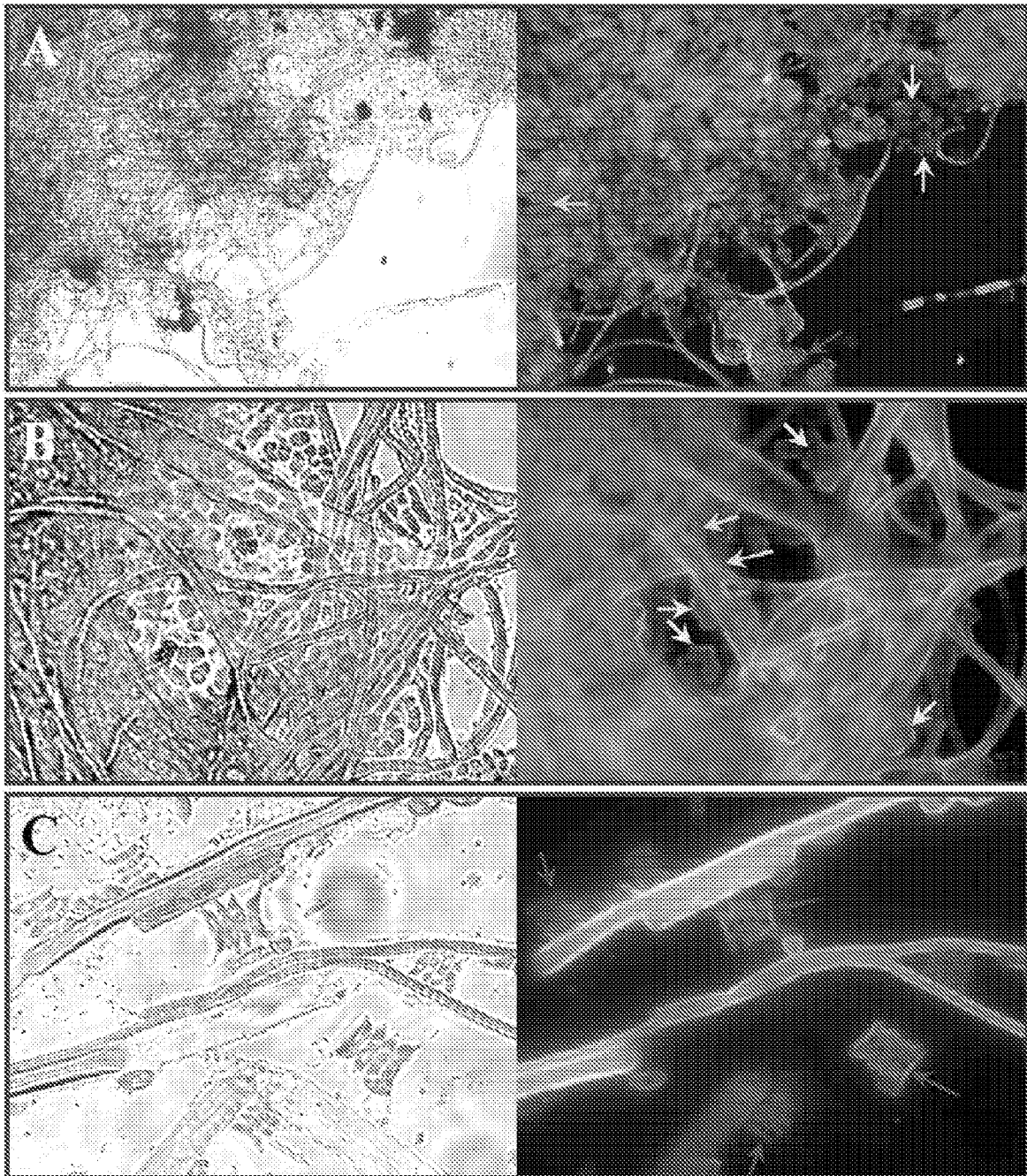


Figure 4

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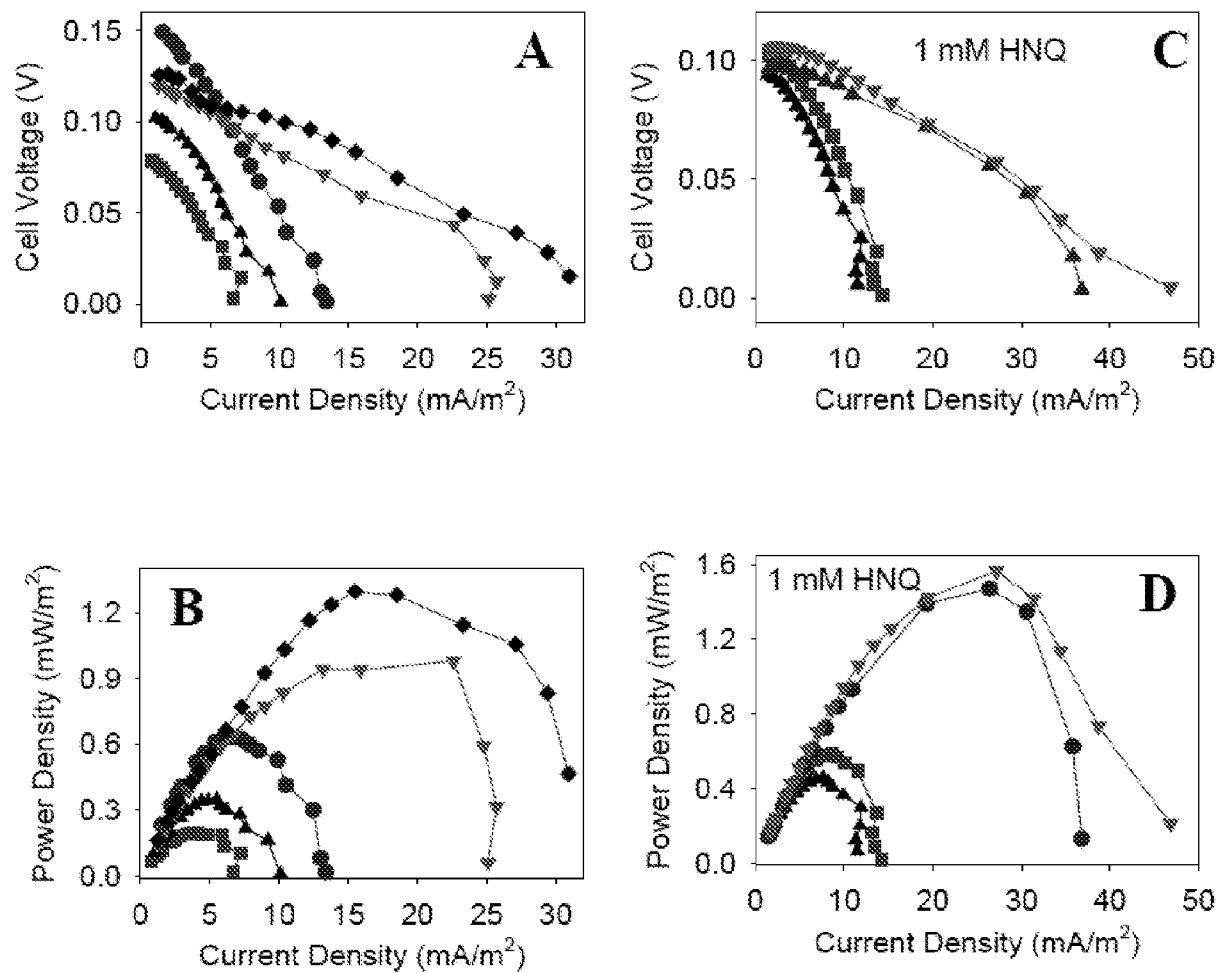
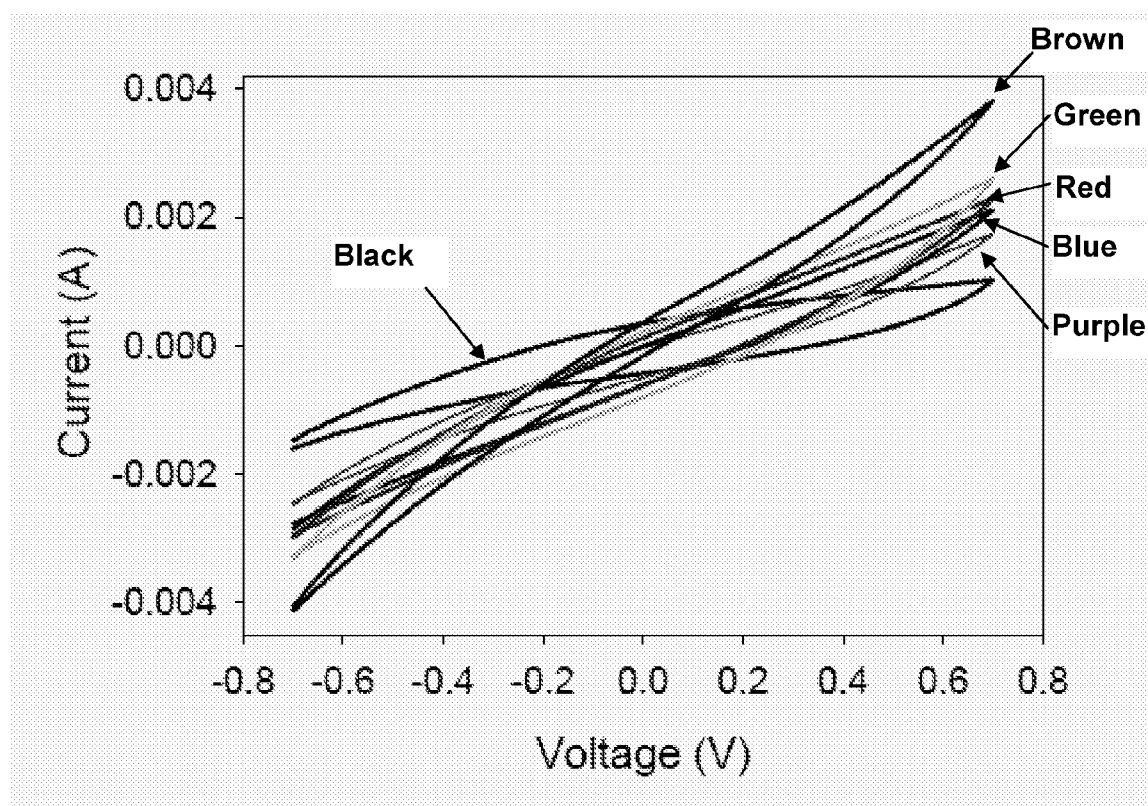


Figure 5

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**Figure 6**

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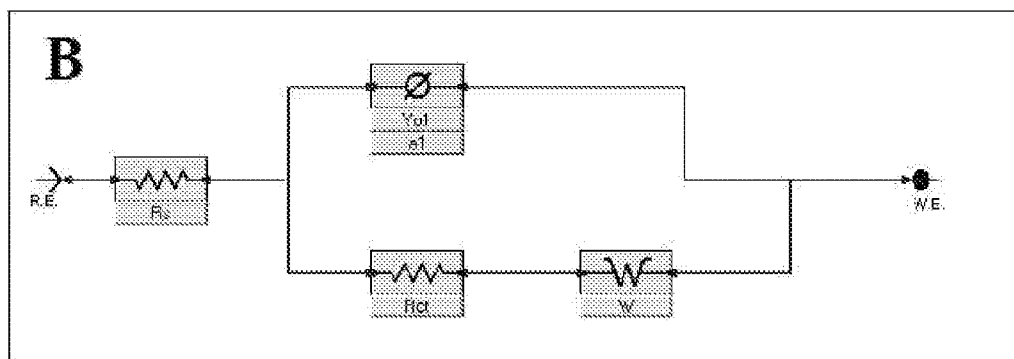
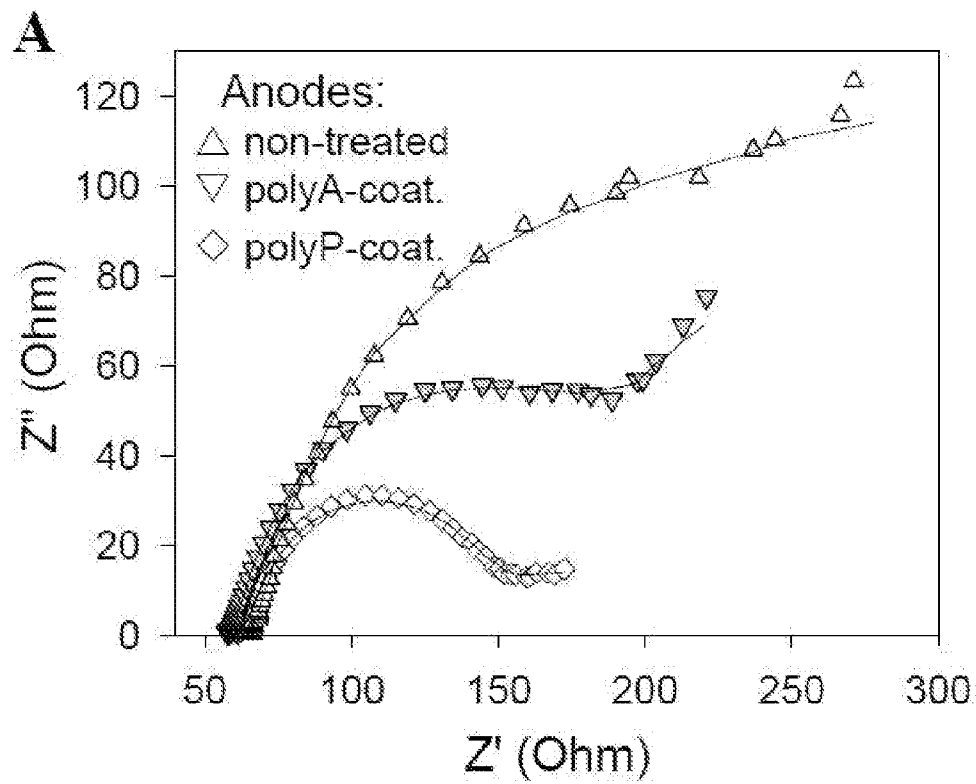
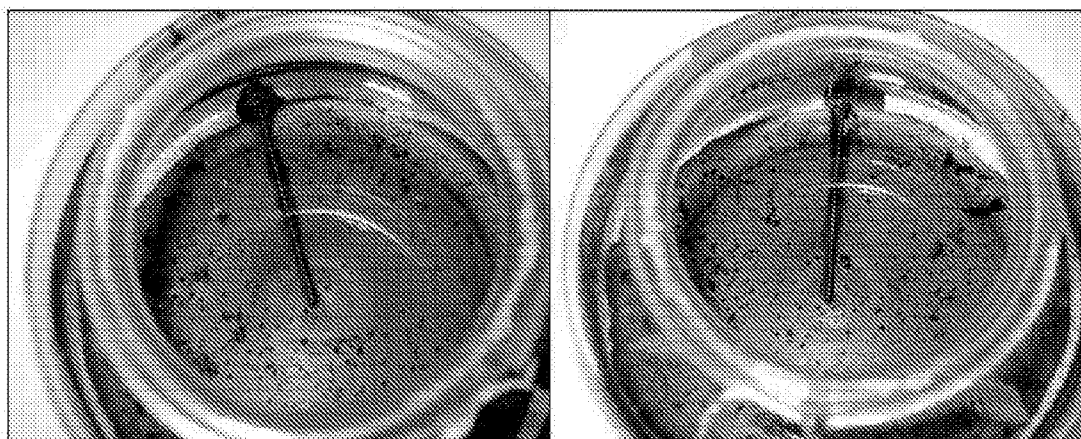


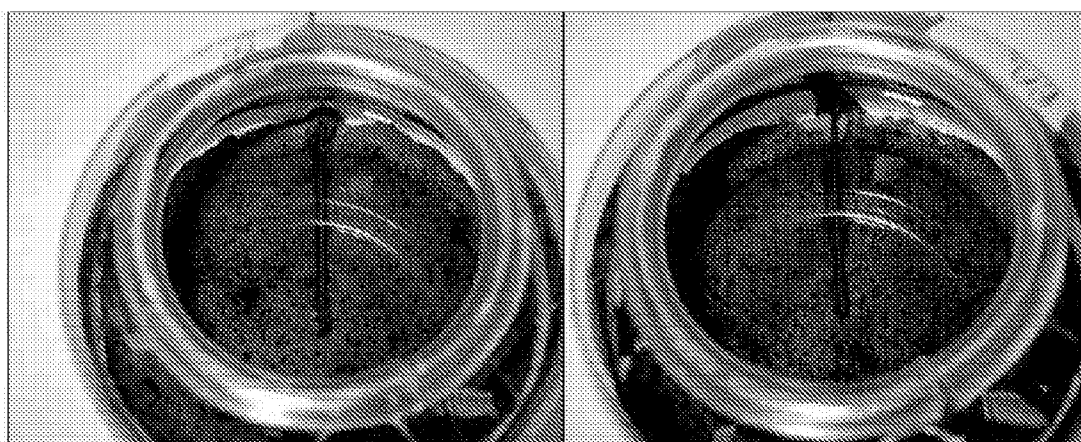
Figure 7

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Carbon Paint



Carbon Paint + Poly A



Carbon Paint + Poly P

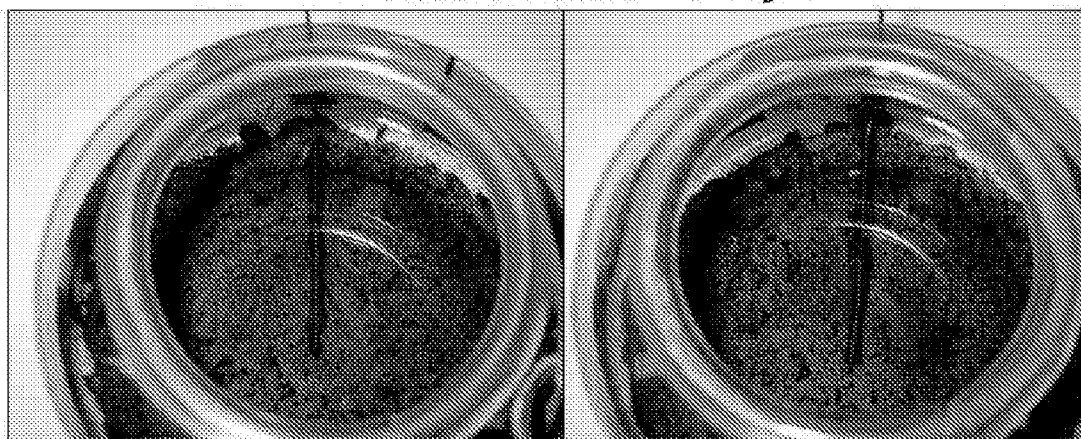
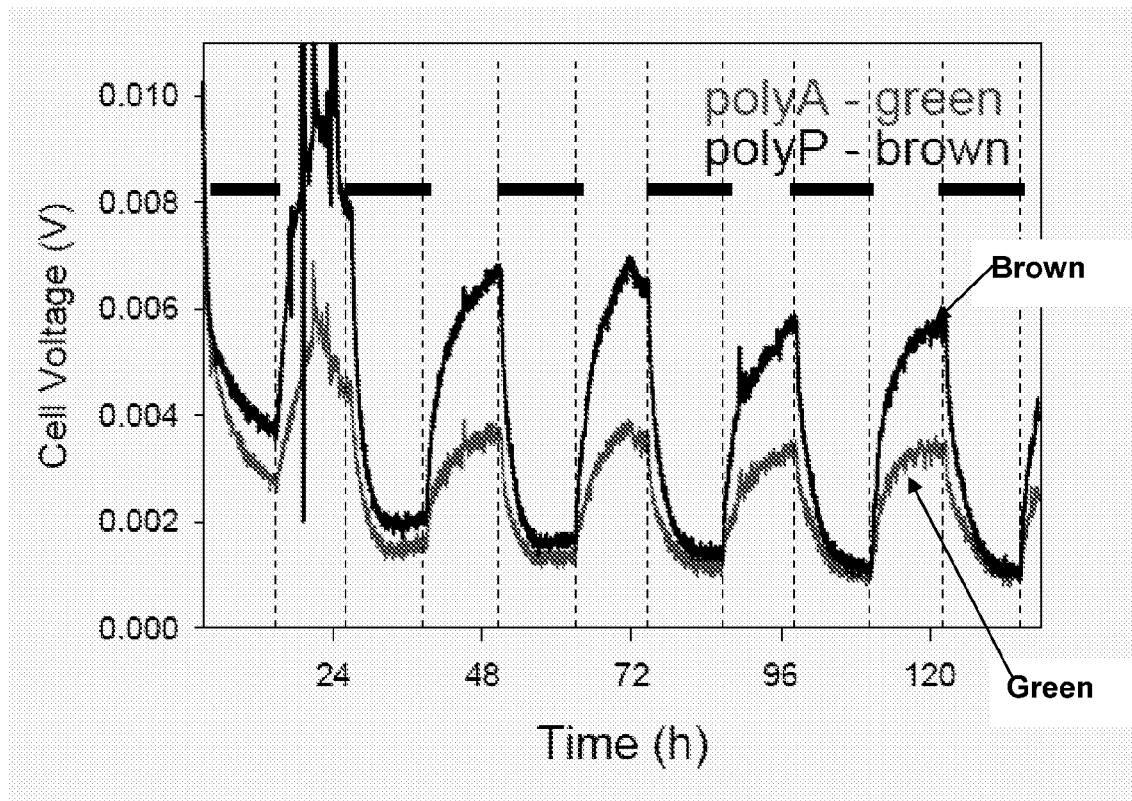


Figure 8

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**Figure 9**

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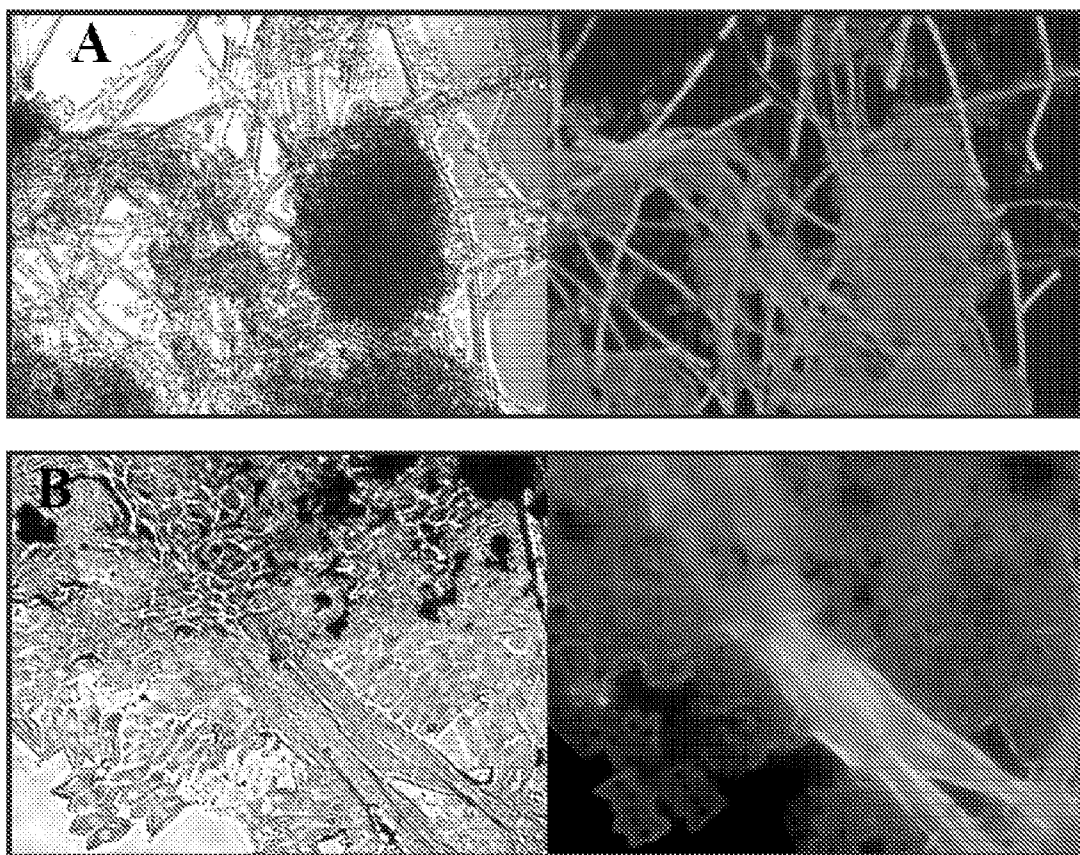
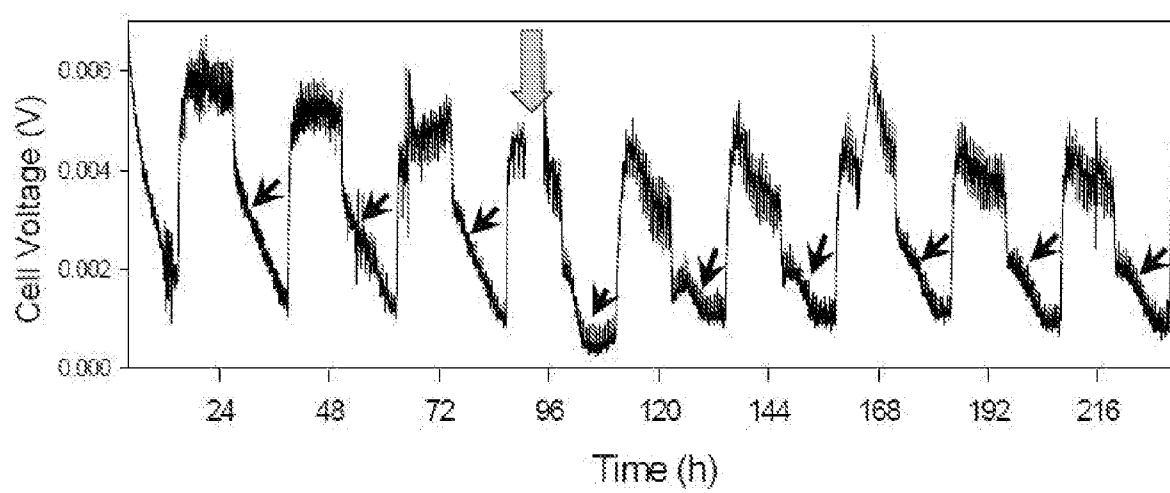


Figure 10

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**Figure 11**

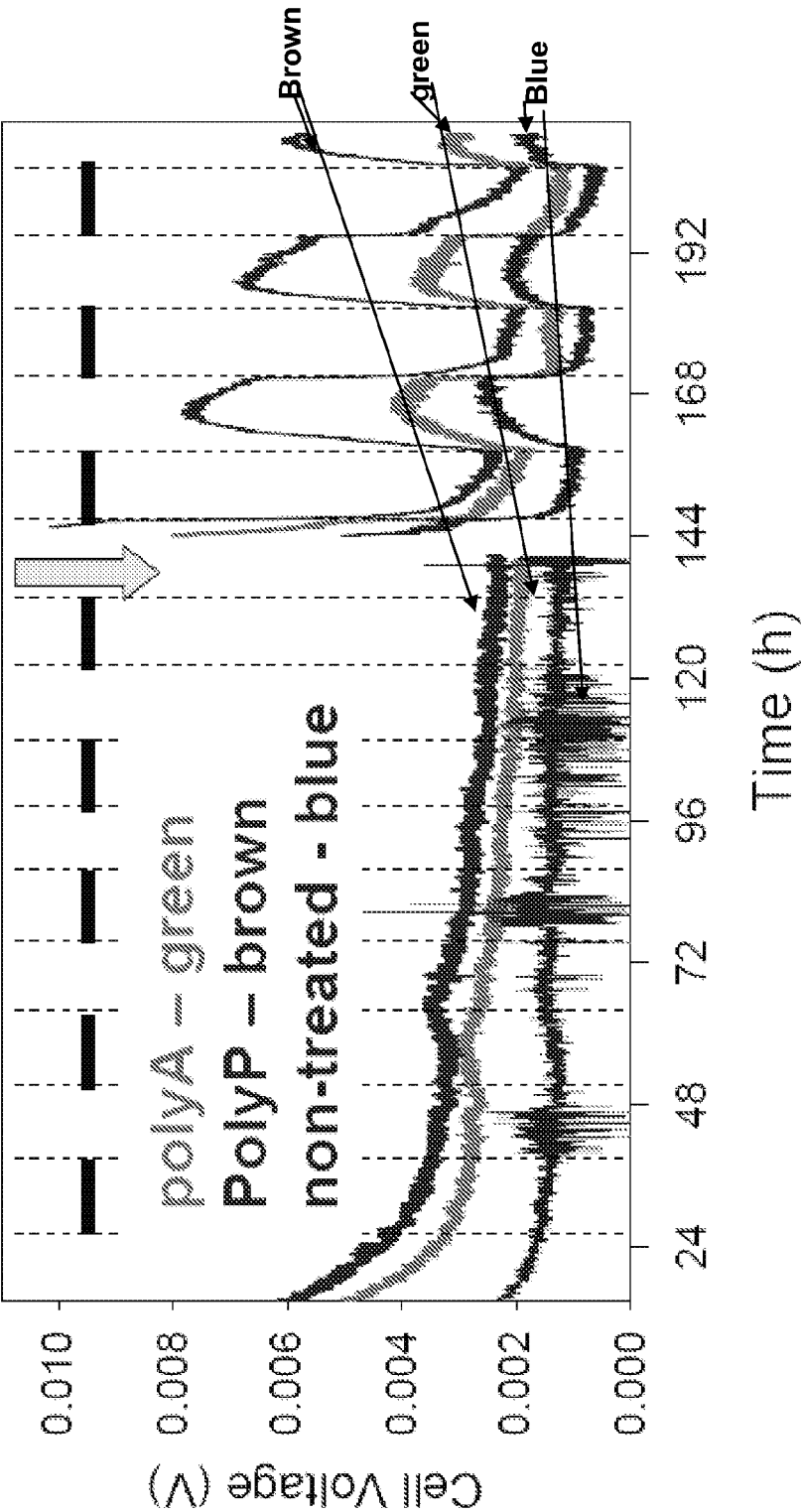
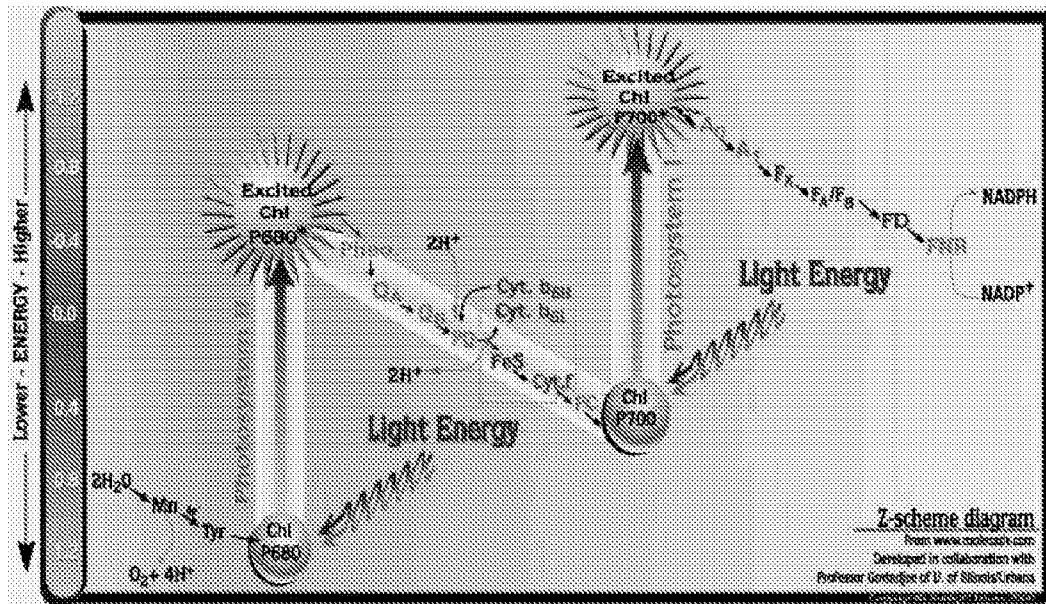


Figure 12

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A



B

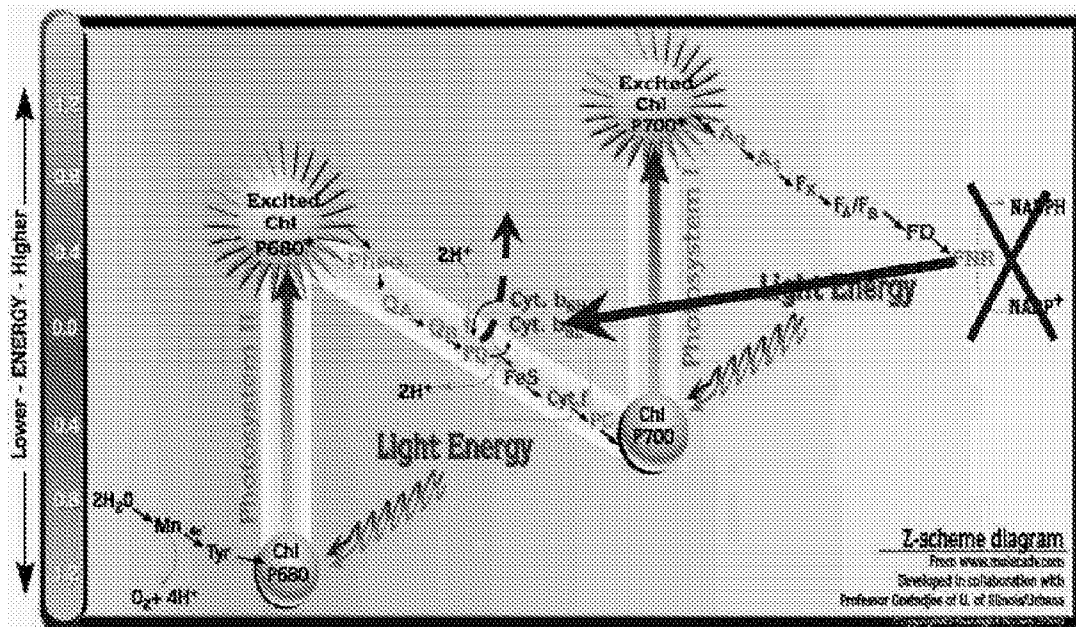


Figure 13

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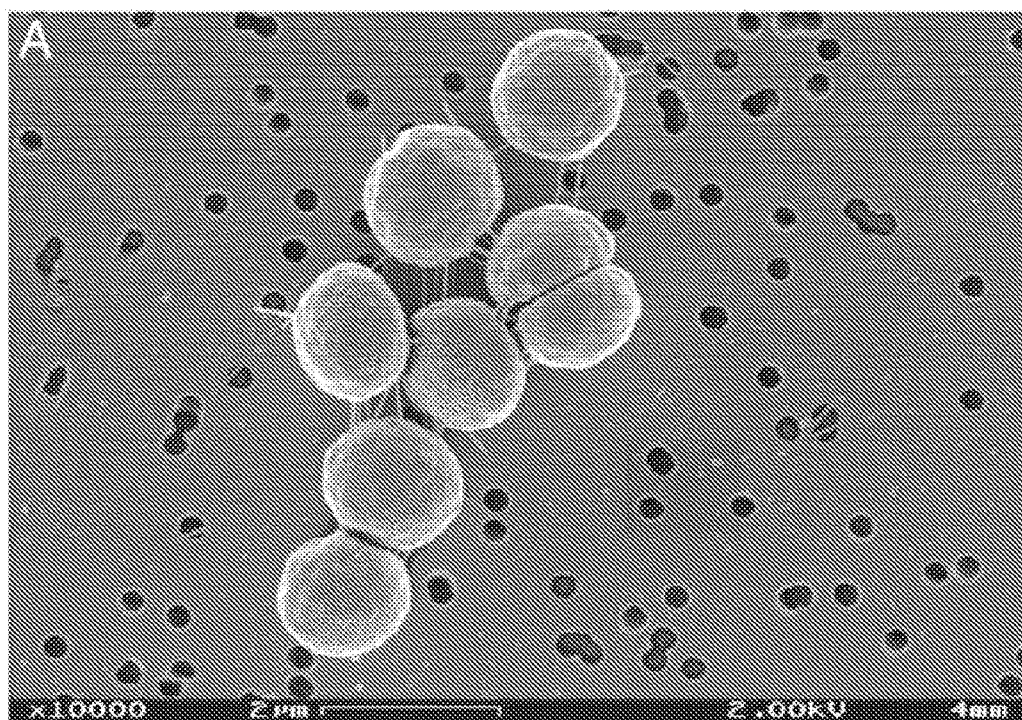


Figure 14

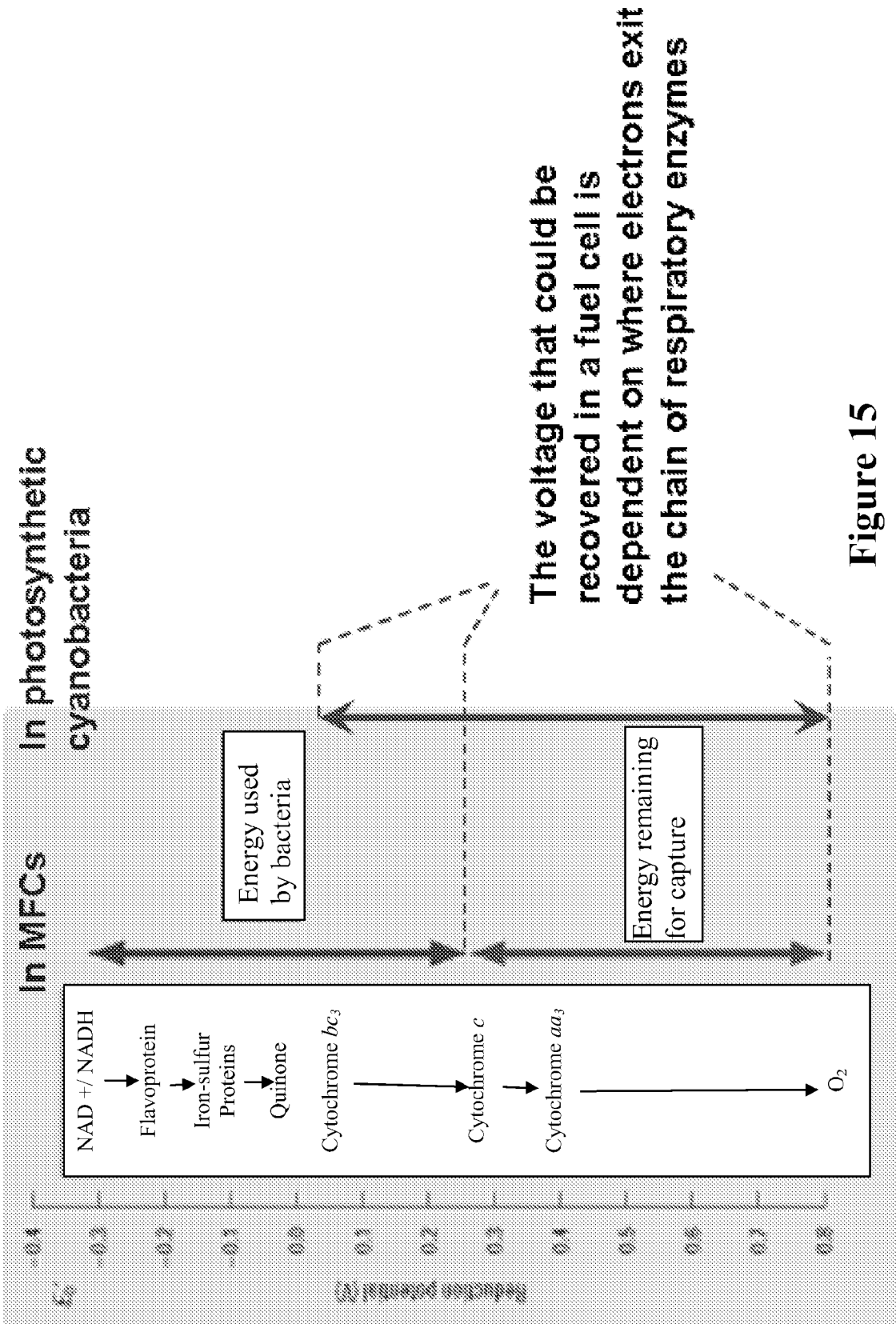


Figure 15