Titania/reduced graphene oxide composite nanofibers for the direct extraction of photosynthetic electrons from microalgae for biophotovoltaic cell applications

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Abstract
Titanium oxide (TiO2)/reduced graphene oxide (rGO) composite nanofibers were synthesized via an electrospinning technique and its potential electrochemical activity constructed its realization as an efficient anode catalyst in biophotovoltaic cells (BPV) with Chlorella vulgaris. The uniform adherence of GO sheets over the hydrolyzed Ti4+ ions, followed by its simultaneous reduction and crystallization, yielded the TiO2/rGO composite nanofibers. The strong interconnection among the nanofibers and the intimate contact between rGO and TiO2 in TiO2/rGO composite improved the efficient electron transportation paths, facilitating the higher oxidation and continuous and stable currents as substantiated, respectively, from the cyclic voltammetry and chronamperometry studies. By coupling the continuous electron conduction paths, proficient cell interaction, and elevated structural and chemical stabilities, TiO2/rGO demonstrated the BPV power density of 34.66 ± 1.3 mW m−2 with excellent durability, outperforming the BPV performances of previous reports. Thus the fundamental understanding achieved on the influences of nanocatalytic system in green energy generation opens up the new horizon of anticipation towards the development of sustainable and high-performance BPVs.

1 Introduction

Global energy demand coupled with the rapid consumption of limited oil reserves and greenhouse effect necessitates the development of prodigious energy technologies for harnessing energy from the renewable energy resources [1]. Among the various renewable energy resources including solar, pure water, wind, and geothermal, the prospective solution for energy crisis is achieved with solar, owing to its massive availability, safe, renewable, clean, and cost-effective characteristics [2]. The annual irradiance level of sun that strikes on Earth (1300 ZJ; 1 ZJ = 1015 J) is ≈ 2300 times higher than that of global energy consumption (0.56 ZJ) [3]. Hence, a number of photovoltaic devices including silicon solar cells [4], polycrystalline thin film solar cells [5], heterojunction [6], and heteroface structure cells [7] have been widely exploited for the generation of electricity from sun light. However, the aforementioned photovoltaic devices exhibit certain constrains including high manufacturing cost, tedious fabrication processes, and limited operational lifespan, delivering a negative impact toward their wide utility [4–7].

To successively encounter the aforesaid obstacles, the biophotovoltaic cell (BPV), a potential green energy device...
that can produce electricity via a photolysis of water using photosynthetic bacteria/microalgae in the presence of sunlight, has been recently developed [8]. On the basis of above, BPVs were developed with Chlamydomonas reinhardtii and Chlorella vulgaris as biocatalysts and the corresponding BPVs demonstrated the maximum power densities, respectively, of 0.036 mW m\(^{-2}\) and 0.014 mW m\(^{-2}\). The relevant power generation process occurred via the exoelectrogenic electron transfer between microalgal cell and electrode surface [9, 10]. Owing to the poor interfacial properties between the electrolyte and current collector via the lower electrical conductivity of bare electrodes, the aforesaid BPVs exhibited inferior power generation performances, which could be increased via the modification of electrodes with highly conductive materials [11]. The electrically conductive materials with considerable biocompatibility and contact points could improve their interaction with microalgae, which may collectively improve the interfacial properties between the electrolyte and electrode for the development of high-performance BPVs [12, 13].

Hence, the BPVs with conductive polymers including polyaniline (PANI) and polypyrrole (PPy)-modified carbon cloth (CC) were constructed with Synechocystis PCC-6803 and the maximum power densities, respectively, of 0.95 and 1.3 mW m\(^{-2}\) were observed for the relevant BPVs. The physical interaction or intercalation between the long chains of conductive polymers and cell membranes enabled the direct and rapid electron transference [14]. Although the conductive polymers facilitated the direct electron transference, the high power output and durability performances of fabricated BPVs were impeded via their sluggish electron transference, shrinkage, swelling, and inferior physiochemical stability [15, 16]. The photosynthetic microalgal-immobilized reduced graphene oxide (rGO) nanosheets on indium tin oxide (ITO) were also exploited as photo-anode in BPVs. Although rGO sheets facilitated a healthier biofilm formation, the inevitable restacking of rGO sheets and lower contact points lead to the inferior BPV power density of 0.1481 mW m\(^{-2}\) [11]. To overwhelm the aforesaid constraints of rGO sheets, the composite formation of rGO sheets with photocatalytic active metal centers, capable of triggering the photolysis of water molecules is essential. Among the various photocatalytic active nanocatalysts, titanium dioxide (TiO\(_2\)) is deemed as a proficient candidate, owing to its chemical inertness, cost effectiveness, environmental friendliness, and high tolerance against the photo and chemical corrosion [17]. Furthermore, TiO\(_2\) nanostructures could interact with the COO\(^-\) group of extracellular polysaccharides of microalgae via the electrostatic and chemical bonding (bridge coordination), leading to the better cell–material interaction [18].

Recently, one-dimensional (1D) nanostructures, in specific, nanofibers have received tremendous attention, owing to its large surface to volume ratio, high porosity, and interconnectivity among the fibers [19]. In general, the transference of electrons from microalgae cells to the electrode surface is initiated via a healthier biofilm. The interconnectivity and porous architectures of nanofibers may assist the effective accommodation of microalgae for the healthier biofilm formation, which may reduce the internal resistance of BPVs. To the best of our knowledge, the research efforts devoted on BPVs have not visualized the electron generation and transference mechanisms with the systematic electrochemical approaches, which hindered the fundamental understanding on the development of high-performance BPVs. Accordingly, we present here the rational development of TiO\(_2\)/rGO nanofiber composite to outfit the adverse effects of existing BPV anode catalysts and analyze their competency to improve the electron transference from microalgae to the electrode surface for the development of high-performance BPVs.

2 Materials and methods

2.1 Materials

Titanium (IV) isopropoxide (TIP, 97%), graphite powder (98%), ethanol (AR, > 99%), acetic acid (AR, > 99.8%), poly(vinlypyrrolidone) (PVP; \(M_w = 1,300,000\) g mol\(^{-1}\)), potassium ferricyanide (K\(_3\)[Fe(CN)\(_6\)]) (AR, > 99%), phosphate buffer saline (PBS) (AR, pH 7.2) and polytetrafluoroethylene (PTFE) (60 wt% dispersed in water) were procured from Sigma-Aldrich and exploited without any further purification. Nafion 115 membrane (Perfluorinated, 0.005 in. thick) derived from Sigma-Aldrich was pretreated with a procedure described elsewhere [20]. The CC received from Electrosynthesis Co. Inc. Lancaster N.Y. GC-14 was ultrasonically cleaned with ethanol and de-ionized water, and dried at 80 °C.

2.2 Preparation of PVP/TIP solution

16 wt% TIP and 3.87 M acetic acid were subsequently added with 16 wt% PVP solution and magnetically stirred for 30 min.

2.3 Preparation of PVP/TIP/GO solution

GO dispersion (0.5 mg mL\(^{-1}\)) was gradually added with 16 wt% PVP solution and the mixture was stirred until a homogenous suspension was obtained. 16 wt% TIP and 3.87 M acetic acid were subsequently added into the above colloidal solution and blended for 2 h at room temperature.
2.4 Preparation of electrospun nanofibers

The prepared polymeric solution was subjected to electrospinning process under the operating conditions of 0.3 mL h\(^{-1}\) feeding rate, 20 kV applied voltage, and 15 cm working distance between the needle and drum collector. The collected nanofiber mat was initially dried at 100 °C and kept into calcination for 3 h at 700 °C.

2.5 Modification of electrodes

The catalyst slurry was prepared by dispersing the appropriate amount of TiO\(_2\) or TiO\(_2\)/rGO nanofibers in 1 wt% PTFE solution and the resultant slurry was sprayed on CC (1 × 1 cm\(^2\)) using a spray technique and dried at 100 °C. The subsequent spraying and drying processes were repeated until the uniform coating with the catalyst loading of 1 mg cm\(^{-2}\) was achieved.

2.6 Characterizations

The Rigaku X-ray powder diffractometer (XRD) with Cu K\(_\alpha\) radiation (\(\lambda = 1.54178 \text{ Å}\)) was used to perform the XRD studies. The scanning electron microscopy (SEM) images were performed with JSM5410LV scanning electron microscope and the transmission electron microscopy (TEM) images were taken with JEOL-JEM-2010 electron microscope at an accelerating voltage of 200 kV. The Raman studies were performed with JY-HR800 Raman coupled with an Ar ion laser (fixed wavelength of 514.5 nm) and Fourier transform infrared (FT-IR) analyses were examined using Perkin Elmer 580B FT-IR spectrometer with KBr pellet technique.

2.7 Cultivation of Chlorella vulgaris

The freshwater unicellular Chlorella vulgaris derived from University of Malaya Algae Culture Collection (UMACC) was cultivated as per the procedure described elsewhere [21]. 100 mL exponential phase culture at an optical density (OD) of 1.5 at 620 nm (OD620) was grown in BG-11 medium at 25 °C with the irradiation of a white fluorescent lamp (30 µM photons m\(^{-2}\) s\(^{-1}\)). The fuel cell performance of constructed BPVs were measured by applying the different external resistances ranging from 10 MΩ to 1 KΩ and the results were monitored via a data acquisition system (Agilent multimeter, Cleveland, USA) connected with a personal computer. All the experiments were carried out in triplicates to improve the experimental accuracy and reliability.

2.8 Electrochemical characterizations

The electrochemical performances of prepared nanostructures were studied with a CHI-650E analytical system, connecting with a single compartment cell comprising of three-electrode configuration with bare/modified CC (1 × 1 cm\(^2\)) as a working electrode, Ag/AgCl (1 M KCl) as a reference electrode, and a platinum (Pt) wire as a counter electrode. This electrode assembly was positioned in Chlorella vulgaris-inoculated BG-11 medium. The effect of light illumination over the current generation was performed by placing the above-constructed electrochemical cell under the irradiation of 30 µM photons m\(^{-2}\) s\(^{-1}\). For the operation of BPV under dark condition, the system was wrapped with a black cloth and the concerned experiment was achieved in a light-off mode.

2.9 BPV performances

The dual-chamber BPV reactor, comprising of two identical transparent cylindrical glass tubes (\(h = 1.5 \text{ cm}, d = 10 \text{ cm},\) and working volume = 10 mL) separated by a pretreated Nafion 115 membrane was used to evaluate the BPV performance of prepared catalysts. The bare CC/nanostructure-modified CC was positioned in the anodic chamber containing cultivated cell culture (OD = 1.5), while bare CC was introduced into the cathode chamber containing 50 mM K\(_3\)[Fe(CN)\(_6\)]. The BPV experiments were performed in a temperature-controlled environment at 25 ± 2 °C for 12:12 h in light:dark cycles and the light cycle was performed under the white fluorescent lamp irradiation of 30 µM photons m\(^{-2}\) s\(^{-1}\). The fuel cell performance of constructed BPVs were measured by applying the different external resistances ranging from 10 MΩ to 1 KΩ and the results were monitored via a data acquisition system (Agilent multimeter, Cleveland, USA) connected with a personal computer. All the experiments were carried out in triplicates to improve the experimental accuracy and reliability.

3 Results and discussion

3.1 Morphological studies

The TEM images of prepared TiO\(_2\) nanostructures display the 1D uniform, continuous, interconnected, and rough-surfaced nanofibers with a mean diameter of 80 nm. It is also evident that TiO\(_2\) nanostructures consist of randomly interconnected crystalline domains, forming a continuous fibrous morphology and the mean diameter of individual nanoparticles in the nanofibers is found to be 18 nm (Fig. 1a, b). The high-resolution TEM (HR-TEM) image of TiO\(_2\) nanoparticles exhibits the distinct set of lattice fringes with the spacing distance of 0.1 nm, which is indexed to the (101) reflection plane (Fig. 1c). The hydrodization reaction of TIP catalyzed with acetic acid yielded the titanium hydroxide and the subsequent condensation reaction lead to the formation of TiO\(_2\)·\(x\)H\(_2\)O/PVP nanofiber mat. The calcination of aforesaid mat at 700 °C generated TiO\(_2\) nanofibers via the thermal decomposition of a polymer matrix and the crystallization of TiO\(_2\) nanoparticles [22]. The mean diameter
of TiO$_2$/rGO nanofibers is found to be 58 nm (Fig. 1d, e), which is lower than that of bare TiO$_2$ nanofibers. The electrical conductivity of PVP/TIP solution is increased with an inclusion of GO, which stimulates a ceiling expansion of a jet along its axis, yielding the lower mean diameter for the corresponding nanofibers. The HR-TEM image of TiO$_2$/rGO composite (Fig. 1f) with a yellow frame represents the TiO$_2$ nanograin and rGO is observed at the edge of TiO$_2$ nanograin, which ensures the bridging of TiO$_2$ with rGO. The GO sheets with negatively charged oxygen-labile surface functional groups were electrostatically interacted with the positively charged Ti$^{4+}$ ions. Upon the calcination process, the simultaneous reduction and crystallization of GO and hydrolyzed Ti$^{4+}$, respectively, generated the TiO$_2$/rGO composite nanofibers [23, 24]. The interplanar lattice spacing of 0.1 nm, ascribing to the (101) reflection plane of TiO$_2$ is clearly observed in TiO$_2$/rGO, which is similar to the bare TiO$_2$ nanofibers. It enunciates that the inclusion of rGO does not alter the crystalline structure of TiO$_2$ nanoparticles in the prepared composite.

### 3.2 XRD studies

The XRD peaks observed for TiO$_2$ nanofibers at 25.1°, 37.5°, and 47.8°, corresponding, respectively, to the (101), (004), and (200) reflection planes of anatase phase (JCPDS No. 21-1272). The diffraction peaks at 27.1°, 35.8°, 41°, 54.2°, 62.4°, 68.9°, 74.8°, and 82.4° are ascertained, respectively, to the (110), (101), (111), (211), (002), (301), (320), and (321) reflection planes of rutile phase (JCPDS No. 21-1276) (Fig. 2a) [25]. With respect to the acquired diffraction patterns, it is confirmed that the TiO$_2$ nanofibers are composed by a mixture of anatase and rutile phases. The aforesaid diffraction peaks of TiO$_2$ were retained in TiO$_2$/rGO composite nanofibers with weakened intensities (Fig. 2b), enunciating that the anatase and rutile structures of TiO$_2$ are not changed even after the composite configuration with rGO. The characteristic diffraction peak of rGO at 24.5° [26] is not separately seen in TiO$_2$/rGO composite, owing to the shielding of the strong diffraction peak of anatase TiO$_2$ pronounced at 25.1°.
3.3 Raman studies

The Raman spectrum of TiO$_2$ nanofibers reveals the characteristic bands at 148, 411, 511, and 626 cm$^{-1}$, representing, respectively, the E$_{1g}$, B$_{1g}$, A$_{1g}$, and E$_g$ modes (Fig. 3a) [27]. Along with the above-characteristic bands of TiO$_2$, TiO$_2$/rGO pronounces the two additional carbonaceous bands at 1360 and 1596 cm$^{-1}$, which are ascribed, respectively, to the carbonaceous D and G bands of rGO sheets (Fig. 3b and inset of Fig. 3b) [27]. The acquired D band corresponds to the breathing mode of k-point photons of A$_{1g}$ symmetry, while the G band in E$_{2g}$ mode is related to the ordered sp$^2$-bonded carbon atoms in rGO [24].

3.4 Photoluminescence (PL) studies

To identify the electron–hole (e–h$^+$) recombination process of prepared nanofibers, the photoluminescence (PL) spectra were recorded and depicted in Fig. 4. The PL spectra of both the TiO$_2$ (Fig. 4a) and TiO$_2$/rGO (Fig. 4b) nanofibers exhibit a strong peak at 389 nm, articulating with the band gap transition of TiO$_2$ nanostructures. The other peaks centered in the wavelength ranging from 430 to 500 nm are related to the transition of surface oxygen vacancies [28]. However, the intensity of the PL spectrum of TiO$_2$ is considerably quenched by its composite formation with rGO, attributed to the electron shuttling features of rGO [29]. Owing to the formation of d–π electron coupling between the well-matched energy levels of TiO$_2$ and rGO [30] in TiO$_2$/rGO composite, the electrons excited from the valence band to the conduction band of TiO$_2$ is rapidly transferred to rGO. Hence, the rapid migration of electrons from TiO$_2$ to rGO inhibits the recombination rate of e–h$^+$ in TiO$_2$/rGO, which improves the interfacial charge transference of TiO$_2$/rGO composite.

3.5 Electrochemical performance analysis

Cyclic voltammetry (CV) studies of fabricated electrodes were performed under the presence of Chlorella vulgaris inoculated in BG-11 medium at 50 mV s$^{-1}$ (Fig. 5a). An oxidation peak was observed at 0.9 V vs. Ag/AgCl for bare CC, owing to the electro-active proteins located over the cellular surface or electro-active species present in the electron-rich metabolic pool of Chlorella vulgaris [31, 32]. Under illumination conditions, the light energy captured by the chlorophyll antennae of algae has photosynthetically driven the photolysis of water molecules, which generates the electrons, protons, and oxygen. On the other side, the electrons are generated by the metabolism of organic substrates under dark conditions [9, 33]. The light-driven photolysis of water molecules pronounces the high electrochemical responses than that of other metabolism of organic substrates. The modification of CC with TiO$_2$ nanofibers improves the electrochemical performance of CC, which is confirmed from its enhanced oxidation current. The COO$^-$ group of extracellular polysaccharides of algae cell interacts with TiO$_2$/rGO composite via the electrostatic interaction and chemical bonding (bridge coordination) [18]. The interconnected cavities and extended porosity of the individual nanofibers facilitate the better algae colonization and diffusion of growth medium, leading to the better electron transference from microalgal cells to the electrode. The electrochemical performance is further enhanced at TiO$_2$/rGO/CC, revealing the positive influence.
of an active carbon support. It is attributed to the existence of functional groups on the surface of rGO, which allows the better microenvironment for the adhesion and growth of a biofilm. Furthermore, the enhanced interfacial charge transference observed for the TiO$_2$/rGO composite fibers govern a foremost role in determining their high electrochemical performances.

The electrokinetics involved in the electrochemical reactions occurring at TiO$_2$/rGO/CC is identified as a function of scan rate ($\nu$) ranging from 10 to 100 mV s$^{-1}$ (Fig. 5b, c). The anodic peak current ($I_{pa}$) is linearly increased with an increase in $\nu$ along with the positive shift in anodic peak potential. The $I_{pa}$ and $\nu^{1/2}$ exhibit a good linearity with high correlation coefficient ($R$) (inset Fig. 5b, c) under the dark and light conditions, specifying that the electrochemical redox reactions occur at the electrode surface are the diffusion controlled processes.

### 3.6 Chronoamperometry studies

The electrochemical stabilities of prepared nanostructures were investigated by performing the chronoamperometry studies in Chlorella vulgaris-inoculated BG-11 medium at an applied of 0.9 V vs. Ag/AgCl (Fig. 5d). The current generation observed for the studied CCs in the presence and absence of light illumination is attributed, respectively, to the photolysis of water molecules and metabolization of an organic substrate. The photocurrent generation of studied electrodes is higher under the illumination of light, which is due to the progression of light-driven water photolysis reaction. Even under the presence of illumination conditions, bare CC does not show any significant current improvement, owing to its electro-inactive behavior. However, TiO$_2$/CC and TiO$_2$/rGO/CC articulate the continuous, stable, and higher photo-oxidation currents toward algae metabolic.

![Fig. 5](image-url)
reactions. In specific, TiO$_2$/rGO/CC demonstrates the two-fold increased stable current over the TiO$_2$/CC, owing to the substantial support of rGO for the photolysis of water molecules via the robust structural and chemical stabilities of TiO$_2$/rGO composite.

### 3.7 BPV performance studies

The fuel cell performances of CC/modified CCs equipped BPVs are provided in Fig. 6a, b. Bare CC demonstrates the rational power and current densities, respectively, of 8.16 ± 1.2 mW m$^{-2}$ and 104.67 ± 16.8 mA m$^{-2}$, which is comparatively higher than those of previously reported BPVs with ITO [34, 35]. ITO is a flat, smooth, and non-porous surfaced glass electrode and is widely used in BPVs, owing to its features including high conductivity, transparency, and light absorptivity. However, the non-porous architectures of ITO limit the microalgae adhesion/accommodation, and access of nutrients supply for cells, retarding the colonization and healthier biofilm formation over its surface [36]. Furthermore, the accumulation of water molecules over the smooth-surfaced ITO consistently reduces the electrochemically active surface area, which collectively limited the power densities of ITO-equipped BPVs into ~ 6 × 10$^{-5}$ µW cm$^{-2}$ and 6.38 × 10$^{-5}$ W m$^{-2}$, respectively, with Chlorella and Synechococcus elongatus strains [34, 35]. In contrast, the CC with high porosity and void space among the interconnected carbon fibers expedite the better algae colonization, rapid ion diffusion, and biofilm formation in the inner space of electrode, leading to the lower contact resistance and good interfacial properties with improved electron transfer. Furthermore, the high surface roughness (~ 30 nm) and hydrophobic nature of CC supported the removal of water molecules adsorbed over its surface [37, 38] and thus the electrochemically active surface area is preserved from water accumulation constrains. By these ameliorating characteristics, the BPV equipped with CC provokes the maximum fuel cell performance over the ITO. The BPV performance of CC is further improved with the modification of TiO$_2$ nanofibers as envisaged from the obtained maximum power and current densities, respectively, of 18.77 ± 1 mW m$^{-2}$ and 343.59 ± 12.67 mA m$^{-2}$. Under the light illumination, the electrons generated from photo-induced water splitting reactions in microalgae cells are transferred to TiO$_2$, which enables the improved electron transportation at the electrode surface. However, the intrinsically lower electrical conductivity and charge recombination characteristics of TiO$_2$ nanostructures lower the electron transfer efficiency, which collectively limits the BPV performance. The substantial enhancement in electrical conductivity of TiO$_2$ via its composite formation with rGO significantly improves the BPV power and current densities, respectively, to 34.66 ± 1.3 mW m$^{-2}$ and 654.06 ± 13.15 mA m$^{-2}$. From the post-morphological image of TiO$_2$/rGO/CC exploited in BPVs (Fig. 7a), it is clear that the carbon fibers modified with TiO$_2$/rGO nanostructures are completely covered with a biofilm, in which the dense colonies of microalgae are clearly visualized (inset of Fig. 7a). The microalgae cells are easily bound with the TiO$_2$ nanofiber surfaces via the amide, hydroxyl, and carboxylic groups of exopolysaccharide (EPS), amino acids and phosphate groups in phospholipids or nucleic acids (Fig. 7b) [18]. Furthermore, the interaction of TiO$_2$ with microalgae cells generates the oxidative stress, leading to

![Fig. 6 a Polarization and b power density curves of (inverted black triangle) CC-, (filled blue circle) TiO$_2$/CC-, and (filled red square) TiO$_2$/rGO/CC-equipped BPVs under the white fluorescent lamp irradiation of 30 µM photons m$^{-2}$ s$^{-1}$ (error bars represent the standard deviation calculated from the data collected in triplicates)](image-url)
the enhanced lipid production, which serve as the structural components of cell membranes or carbon storage [39, 40]. The efficient interaction between TiO$_2$ and rGO sheets via the formation of Ti–O–C bonds [27] suppresses the charge recombination occurring at TiO$_2$, facilitating the increased electrical conductivity and interfacial charge transfer properties. The oxygen-labile surface functional groups of rGO assist the easier and better attachment of microalgae over the electrode surface and enhance the redox activities, leading to an effective electron transfer from microalgae to the electrode. The 1D and interconnected TiO$_2$/rGO composite nanofibers exhibit a large number of continuous active sites. The existing fiber layers support the formation of a healthier biofilm, whereas the cavities of interconnected fibers serve as the diffusion channels and provide sockets for the cell adhesion and growth. By embracing the substantial features of rGO and TiO$_2$ nanostructures, BPV with TiO$_2$/rGO/CC demonstrates the superior fuel cell performance than those of TiO$_2$/CC and CC.

**3.8 Durability studies**

The electrochemical stabilities of prepared nanostructures in BPVs are evaluated by measuring the cell voltage of studied BPVs as a function of time under the 12:12 h light/dark conditions (Fig. 8). Under the light illumination, the cell voltage of bare CC-equipped BPV is gradually increased and attained the maximum voltage of 0.207 V. The cell voltage is gradually decreased to 0.08 V at dark conditions and the voltage difference between light and dark conditions is found to be 0.127 V. During the second and third cycles, the BPV with CC experiences the drastic decrement in the voltage under both light and dark conditions. By the modification of CC with TiO$_2$, the voltage difference between light and dark conditions is observed to be 0.11 V and is considerably maintained for three cycles. The improved durability performance of BPV with TiO$_2$ is related to the large specific surface area, proficient cell interaction, considerable conduction paths, and prominent morphological features of TiO$_2$ nanostructures. However, the limited electrical conductivity and degradation of surface area upon the repetitive cycles due to its direct exposure in an aqueous environment reduces the voltage difference at fourth, fifth and sixth cycles. On the other side, the voltage of BPV with TiO$_2$/rGO is substantially increased and reached a maximum value of 0.305 V.
under light conditions and dropped slowly to 0.251 V at dark conditions. The voltage difference between light and dark conditions is observed to be 0.055 V and the obtained voltage difference is considerably maintained for six cycles. Under light conditions, the BPV with TiO₂/rGO retained its voltage generation up to 90.16% at sixth cycle, which is comparatively higher than those of TiO₂ (57.5%) (at sixth cycle) and bare CC (38.64%) (at third cycle) equipped BPVs. The existence of rGO in TiO₂/rGO composite prevents the direct exposure of TiO₂ nanoparticles into an aqueous environment, which avoided the agglomeration of individual TiO₂ nanoparticles in nanofibers. The strong interconnection among the nanofibers constructs the elevated mechanical characteristics to the fabricated electrode, and the electrostatic interaction between TiO₂/rGO and microalgae leads to the stable colonization at the nanofiber pores and interconnected cavities. The constructive properties including excellent electrical conductivity, and elevated physio- and electrochemical stabilities of TiO₂/rGO collectively promoted the durability performance of corresponding BPV over the CC and TiO₂/CC-equipped BPVs.

The obtained fuel cell power generation and durability performances of constructed BPV with TiO₂/rGO are suitably compared with the previously reported BPVs (Table 1) [8–14, 32, 34, 35, 42]. The green energy generation of various photosynthetic strains including NOS [12], Synechocystis PCC-6803 [14], and Paulschulzia pseudovolvox [32] in BPVs requires the utilization of artificial redox mediators including 1,4-benzoquinone, 2-hydroxy-1,4-naphthoquinone, and K₃[Fe(CN)₆] for the extracellular electron transference (EET). The utilization of external mediators in BPVs exhibit certain constrains including high cost, toxicity, and sequential replenishments of mediators, which not only limit their high power output but also their durability [41]. To overcome these critical issues, restrict the mediator-less BPV with self-mediated Chlorella vulgaris was developed, exhibiting the high power density over the self-mediated Synechococcus elongatus [35], and Spirulina platensis strains [42]. The increased power performance of BPV with Chlorella vulgaris is ascribed to the high specific growth rate (µ = 0.42 d⁻¹), high lipid production, and superior relative electron transport rate (rETR) (430.730 ± 32.74 µ mol electrons m⁻² s⁻¹) [43]. Furthermore, the considerable non-photochemical quenching (NPQ) effect and the strong photoprotection of Chlorella vulgaris support the removal of excess light energy in the form of heat [35], facilitating the better lifespan performance of BPV, leading to the competent use of Chlorella vulgaris in BPVs.

The BPVs developed with Chlorella vulgaris [10], Spirulina platensis [42], and Synechococcus elongatus [35], demonstrate the peak power densities, respectively, of 14 µW m⁻², 6.5 mW m⁻², and 6.38 × 10⁻⁵ W m⁻². However, the

<table>
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<tr>
<th>Table 1</th>
<th>Comparative profile of the BPV performances</th>
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<tr>
<td>Anode</td>
<td>Cathode</td>
</tr>
<tr>
<td>PET⁺/ITO⁺</td>
<td>Pt⁺-coated gold</td>
</tr>
<tr>
<td>Au⁴-coated Nafion</td>
<td>Au⁴-coated Nafion</td>
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<td>Stainless steel mesh</td>
<td>Stainless steel mesh</td>
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<tr>
<td>rGO⁴/ITO⁺</td>
<td>Pt⁺-coated glass</td>
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<tr>
<td>CNT⁴/CP⁴</td>
<td>Laccase/CNT⁴/CP⁴</td>
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<tr>
<td>PPP⁺/CC⁺</td>
<td>Pt-coated CC</td>
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<tr>
<td>Os⁴/Graphite</td>
<td>–</td>
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<tr>
<td>TiO₂⁴/rGO⁴/CC⁺</td>
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*a Polyethylene terephthalate  
*b Indium tin oxide  
*c Platinum  
*d Gold  
*e Reduced graphene oxide  
*f Carbon nanotubes  
*g Carbon paper  
*h Polypyrrole  
*i Carbon cloth  
*j Osmium polymer  
*k Titania
inferior electrical conductivity, limited microalgae accommodation, insufficient nutrient diffusion, and poor biofilm surface coverage experienced in the bare electrodes (stainless steel mesh, ITO, and Pt) lower the electrode–electrolyte interfacial properties, which limited their BPV performances. Although the electrode materials are modified with polyethylene terephthalate (PET) [8], rGO [11], carbon nanotubes (CNTs) [12], and PPy [14] to promote the electron transfer, their constraints including the restacking of rGO sheets, cellular toxicity of CNTs, swelling, and inferior physio-chemical stabilities prohibit their high BPV performances. The aforesaid obstacles are resolved with the ameliorating characteristics of TiO2/rGO composite nanofibers. Owing to the well-matched energy levels of TiO2 and rGO, the Ti–O–C bond exerted interfacial charge transference and electrical conductivity. Owing to the well-matched energy levels of TiO2 and rGO, the substantial characteristics of rGO and fibers serve as the diffusion channels and provide sockets of a healthier biofilm, whereas the cavities of interconnected microalgae supports the formation of a healthier biofilm, whereas the cavities of interconnected fibers serve as the diffusion channels and provide sockets for the cell adhesion and growth. Along with aforesaid significant features, the substantial characteristics of rGO and TiO2 including chemical inertness, environmental friendliness, and high tolerance against the photo and chemical corrosion, BPV with TiO2/rGO/CC demonstrates the superior fuel cell performances.

Although the BPV fabricated with CNTs/carbon paper (CP) as the anode and laccase-modified CNTs/CP as the cathode demonstrated the comparable power density (35 mW m−2) [12] with the present report, the high cost of CNTs, laccase enzyme, and molecular tethering agent increases the fabrication expenditure of aforesaid BPV, creating a barrier for their scale-up process. The experimental strategies involved in the immobilization of Nostoc sp. (NOS) and laccase comprise multistep protocols with the utilization of various reagents, which are complex and time consuming. The cyclic voltammogram of NOS/CNTs/CP in PBS has not exhibited any redox peaks, revealing the absence of redox proteins over the NOS surface, which uncertain the mechanistic pathways for the electron transportation from NOS to the current collector. Owing to the cytotoxic and genotoxic effects, CNTs inhibit the growth rate and lifespan of NOS, which might be the reason for the absence of durability analysis in the NOS-equipped BPV [45]. Furthermore, the laccase enzyme used in the ORR process exhibits certain constrains including denaturation, surface fouling, and leaching, which leads to the insufficient stability of corresponding electrode, fading their authentic application in long-term green energy generation [46]. The power efficiencies obtained for the aforementioned research was derived by the combinative effects of both the modified electrodes [CNT/CP (NOS—anolyte) (a|c) laccase/CNT/CP (O2—electron acceptor)]. In contrast, the BPV reported in this research effort accomplished the maximum power generation only on the basis of the modified anode [TiO2/rGO/CC (Chlorella vulgaris—anolyte) (a|c) CC (K3[Fe(CN)6]—electron acceptor)]. In general, O2 and K3[Fe(CN)6] are widely used as electron acceptors in dual-chamber biofuel cells. Despite of the lower standard redox potential of K3[Fe(CN)6] compared to O2, it exhibits a lower overpotential with a plain carbon cathode, facilitating a faster reaction rate and bringing a working potential of cathode near its open circuit potential [47]. On the other side, the usage of O2 as an electron acceptor compels its replenishment, degrading the durability of a relevant system, while the BPV with K3[Fe(CN)6] does not require any replenishment. The O2 crossover from cathode to anode leads to the detrimental effect on oxygen-sensitive microalgae and the sluggish reaction kinetics experienced from the poor diffusion and solubility of O2 limited its utilization in BPVs [48]. Furthermore, the utilization of K3[Fe(CN)6] does not require any external catalysts to trigger the reduction reaction, reducing the fabrication cost of BPV. It is reported that the power output of carbon electrode with K3[Fe(CN)6] is 50–80% higher than that of Pt-modified carbon electrode using O2 as an electron acceptor due to the improved mass transference and large cathode potential [49]. Owing to the cost efficiency, high solubility, and production of environmentally benign reduction products, and their negligible influence on the subsequent green energy generation, K3[Fe(CN)6] is used as a favorable electron acceptor in this report. Thus the TiO2/rGO composite nanofibers reported in this study demonstrates the superior BPV and durability performances compared to the previous BPV efforts reported to date (Table 1) [8–14, 32, 34, 35, 42] and provides the simple, and time and cost-efficient strategies for the green energy generation.

4 Conclusions

The direct extraction of electrons from algae cell-catalyzed water photolysis reaction was established with the 1D nanofibers prepared from electrospinning technique. The post-morphological studies demonstrated that the modification of anode with the porous and well-interconnected architecture of TiO2/rGO nanofibers is an elixir to improve the electron transference via the formation of a highly dense and healthier microalgae biofilm. Furthermore, the preservation of the surface degradation of TiO2 nanofibers
in an aqueous environment upon the repetitive cycles was effectively tackled with rGO, facilitating the BPV durability performance for 144 h. Thus, the 1D TiO₂/rGO composite nanofibers presented in this report progressively reduces the energy-harvesting hurdles and provide tremendous impetus for the development of simple, cost-efficient, and sustainable green energy generation systems.

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