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Extraction of photosynthetic electron from mixed photosynthetic consortium of bacteria and algae towards sustainable bioelectrical energy harvesting

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ABSTRACT

Extraction of photosynthetic electron for bioelectrical energy harvesting is an efficient approach to utilize solar energy harvested by photosynthetic microorganisms. To explore this approach in natural and artificial photosynthetic system in which alga and photosynthetic bacteria are usually coexisted, the intracellular electron extraction from mixed photosynthetic consortium of *Chlorella vulgaris* and *Rhodopseudomonas palustris* was investigated under three-electrode mode by holding working electrode at different potentials. The mixed-culture biofilm grown at 0 V exhibited a maximum Coulomb efficiency of 42.12% while the peak current (12.2 mA) was 8.07, 1.5, 2.97 and 4.65 fold higher than that produced at −0.4 (1.5 mA), −0.2 (8.06 mA), 0.2 (4.08 mA) and 0.4 V (2.6 mA), respectively. The electrode potential can regulate the dominant species within the biofilm. Large enrichment of *Rhodopseudomonas palustris* in the biofilm was responsible for the high photosynthetic electron extraction efficiency at 0 V since the photosynthetic electrons extracted by the electrode were mainly derived from photoheterotrophic metabolism of *Rhodopseudomonas palustris*. Extraction of equivalent amounts of intracellular electron from *Chlorella vulgaris* required higher potential than that from *Rhodopseudomonas palustris* and was highly dependent on the presence of exogenous electron mediator. As an electron sacrificer, photosynthetic oxygen released by *Chlorella vulgaris* could complete electron with electrode. Intracellular electrons can also be extracted from dark respiration, but the peak current (6.4 mA) was 47.54% lower than that produced under illumination (12.2 mA) due to low exoelectrogenic activity of biofilm.

1. Introduction

In view of current and foreseeable energy shortage and environmental degradation, there is an urgent need to explore renewable energy sources and decrease the dependence on fossil fuel [1–5]. As solar energy collectors, photosynthetic microorganisms are widely present in natural environment and can be used to produce substantial valuable biochemicals [6–8]. However, extraction of these biochemicals is costly, thereby making it difficult to be competitive with fossil hydrocarbons [9]. Hence, it is urgent to seek a convenient and effective approach to use the solar energy harvested by photosynthetic microorganisms.

Bioelectrochemical system (BES) is a promising technology, which has been applied to obtain electrical power and other value-added chemicals based on microbial catalytic oxidation of organic compounds [10,11]. As a terminal electron acceptor and habitat for microorganisms, anode plays a key role in improvement of BES performance. In the light of thermodynamics, microorganisms can harvest more energy from a terminal electron acceptor with more positive redox potential [12]. Therefore, the anode potential within a BES theoretically determines energy harvesting for microorganism [13] and then further determines current output of the BES [14]. Nevertheless, the gained energy of microorganism is decided

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by kinetics rather than thermodynamics [12]. Recent study has showed that one more positive potential would result in higher and faster current production using *Shewanella oneidenic* MR-1 as exoelectrogens in BES due to the increased expression of extracellular electron transfer (EET) proteins [15]. Similarly, electricity generation from *Geobacter Sulfurreducens* in BES is benefited from more positive potential when set anode potential range from −0.46 V to 0.6 V (vs. Ag/AgCl) [16]. However, there is a completely opposite conclusion derived by using mixed bacteria inoculation which showed that lower potential would lead to higher current production, faster boot time as well as thicker biofilm although the biofilm was dominated by *Geobacter Sulfurreducens* [17]. One possible reason may be attributed to the fact that non-dominant exoelectrogens could colonize the electrode first at higher potentials in the mixed bacterial cultures, disturbing to the capability of dominant exoelectrogens to conduct direct contact with electrode [17]. Zhu et al. [18] held that the decline in electricity generation performance could be caused by the loss of effective electron transfer pathway, substitution of biofilm community or production of damaging substance under higher potentials with a mixed culture. Thus, optimal applied potential cannot be forecasted in a mixed culture system due to complicated interaction among multiple microorganisms [12].

Chlorella vulgaris (*C. vulgaris*) and *Rhodopseudomonas palustris* (*R. palustris*) are typical representatives of photosynthetic algae and photosynthetic bacteria in the natural environment. Both of them dominantly coexist in the sewage lagoons from industrialised poultry and swine farms [19]. A wide variety of organic substrates can be utilized for electricity production by *R. palustris*, which have also been demonstrated a higher power output than other kinds of exoelectrogens under the same conditions [20]. *R. palustris* has strong reductive cytochrome protein complex mainly comprised by bacteriochlorophylls, carotenoid and apoprotein which can be stimulated by photons to promote electron transport from intracellular to electrode in photo-BES [21]. Besides, *R. palustris* can also conduct EET by endogenous respiration and using hydrogen as mediator produced from photosynthesis at the interface between biofilm and electrode [21]. *C. vulgaris* is the fast growing photosynthetic microalga with powerful tolerance to extreme environment and has been increasingly incorporated into BES for electricity generation by means of organic carbon feeding to exoelectrogens in the anode [22,23] and/or in-situ photosynthetic oxygen supply for the cathode [24,25]. More recently, *C. vulgaris* was demonstrated to possess exoelectrogenic activity for electricity generation in the anode of the BES without the aid of artificial electron-shuttling mediator [26,27]. The most intensive researches to date have focused on electricity generation using non-photosynthetic bacteria [28,29]. Optimizing the harvesting of electricity from photosynthetic microorganisms, especially for mixed photosynthetic microalga and photosynthetic bacteria by regulation of electrode potential, is still not fully explored, which seriously hinder our cognition for synergistic interaction between photosynthetic microorganisms and electrode in BES for sustainable bioelectrical energy harvesting.

In the present study, we aim to investigate the efficiency and mechanisms of intracellular electron extraction from co-culture of *R. palustris* and *C. vulgaris* by using working electrodes as extracellular electron acceptors in a three-electrode photo-bio-electrochemical system (photo-BES). The current, Coulomb efficiency (CE) and substrate degradation were monitored to investigate the influence of electrode potentials and illumination on photosynthetic electron extraction. CV and EIS were performed to characterize the mechanisms of EET and electrochemical reaction at the interface between biofilm and the working electrode. The structure and morphology of biofilm were analyzed by SEM and CLSM to examine the effects of electrode potentials and illumination on biofilm formation and viability.

2. Materials and methods

2.1. Photosynthetic microorganisms and culture condition

The pure cultures of *R. palustris* was isolated from an aquaculture fishpond while *C. vulgaris* was acquired from the Institute of Hydrobiology, Chinese Academy of Sciences. Both of them were separately precultured in a nutrient medium containing (per liter): 1 g CH_3COONa, 7.76 g $K_2HPO_4 \cdot 3\ H_2O$, 2.53 g KH_2PO_4, 0.006 g Ferric citrate, 0.006 g Citric acid, 0.001 g EDTA-Na_2, 0.31 g NH_4Cl, 0.13 g KCl, 12.5 ml of mineral solution and 12.5 ml of vitamin solution. The formulation of mineral solution and vitamin solution was based on a previous study [30].

2.2. Photo-BES set-up and operation

Three-electrode photo-BES was constructed with one 256 ml (8 × 4 × 8 cm) plane plexiglas cubical device and six glass bottles (empty bed volume of 144 ml) devices (Fig. 1). The former equipped with a working electrode (3 × 3 × 0.5 cm) was used to measure the electrochemical performance while the latter equipped with a working electrode (2 × 2 × 0.5 cm) was used to characterize the photosynthetic biofilm. Carbon felt and titanium wire were used as working and counter electrodes, respectively. Prior to use, carbon felt were ultrasonically immersed in acetone for 10 min and hydrochloric acid solution (5 mol/L) for 1 min, then soaked in deionized water [31]. The working and counter electrodes were maintaining parallel with approximately 1 cm distance in the

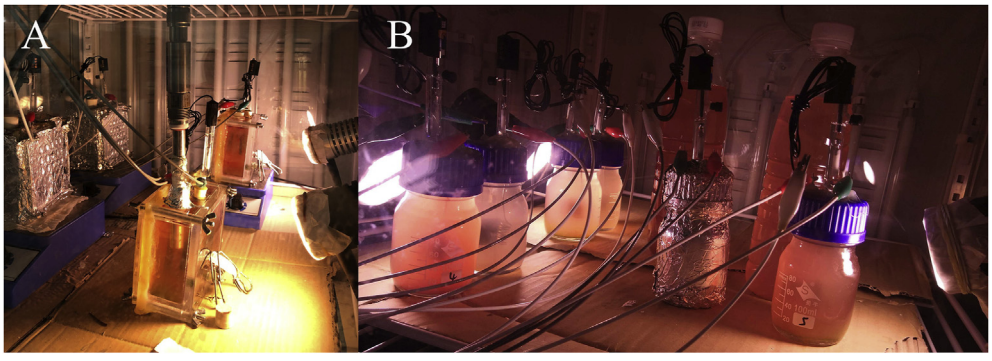

Fig. 1. Pictures of the photo-BES used in this study: (A) Plexiglas devices; (B) glass bottles devices.

middle of devices. Saturated calomel electrode (SCE) was used as reference electrode and inserted between the working and counter electrodes. All holes of these devices were sealed with rubber stoppers except outfalls.

25 ml of *R. palustris* and *C. vulgaris* during their logarithmic growth phase were added to the photo-BES as inoculums. The medium used in the photo-BES was same to the preculture medium for the two photosynthetic microorganisms mentioned above. In these photo-BES, the working electrodes were successively poised at −0.4, −0.2, 0, 0.2 and 0.4 V by using a multi-channel potentiostat (CHI 1000C, Chenhua, China) under batch mode. The whole experiment process was sustained in a constant-temperature incubator (27 ± 1 °C) and illuminated by a cool white fluorescent (3000 lux) unless otherwise stated.

2.3. Electrochemical measurements

The current generated at a selected poised potential was recorded using a potentiostat mentioned above. CE was calculated as previously described [32]. CV was performed under turnover conditions (i.e. at the maximum current) and non-turnover conditions (i.e. after depletion of sodium acetate), respectively. CV was scanned from −0.6 to 0.5 V then back to −0.6 V with scan rates of 5 mV s^{-1}. EIS was performed under non-turnover condition, which was conducted from 0.1 MHz to 5 mHz with a perturbation amplitude of 5 mV. Furthermore, ZSimpwin 3.10 software (Echem, US) was used to fit EIS data with the equivalent circuit of R(Q(RW)) to obtain the value of each circuit element.

2.4. Degradation of sodium acetate

In order to investigate the consumption of sodium acetate and calculation of CE, the concentration of sodium acetate was measured from the beginning to the end of a complete cycle every 4 h. Samples derived from devices were filtered through 0.22 μm microporus filters and then analyzed using high performance liquid chromatography (HPLC, LC-16, Jiangsu, China).

2.5. Microscopy analyses of photosynthetic electrodes biofilm

SEM (JSM-6330 F, JEOL, Japan) and CLSM (TCS SP8, Leica, Germany) were separately used to analyze the plane and three dimensional distribution of photosynthetic biofilm adhering to the working electrodes surface. Before performing SEM examination, a series of preprocessing steps were applied, including fixation, dehydration, drying, and gold spraying. The LIVE/DEAD BacLight Bacterial Viability Kit (L7012) (Molecular Probes, Invitrogen, Carlsbad, CA, USA) was used to stain photosynthetic biofilm on the basis of the manufacture's manual before CLSM analysis.

3. Results and discussion

3.1. Intracellular electron extraction under different electrode potentials

In order to investigate the performance of intracellular electron extraction from the co-culture of *R. palustris* and *C. vulgaris* by the electrode, current and CE were measured for probing the effects of electrode potentials, carbon source and illumination. As shown in Fig. 2A, the current increased after replacing the fresh medium and declined with the gradual depletion of substrate, which was accorded with typical fed-batch system. The current at −0.2 (8.06 mA) and 0 V (12.2 mA) were significantly higher than those at −0.4 (1.5 mA), 0.2 (4.08 mA) and 0.4 V (2.6 mA). Obvious increase of maximum current at 0 and -0.2 V was likely ascribed to high electron transfer efficiency [16] or the self-regulation of EET

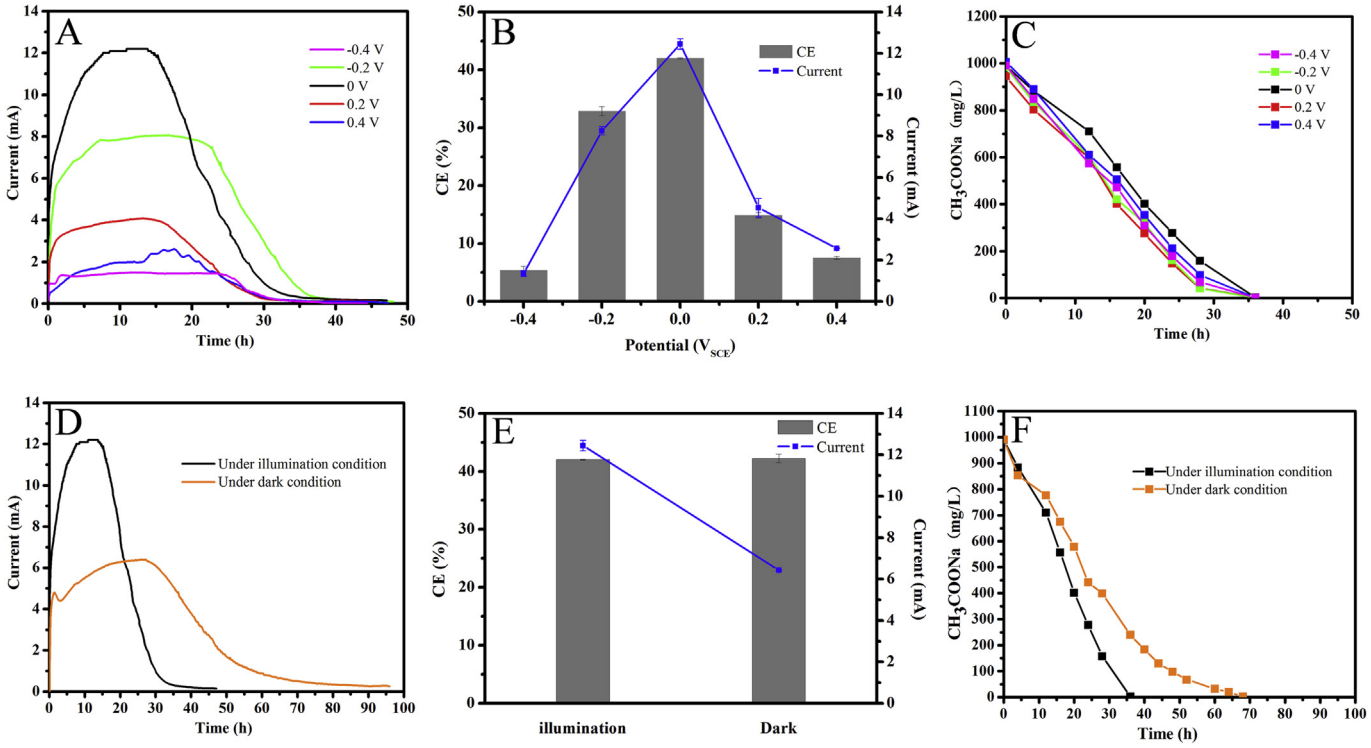

Fig. 2. A–C: The effects of electrode potential on current production (A), CE compared with the peak current (B) and acctate consumption (C) under illumination; D–F: The effects of illumination condition on current production (D) CE compared with the peak current (E) and acetate consumption (F) at a poised potential of 0 V.

pathways [18]. CE was positively correlated with the change of maximum current production at different applied potentials (Fig. 2B), and the maximum value of CE run up to 42.12% at 0 V, which was similar to the research of TerAvest et al. [33] who also illustrated the optimal EET appeared at moderate electrode potentials. A huge difference in CE at different applied potential may be ascribed to the shift of energy from current generation to organism growth [34]. According to Fig. 2C, acetate was linearly degraded and completely exhausted after 28–36 h under all applied potentials, indicating that there were few effects for consumption of acetate under different poised potentials. However, the amount of intracellular electron extraction was significantly different at different potentials, which further verified the competitiveness for the utilization of acetate between current production and organism growth. As shown in Fig. 2D–F, double higher current and faster degradation rate of acetate were exhibited in illumination than in dark, however, the CE of both were similar at the end of the respective cycle. The phenomena can be explained that higher current production and faster degradation rate under illumination may be ascribed to synergistic effect of photosynthetic and respiratory electron transfer from organism to electrode [21,35]. Xiang Qi et al. [21] also proved that the cytochrome protein complex from the reaction center of anoxygenic phototrophic bacteria (APB) can be stimulated by photons and further promote EET from APB to electrode. Therefore, photosynthesis process can boost degradation of acetate faster and then stimulate the generation of photosynthetic electron more quickly under the same applied potential. Moreover, the similar CE of both illustrated that depletion of acetate required lower time along with identical intracellular electron extraction during illumination compared with in dark. If applied to the removal of organic pollutants, the degradation efficiency can be rapidly improved by illumination.

In order to illustrate the contribution of each of the photosynthetic microorganisms to current generation, the current generated by the pure culture of *R. palustris* and *C. vulgaris* was recorded at 0.2 V, and under illumination and dark conditions (Fig. 3A). It was found that contribution of *C. vulgaris* for positive current generation was insignificant at 0.2 V and absent at 0 V as compared to that of co-culture of *R. palustris* and *C. vulgaris* regardless of illumination or dark condition. Thus, it can be concluded that *R. palustris* played a predominant role in intracellular electron extraction in the binary co-culture system. Moreover, the pure culture of *R. palustris* produced higher current than co-culture of *R. palustris* and *C. vulgaris*. There are two possible reasons for the observed result. The first one is that the amounts of *R. palustris* on the surface of the electrode were higher than in the pure culture of *R. palustris* than that in co-

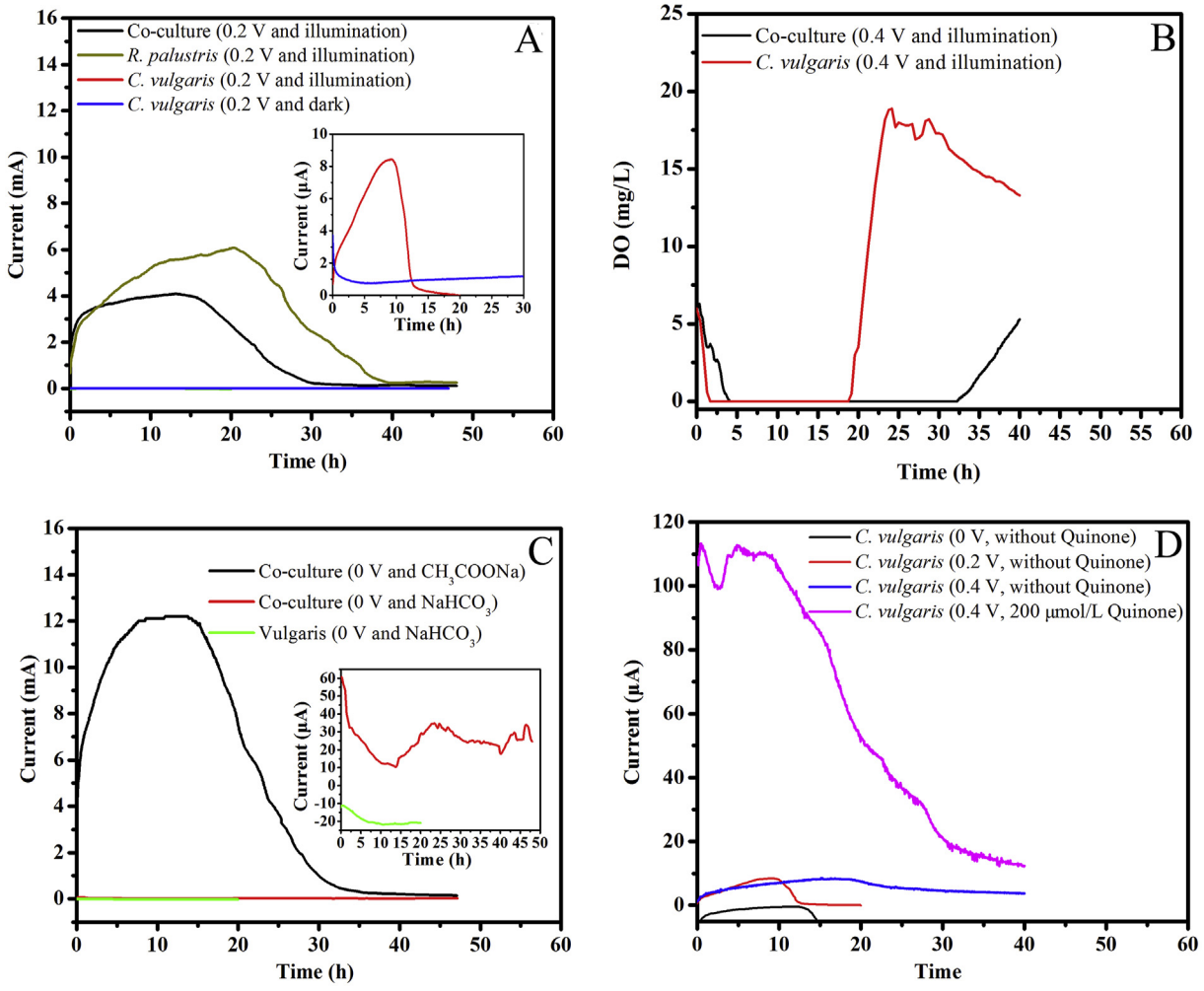

Fig. 3. (A) Representative current-time cycles of pure culture of *C. vulgaris*, *R. palustris* and co-culture of *C. vulgaris* and *R. palustris* at 0.2 V and illumination regimes; (B) Variation of photosynthetic oxygen concentration with time in the pure culture of *C. vulgaris* and co-culture of *C. vulgaris* and *R. palustris* at a poised potential of 0.4 V; (C) Representative current-time cycle of the co-culture of *C. vulgaris* and *R. palustris* using sodium bicarbonate and sodium acetate as substrate at a poised potential of 0 V and under illumination condition, the larger images of current-time cycles were shown in insets of (A) and (C); (D) Current-time cycles of pure *C. vulgaris* in the presence and absence of quinine.

culture of *R. palustris* and *C. vulgaris* and *R. palustris* mainly contributed to the current production. The other one could be resulted from the electron consumption by the photosynthetic oxygen released by *C. vulgaris* since the oxygen is a strong electron sacrificer which could compete with the electrode for the electron. Fig. 3B clearly evidented the increase in concentration of dissolved oxygen due to the photosynthesis of *C. vulgaris*. However, the sharp increase in dissolved oxygen was observed after 20 h which could be due to the depletion of electron donor (Fig. 2C). In addition, the current generated by using sodium bicarbonate (2 g/L) as sole carbon source (Fig. 3C) was remarkably lower than that using sodium acetate, indicating low efficiency of photosynthetic electron extraction using inorganic carbon source. *R. palustris* is unique among characterized non-sulfur purple bacteria because of its capacity for anaerobic photoheterotrophic growth using organic carbon source and large amounts of electrons can be produced during photoheterotrophic metabolism. Considering of the extremely low intracellular electron extraction efficiency from *C. vulgaris*, exogenous electron mediator (quinone) was added to the pure culture of *C. vulgaris* in the photo-BES. As shown in Fig. 3D, addition of quinine resulted in more than 10-fold increases in current, indicating that the intracellular electron extraction from *C. vulgaris* was highly dependent on the presence of exogenous electron mediator. However, the current intensity was still significantly lower than that produced by *R. palustris*. Enhanced intracellular electron extraction from *C. vulgaris* can be attributed to increases in the proton conductivity and electron leak with the aid of exogenous electron mediator [7]. There was no any exogenous mediator addition in present system which was in line with the conclusion of the lack of intracellular electron extraction capacity of *C. vulgaris* described above.

3.2. Electrochemical characterization of the photosynthetic biofilm grown at different electrode potentials

In order to explain the difference of the current profiles and clarify extracellular electron transfer mechanisms in the photo-BES at different applied potentials, CV and EIS were performed. As shown in Fig. 4A1, the currents at applied potentials of −0.2 and 0 V were evidently higher than that at other applied potentials, which was consistent with the law of current-time profiles at different applied potentials. Although high electrode potential can provide larger driving force for photosynthetic electron extraction, however, the EET protein could be damaged by the oxidating condition on the electrode surface at high applied potential [36] which could partly contribute to the low electrocatalytic response of the photosynthetic biofilm grown at +0.2 and + 0.4 V. Moreover, the applied electrode potential can regulate the EET mode of electroactive microorganism and more negative potential appears to induce the electroactive microorganism to use electron mediator

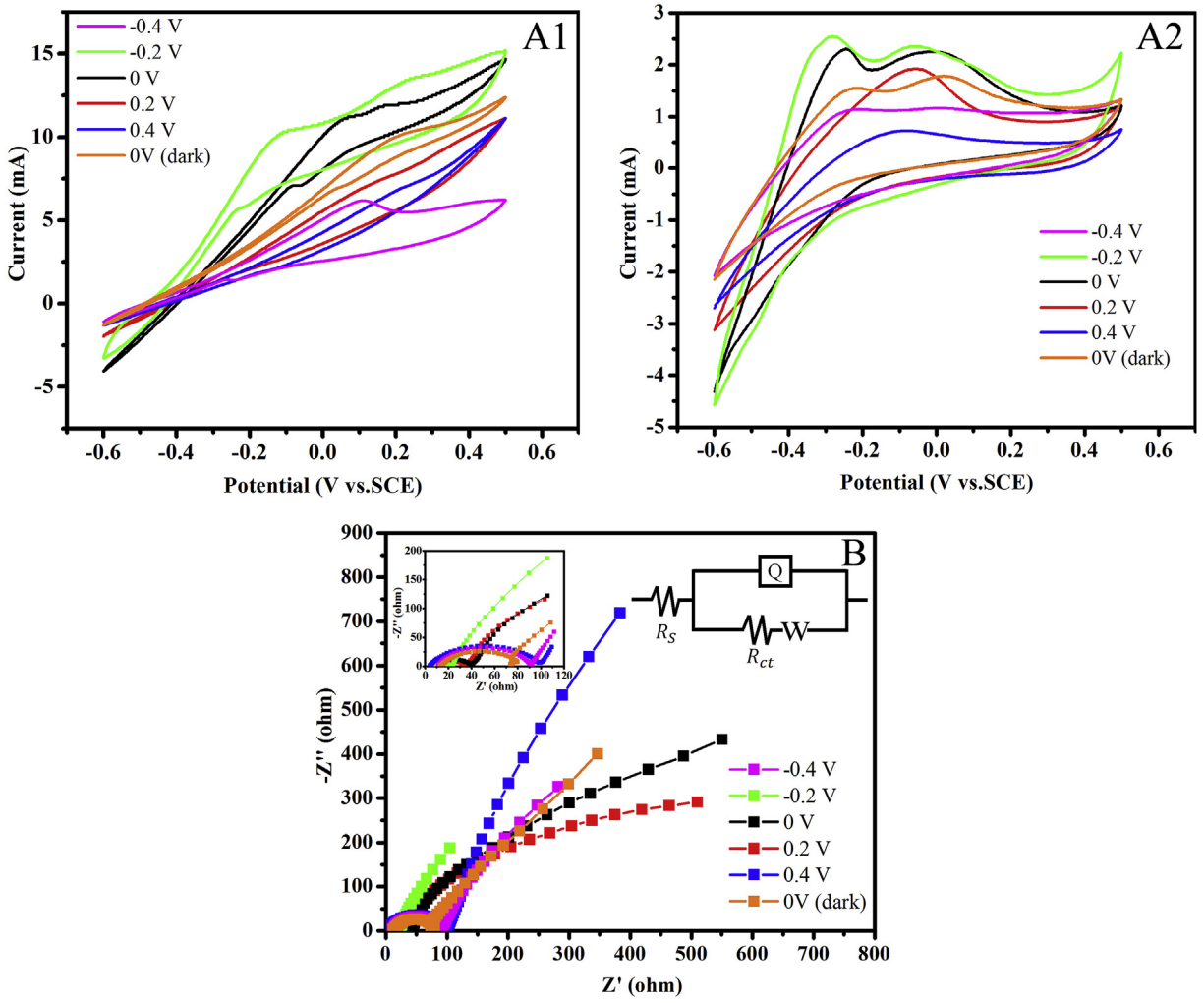

Fig. 4. Representative cyclic voltammograms (A1: under turnover condition; A2: under non-turnover condition) and Nyquist plots (B) of co-culture of *C. vulgaris* and *R. palustris* at different electrode potentials.

for EET [37] which could be responsible for the high electrocatalytic response of the photosynthetic biofilm at −0.2 and 0 V. To investigate the EET mechanisms at the interface between the photosynthetic biofilm and the electrode in detail, non-turnover CVs were recorded (Fig. 4A2). It can be clearly seen that an oxidation peak located at −0.049 ± 0.032 V was present at all applied potentials. This peak was similar to the reports of Krishnaveni Venkidusamy et al. [38] and Xing et al. [20], which confirmed the existence of a direct EET mediator within the biofilm. The oxidation peak at −0.245 ± 0.032 V was only found at poised potentials of −0.4, −0.2 and 0 V, which might be resulted from the secretion of soluble electron shuttle by photosynthetic microorganisms [39]. It was reported that *R. palustris* cell grown anaerobically in the light can excrete large amounts of redox-active porphyrins which has been demonstrated to mediate the extracellular electron transfer from microbes to solid electrode [40,41]. Thus, the higher current produced at poised potentials of −0.2 and 0 V could be explained by the enhanced extracellular electron transfer mediated by the self-secreted soluble electron shuttle. The low electron extraction efficiency at −0.4 V could be ascribed to the absence of the driving force for sodium acetate oxidation [39]. The absence of this peak at high potential conditions might be resulted from the inactivation of redox mediators (e g. cytochrome c) [36] or the choice of organisms itself for redox mediators with lower activation energy [42]. It is well recognized that the c-type cytochromes, such as MtrC and OmcA, play an important role in promoting EET from microorganisms to electrode [38,43,44]. The oxidation peak located at −0.245 ± 0.032 V was close to those of MtrC and OmcA reported by other literature [45,46]. Additionally, Krishnaveni Venkidusamy et al. [38] also demonstrated the role of OmcA in photosynthetic current generation from *R. palustris* strain RP2. This peak was also found under dark conditions, but the peak height was obviously lower than that under illumination, indicating that the illumination was more beneficial to promote the generation of this redox mediator. In addition, CV was also measured under non-turnover condition using sodium bicarbonate as a sole carbon source (Fig. S1A). No obvious redox peak was observed, indicating low electrochemical activity using inorganic carbon source, which was consistent with the faint current generation under such condition.

EIS is regarded as a simple and effective method for studying the EET process between exoelectrogens and electrode [47]. Fig. 4B showed a Nyquist plot of biofilm electrodes with different poised potentials under non-turnover conditions. The equivalent circuit simulated by our Nyquist plot consisted of three resistances, i.e. the solution resistance (R_s), charge transfer resistance (R_{ct}) and Warburg's diffusion element (WDE), shown as an inset in Fig. 4B. The values of the circuit elements were displayed in Table 1S. It was clearly that the R_s and WDE were small under all conditions, while the R_{ct} was highly dependent upon the poised potentials of the working electrodes. The least R_{ct} (11.4 Ω) was found at −0.2 V, which decreased by 58.23%, 78.37%, 85.55% and 86.33% compared to 0, 0.2, 0.4 and −0.4 V, respectively. Thus, the best EET performance between the photosynthetic biofilm and the electrode was found at −0.2 V. However, the highest current produced at 0 V could be explained as a larger driving force for sodium acetate oxidation at 0 V than at −0.2 V. Further, the R_{ct} was nearly twice higher in dark than that under illumination, which could be caused by the loss of photosynthetic EET through the promotion of photon under illumination [48]. This result was consistent with the analysis of CV which indicated the appearance of higher concentration of redox mediators under illumination. When sodium bicarbonate was used as an inorganic carbon source under a poised potential of 0 V, a higher R_{ct} (100.3 Ω) was found compared with the aforementioned conditions (Fig. S1B). Thus, it further coupled the statement of the faint current generation and the absence of redox peak when using sodium bicarbonate as the sole carbon source.

3.3. Microscopic characteristics of the photosynthetic biofilm

The structure and morphology of the biofilm grown at different applied potentials under illumination and at 0 V under dark conditions were characterized by SEM. As shown in Fig. 5, globular and

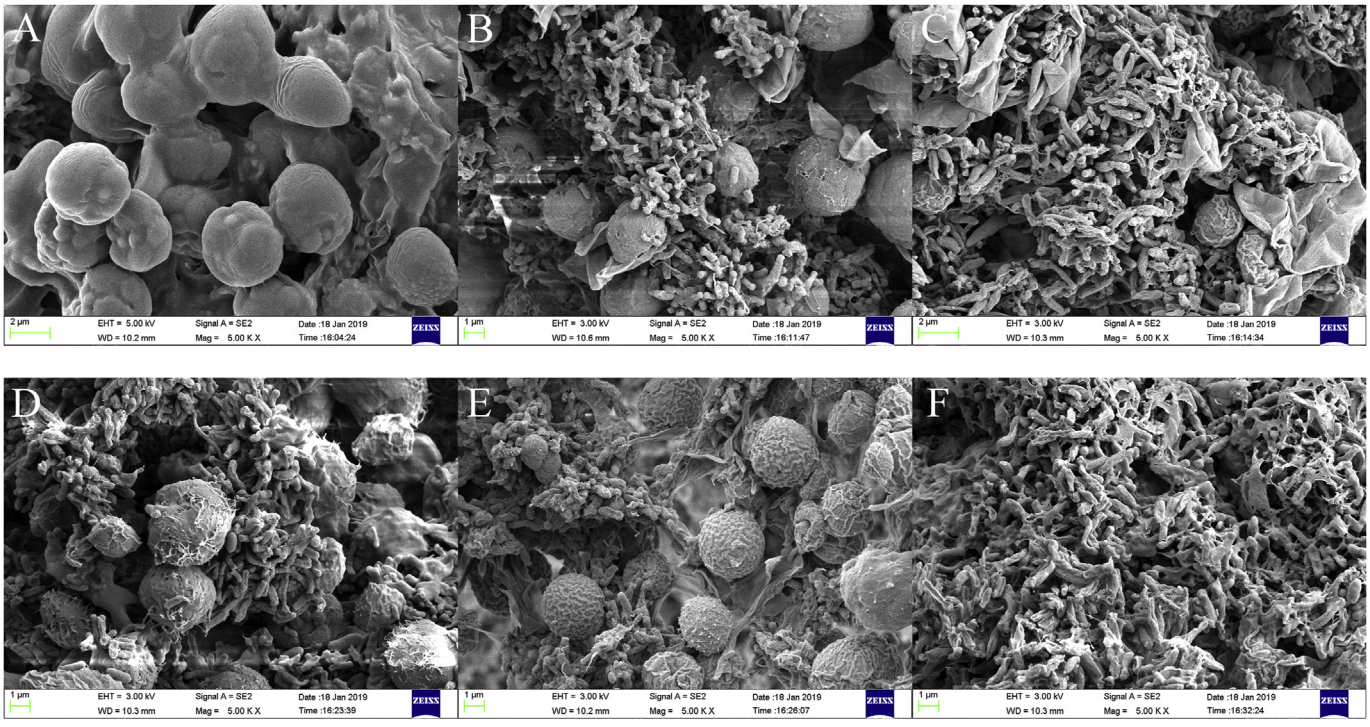

Fig. 5. SEM micrographs of biofilm grown at different applied potentials (A: −0.4 V, B: −0.2 V, C: 0 V, D: 0.2 V, E: 0.4 V) during under illumination, and at 0 V in dark.

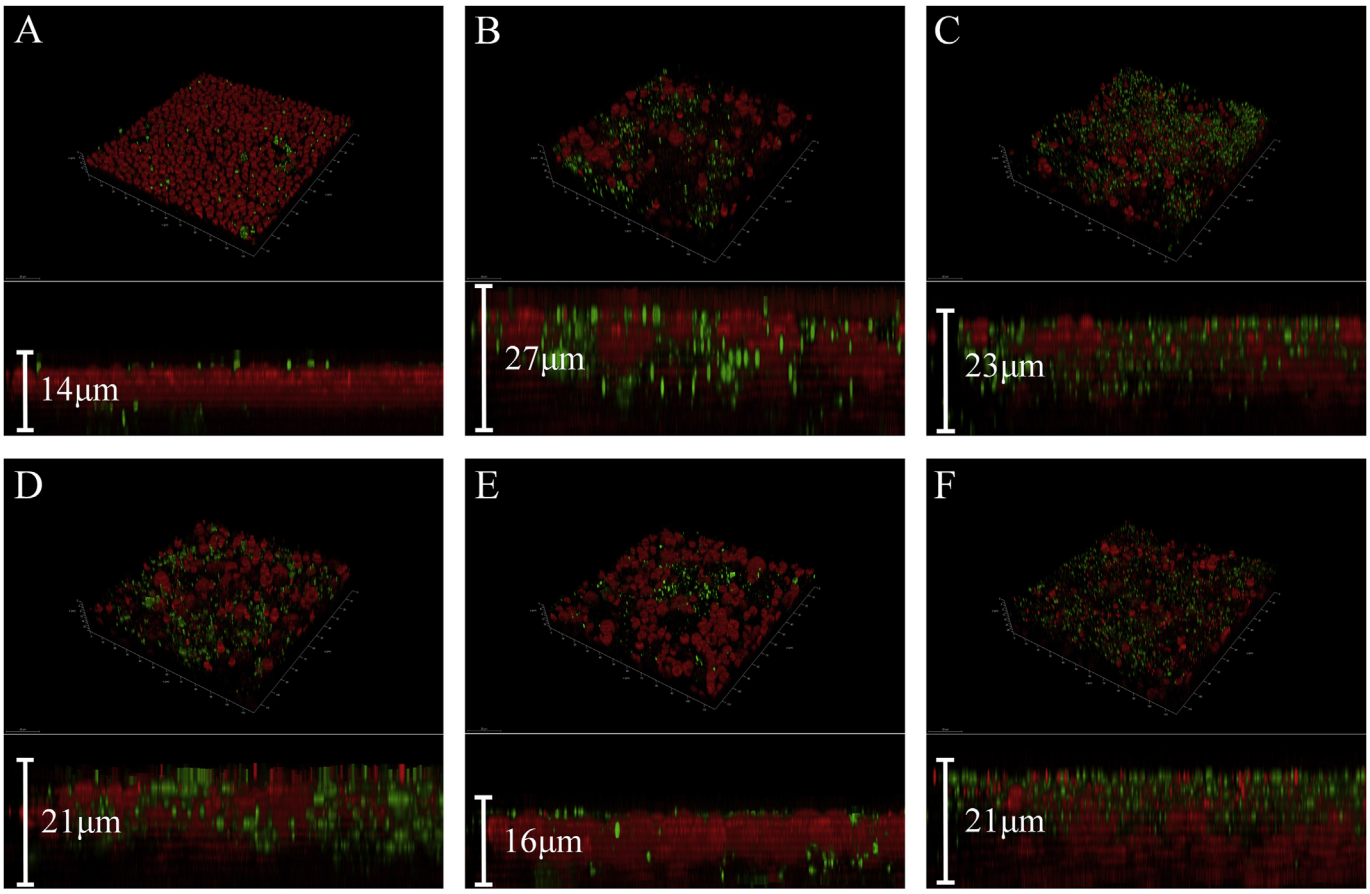

Fig. 6. Three dimensional and thickness micrographs of CLSM of the biofilm grown at different applied potentials (A: −0.4 V, B: −0.2 V, C: 0 V, D: 0.2 V, E: 0.4 V) under illumination and at 0 V in dark. Color assignment: inactivated cells (red), active cells (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

rod cells represented chlorella algae and *R. palustris*, respectively. The cells were densely attached to the biofilm under all experimental conditions, and *R. palustris* showed an overwhelming advantage at poised potential of 0 V while *C. vulgaris* was dominant under −0.4 and 0.4 V. Based on this, the highest power generation at 0 V could be attributable to considerable *R. palustris* attached to the working electrodes. In other words, the large amount of adhesion of *C. vulgaris* on electrodes decreased the power generation which may be attributable to more oxygen level restraining EET from organisms to electrode [7]. Moreover, the contribution of *C. vulgaris* to the intracellular electron extraction could be ignored as evidenced by the insignificant current production by the pure culture of *C. vulgaris* which further confirmed the high electricity-generating capacity of *R. palustris*. The amount of *R. palustris* attached on electrode in dark was comparable to that under illumination condition.

In order to observe the three-dimensional distribution of biofilm adhering to the electrodes, CLSM measurement was performed. As shown in Fig. 6, green and red fluorescence symbolized active and dead cells, respectively. Obviously, the electrode at poised potentials of −0.2, 0 or 0.2 V was attached by more cells and simultaneously displayed more living cells on the basis of the three-dimensions and thickness images of Fig. 6. Furthermore, the living cells at 0 V were more intensive than those at −0.2 and 0.2 V (Fig. S2), demonstrating that high biofilm microbial activity can be achieved by holding electrode potential at 0 V. The density of active cells (i.e. a lower electrochemical activity) within the biofilm grown in dark was much lower as compared to that grown under illumination which is in accordance with the results of the current production and CV.

4. Conclusions

Intracellular electrons extraction from co-culture of *C. vulgaris* and *R. palustris* was achieved by using electrode as extracellular electron acceptor. A moderate electrode potential of 0 V was more favor for photosynthetic electron extraction than other electrode potentials (−0.4, −0.2, 0.2 and 0.4 V) which might be caused by the inactivation of redox mediator at higher potentials and inadequate driving force for oxidation of electron donor at lower potentials. The electrode potential had a great impact on the dominant species within the photosynthetic biofilm. The most active cells adhering to the electrode were *R. palustris* at 0 V which was responsible for the higher photo-current intensity at 0 V compared to those at other potentials in which the amount of *C. vulgaris* was increased. The respiratory electron can also be extracted by the electrode in dark but the current was much lower than that under illumination, owing to the reduction in the amount of microbial derived electron mediator and less active cells attached to electrode.

Credit author statement

Jian Sun: Data curation, Writing- Original draft preparation, wring responses to reviewers, **Ping Yang**: Conceptualization, Methodology, Software **Nan Li**: Visualization, Investigation., **Mengmeng Zhao**: Software, Validation., **Xubin Zhang**: Software,

Validation., **Yaping Zhang:** Reviewing and Editing, **Yong Yuan**: Supervision, Reviewing and Editing, **Xingwen Lu**: Reviewing and Editing, **Xun Lu**: Original draft preparation, Reviewing and Editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.electacta.2020.135710.

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