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论文题目：Titanium dioxide (TiO_2) enhances the photocurrent generation of cyanobacteria (二氧化钛增强蓝藻的光电流产生)

Titanium dioxide (TiO_2) enhances the photocurrent generation of cyanobacteria

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Abstract

Photosynthetic organisms such as algae can convert photons into electrons, providing ideal eco-friendly materials for converting solar energy into electricity. However, the electrons are hardly transported outside the algae cells due to the insulation feature of the cell wall and cell membrane. Various nanomaterials have been reported to enhance extracellular electron transfer of electroactive microorganisms, but its effect on photosynthetic microorganisms has not been studied. This study investigated the effect of six different nanomaterials on the photocurrent generation of cyanobacterium *Synechocystis* sp. PCC6803, a prokaryotic algae. Among the nanomaterials tested, titanium dioxide (TiO_2) increased the photocurrent generation of cyanobacteria up to four-fold at the optimum concentration of 2 mg/mL. Transmission electron microscopy showed that TiO_2 bound to cyanobacterial cells. Photochemical analysis showed that TiO_2 decreased the activity of photosystem II but increased the activity of photosystem I. This study provides an alternative new approach for enhancing the photocurrent generation of cyanobacteria.

Keywords: cyanobacteria, photocurrent, nanomaterials, titanium dioxide (TiO_2), photosynthesis

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1 Introduction

The sun is the largest source of energy on the earth's surface [1]. Therefore, development of efficient, cost-effective, and environmentally-friendly solar energy utilization technology is of great significance to achieve sustainable development of the current society. Photovoltaic technology has been widely used for converting solar energy into electricity, and the key element of photovoltaics is semiconductor materials which transform light into electricity [2]. However, semiconductor materials may pose threats to our environment due to its potential toxicity. In this connection, developing environmentally-friendly, readily-available photoelectric conversion materials is of great interest.

Photosynthesis is the most important biochemical reaction on Earth. Photosynthesis is also the most important way for living organisms in nature to use solar energy. Photosynthesis begins with charge separation at the photoreaction center, with a very high quantum efficiency (close to 100%) [3]. However, the overall energy efficiency of photosynthesis is rather low – less than 1% for most of the plants [4]. This is because photosynthesis is a very complicated process, where stepwise energy lost occurs [4].

Since the quantum efficiency of the charge separation at the photoreaction center is very high, theoretically the photoreaction center of photosynthetic organisms should be good materials for solar energy utilization. Back to 1980, scientists demonstrated it was possible to generate electricity from light by using a "living electrode" [5]. They immobilized a blue-green algae on a transparent electrode and observed a photocurrent of 1.2 $\mu A/cm^2$. Later, scientists found different algae may have different photoelectric conversion activity, but the overall photocurrent was low, due to the insulation feature of the cell wall and cell membrane of algae. Many researchers have tried to fabricate electrodes with different structures or materials, with the hope to extract more electrons from algae by increasing the contact area [6]. However, these approaches could not fundamentally solve the low photocurrent problem caused by the insulation feature of cell wall/membrane of photosynthetic microorganisms.

Recently, it was shown that nanomaterials can improve microbial extracellular electron transfer of heterotrophic electroactive microorganisms [7, 8], but the effect of nanomaterials on photosynthetic microorganisms have not been studied. I therefore wanted to test whether I can find a nanomaterial that can boost the photocurrent generation of photosynthetic microorganisms. For this purpose, I tested the effect of six different nanomaterials on the photocurrent generation of cyanobacteria, and found titanium dioxide (TiO_2) showed the best effect for enhancing the photocurrent generation of cyanobacteria.

2 Materials & Methods

2.1 Strains and culture media

Cyanobacterium *Synechocystis* sp. PCC6803 was used in this study as a photosynthetic microorganism to be tested. Cyanobacterium PCC6803 was cultivated in a medium termed as BG11, with the following composition (per liter): 1.5 g $NaNO_3$, 0.047 g $K_2HPO_4 \cdot 3H_2O$, 0.036 g $MgSO_4 \cdot 7H_2O$, 0.027 g $CaCl_2 \cdot 2H_2O$, 0.02 g Na_2CO_3, 0.006 g citric acid, 0.006 g ammonium ferric citrate, 0.001 g EDTA-2Na, 1 mL trace element stock solution, pH 7.4. Trace element stock solution (per liter): 2.86 g H_3BO_3, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.222g $ZnSO_4 \cdot 7H_2O$, 0.079 g $CuSO_4 \cdot 5H_2O$, 0.391 g $Na_2MoO_4 \cdot 2H_2O$ and 0.04 g $CoCl_2 \cdot 6H_2O$. *Shewanella oneidensis* MR-1, which can generate electricity by oxidizing organic compounds, was used as a negative control for photocurrent generation. Strain MR-1 was grown at 30°C in Luria Broth (LB) medium composed of (per liter): 10 g NaCl, 10 g tryptone and 5 g yeast extract.

2.2 Nanomaterials tested in this study

Six different nanomaterials, including tin oxide (ITO), antimony tin oxide (ATO), silicon carbide (SiC), titanium dioxide (TiO_2), amino nanosilver (AgNPs), copper nanoparticles (CuNPs), were used in this study. ITO was purchased from Sigma-Aldrich (MA, United States), ATO was purchased from Aladdin (Shanghai, China), SiC was purchased from J&K

Scientific (Beijing, China), TiO_2, AgNPs, and CuNPs were purchased from Macklin (Shanghai, China).

2.3 Bioelectrochemical system

The bioelectrochemical system used in this study is a three-electrode configuration (Fig. 1a) consisting of a working electrode (ITO conductive glass), a counter electrode (platinum wire) and a reference electrode (Ag/AgCl electrode). The BG11 medium was used as electrolyte. The light source applied to cyanobacteria is a red LED light with an intensity of 400 μmol photons·m^{-2}·s^{-1}.

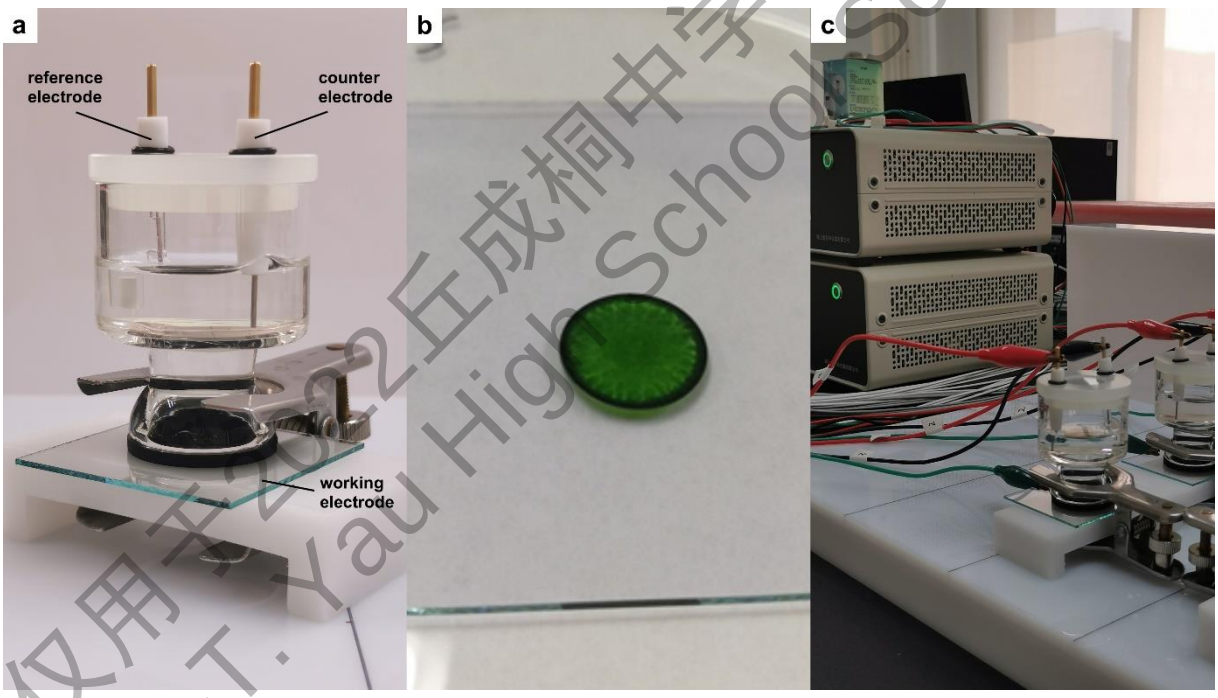

Figure 1. The photographs of bioelectrochemical system used in this study. a, Three-electrode system composed of working electrode, counter electrode and reference electrode. **b**, The cyanobacteria dropped onto the ITO conductive glass (working electrode). **c**, the bioelectrochemical systems were connected with a CHI1030C potentiostat for photocurrent measurement.

2.4 Incubation of cyanobacterial cells with nanomaterials

Cyanobacterium PCC6803 was cultivated in a light incubator (3% CO_2 and light intensity of 150 μmol photons·m^{-2}·s^{-1}) at 30°C for 3-4 days. One milliliter (mL) of cyanobacterial culture was pipetted from the conical flask to 2-ml centrifuge tubes, and centrifuged at 12000 rpm for 2 minutes. The supernatant was removed with a pipette. The precipitated cyanobacteria pellet in eight tubes was resuspended in 1 mL fresh BG11 medium. The optical density at 730 nm (OD_{730}) of the concentrated cyanobacterial cells was measured on a spectrophotometer (Persee, Beijing, China) after diluting for about 100 times (10 μL of concentrated cyanobacteria + 990 μL of BG11 medium), using BG11 medium as blank control.

For testing the effect of the nanomaterials, a certain volume of the concentrated cyanobacterial cells, equivalent to an OD_{730} of 2.5 (the required volume equals to 2.5 divided by the OD_{730} of the concentrated cyanobacterial cells) was transferred to a 2-ml centrifuge tube. Different nanomaterials were added to different centrifuge tubes at a designed concentration, and the final volume was adjusted to 100 μL using BG11 medium. The tubes containing cyanobacterial cells and nanomaterials were mixed by using a pipette, incubated at a low light condition (50 μmol photons·m^{-2}·s^{-1}) for 1 hour to allow sufficient interaction between nanomaterials and cells.

2.5 Photocurrent measurement

After incubation, 100 μL of cyanobacteria-nanomaterial mixture was dropped on an ITO conductive glass (Fig. 1b). The ITO conductive glass was left for about 2 hours at room temperature until cyanobacteria was dried. And then, ITO conductive glass was installed into a bioelectrochemical system by sequentially placing the ITO conductive glass containing cyanobacteria and nanomaterial, sealing ring, and electrolytic cell on the top of device base, and fixing all these components with a clamp. The electrolyte was added to the electrolytic

cell through a peristaltic pump. The counter electrode and reference electrode were placed in an optically transparent quartz lid (Fig. 1a). Repeat the above steps to make other identical devices, including negative controls.

Photocurrent measurement was conducted by using a CHI1030C potentiostat (CH Instruments, Shanghai, China). The three electrodes of bioelectrochemical system were electrically connected with the potentiostat (Fig. 1c). The photocurrent was measured using chronoamperometry technology, and the potential of working electrode was controlled at +0.7 V vs. Ag/AgCl. The light-dark cycle (ON 100 s/OFF 100 s) was applied during the photocurrent measurement.

2.6 Measurements of photochemical activities of photosystems

The photochemical activities of photosystem II (PSII) and photosystem I (PSI) of cyanobacteria were determined by measuring chlorophyll fluorescence parameters using a Dual-PAM-100 fluorometer (Walz, Germany). In brief, 2 ml cyanobacteria suspension at an OD_{730} of 5.0 with 0, 0.5 or 2.0 mg/mL TiO_2 were measured. Slow induced curve and light response curve were monitored to get the chlorophyll fluorescence kinetic parameters, including the relative electron transport rate (rETR(II) and rETR(I)) and the effective quantum yield (Y(II) and Y(I)).

2.7 Determination of oxygen evolution rate

The photosynthetic oxygen evolution rate was determined using a Chlorolab2 Liquid-Phase Electrode (Hansatech, UK). Cyanobacterial cells at exponential growth phase were harvested and resuspended in 2 mL BG11 medium containing 100 mM bicarbonate at an OD_{730} of 5.0. The light was provided at intensity of 500 μmol photons m^{-2} s^{-1}. The O_2 evolution rates were measured at the constant illumination for 5 min after 3 min darkness. TiO_2 was added with final concentration of 0, 0.5 or 2.0 mg/mL.

2.8 Transmission electron microscope (TEM) observation

Cyanobacterial cells incubated with or without TiO_2 were subjected to transmission electron microscopy (TEM) observation using HT7800 (Hitachi, Japan) at an accelerating voltage of 80 kV.

2.9 Other equipment or materials used in this study

Light incubator (with shaker inside), spectrophotometer, bench-top high-speed centrifuge, ultra-clean bench, scales, test tubes, centrifuge tubes, pipette.

3 Results

3.1 TiO_2 increased the photocurrent generation of cyanobacteria

Cyanobacterium PCC6803 is able to generate photocurrent upon exposure to light, but the light-induced photocurrent was quite weak (Fig. 2). This is in consistent with what reported in the literature [9], indicating the poor electron export ability of PCC6803. The aim of this study was to investigate whether nanomaterials can boost the light-induced photocurrent generation. Interestingly, incubation cyanobacterium PCC6803 with 1.0 mg/mL TiO_2 showed a three-fold higher photocurrent generation as compared to that of the control, and such increase can be stably maintained for the five cycles tested (Fig. 2a). Incubation cyanobacterium PCC6803 with ITO also showed a two-fold higher photocurrent generation initially, but such effect was reduced to less than one-fold after five cycles (Fig. 2b). Incubation with ATO or SiC did not increase photocurrent generation (Fig. 2c, 2d), nor for CuNPs or AgNPs (Fig. 2e, 2f, considering the baseline shift).

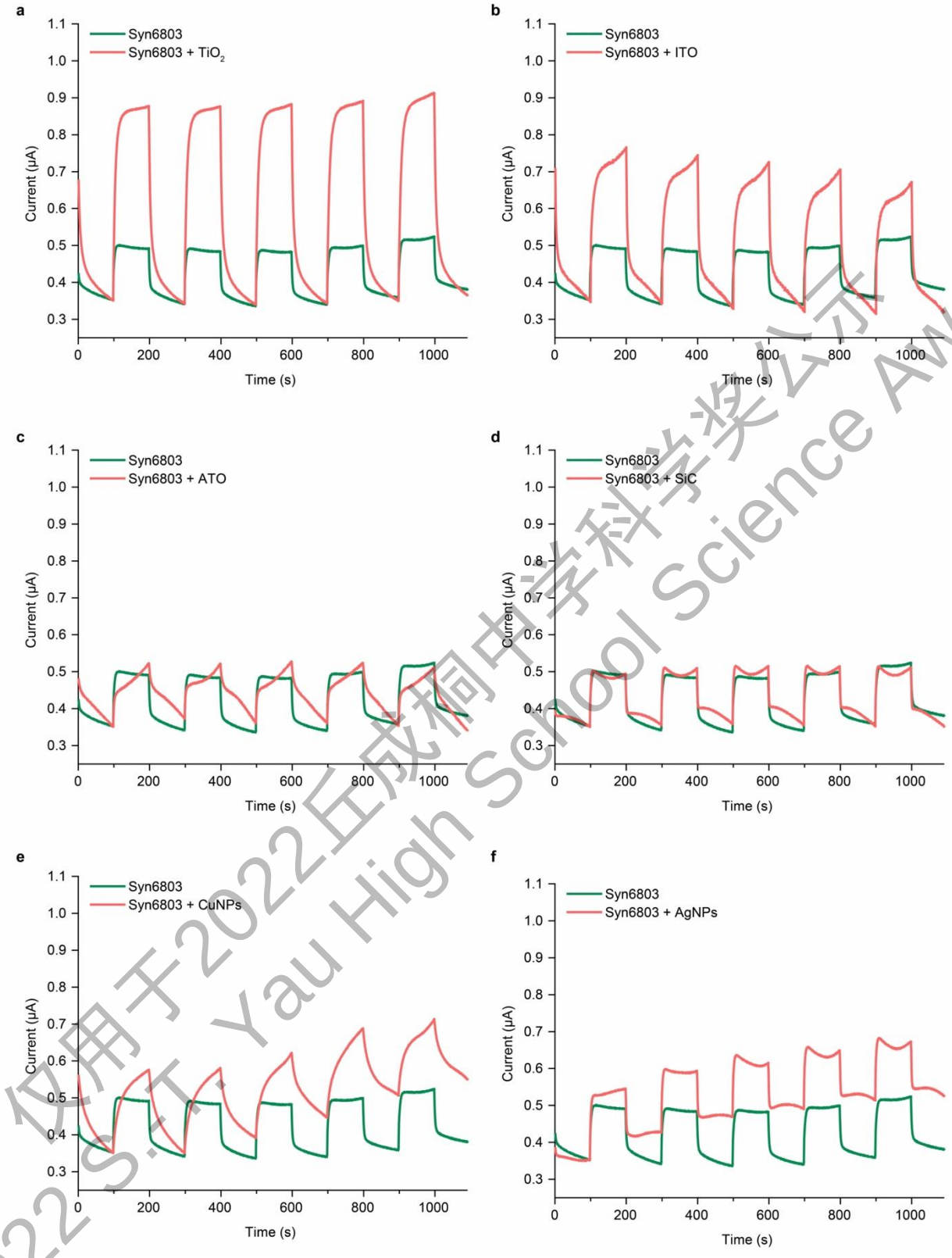

Figure 2. Effect of different nanomaterials on the photocurrent generation of cyanobacteria. Certain volume of nanomaterials suspension was added to a tube containing 2.5 OD_{730} of cyanobacteria cells. The final concentration of nanomaterials are as follows: TiO_2-1.0 mg/mL;

ITO-2.0 mg/mL; ATO-1.0 mg/mL; SiC-1.0 mg/mL; CuNPs-1.0 mg/mL; AgNPs-1.0 mg/mL. A tube only containing 2.5 OD_{730} of cyanobacterial cells was used as a negative control. Light was turn on/turn off every 100 seconds and the change of photocurrent was recorded automatically.

3.2 Determination of the optimal TiO_2 concentration

As TiO_2 showed positive effect in boosting the photocurrent generation of PCC6803 cells, I wondered what the optimum concentration of TiO_2 was. To answer this question, I incubated PCC6803 cells with different concentration of TiO_2 and determined its effect on photocurrent generation. As shown in Fig. 3a, adding TiO_2 at a concentration of 2 mg/mL resulted in a stable and high photocurrent, which is about a four-fold increase as compared to the negative control. Increasing the TiO2 concentration to 5 mg/ml resulted in a less steep increase of photocurrent. When the concentration of TiO_2 was increased to 10 mg/mL or even higher, the increment of photocurrent starts to decline (Fig. 3b). Therefore, I choose 2 mg/mL TiO_2 as the optimum concentration for following studies.

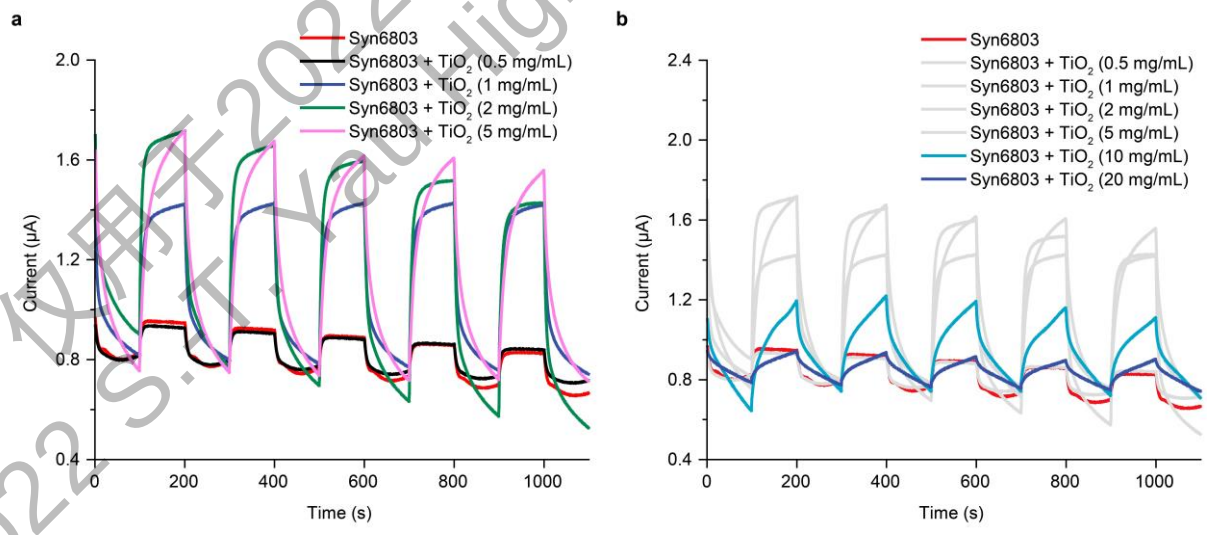

Figure 3. Effect of different concentrations of TiO_2 on the photocurrent generation of cyanobacteria. Different volume of TiO_2 (100 mg/mL) was mixed with 2.5 OD_{730} of cyanobacterial cells. The final concentrations of TiO_2 were labeled in the figure.

3.3 Observation of the binding of TiO_2 to cyanobacterial cells

In order to better understand how TiO_2 interacts with the cyanobacterial cells, samples of PCC6803 cells incubated with or without TiO_2 were prepared and subjected to transmission electron microscopy (TEM) observation. Fig. 4 shows clearly that TiO_2 bound to the cell, apparently cross the cell wall and inserted into cell membrane. When the TiO_2 was increased from 2 mg/mL to 20 mg/mL, the number of particles that bound to the cell also increased (Fig. 4).

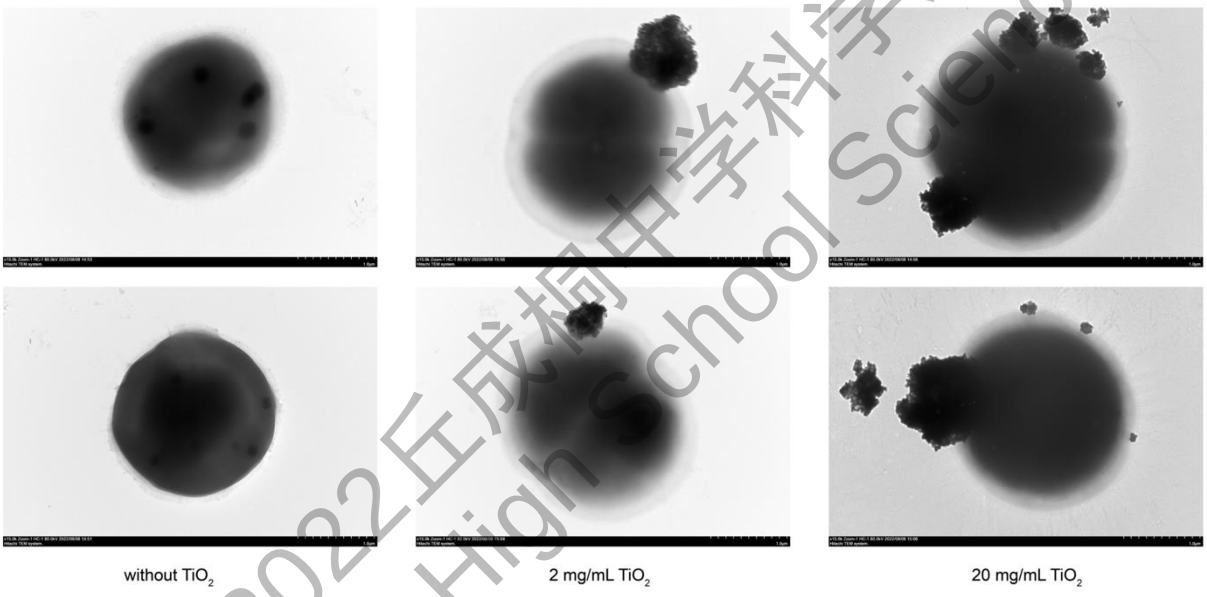

Figure 4. Transmission electron microscopy to observe the degree of binding of TiO_2 to cyanobacterial cells (non-sectioned). Different volume of TiO_2 (100 mg/mL) was mixed with 2.5 OD_{730} of cyanobacterial cells. The final concentrations of TiO_2 were labeled in the figure.

3.4 Determination of the photosynthesis parameters of PCC6803 cells

Addition of 2 mg/mL TiO_2 resulted in up to four-fold increased photocurrent generation of PCC6803 cells. Since the photocurrent generation is closely related with the photosynthesis, it would be natural to ask a question what the effect of TiO_2 on the photosynthesis is. Photosystem II (PSII) and photosystem I (PSI) are the two key systems of photosynthesis, and

the photochemical activities of these two photosystems can be determined by using a photochemical analyzer. The relative electron transport rate of PSII and PSI (rETR(II) and rETR(I)) and the effective quantum yield of PSII and PSI (Y(II) and Y(I)) of PCC6803 and PCC6803 adding different concentrations of TiO_2 were determined. It is quite interesting that addition of TiO_2 decreased the relative electron transport rate and the effective quantum yield of PSII (Fig. 5a, 5b), but increased that two parameters of PSI (Fig. 5c, 5d).

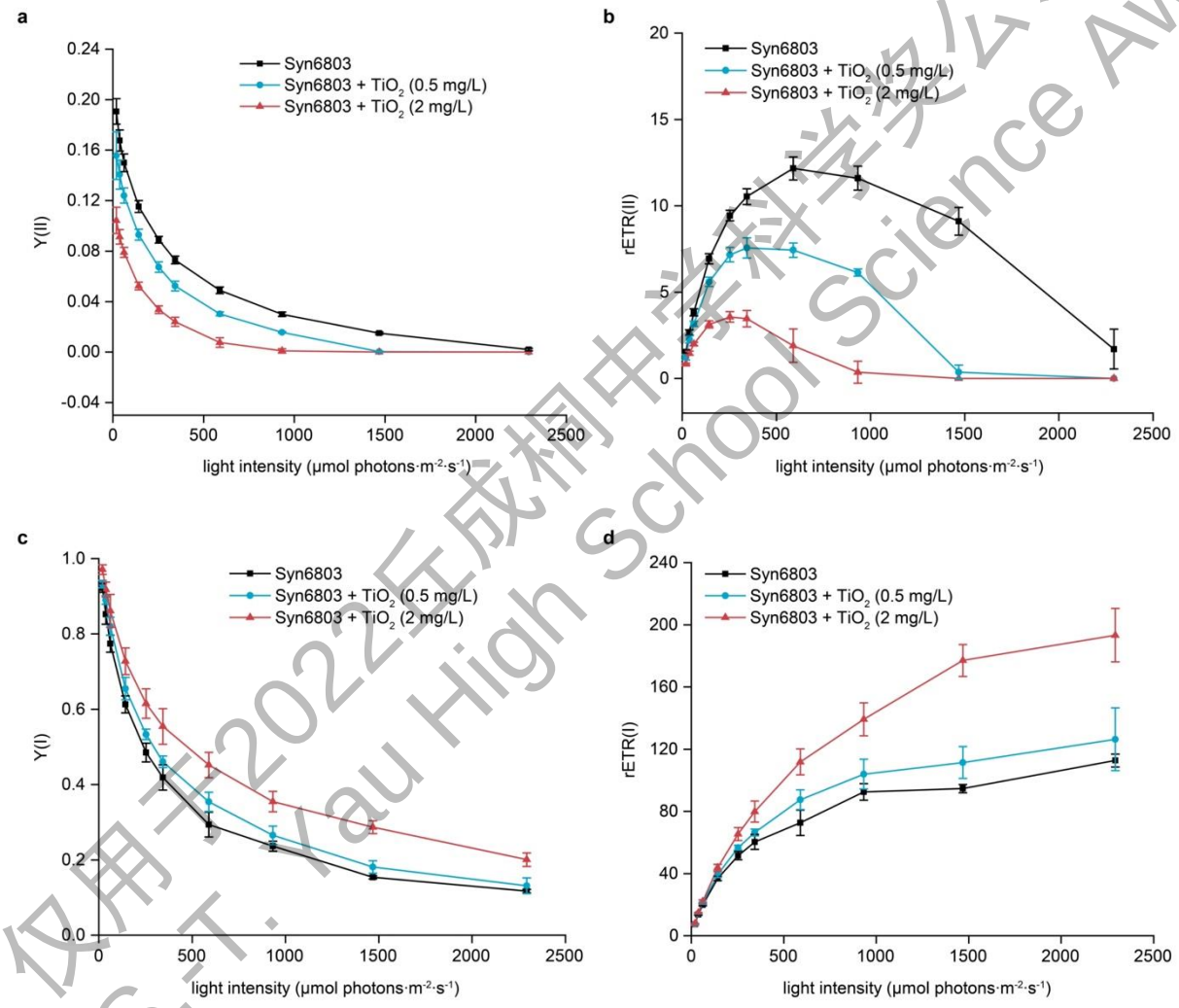

Figure 5. Photochemical quantum transfer rates of PSII and PSI. Different volume of TiO_2 (100 mg/mL) was mixed with 2.5 OD_{730} of cyanobacterial cells. The final concentrations of TiO_2 were labeled in the figure.

Another important parameter to reflect the activity of photosynthesis is the oxygen

evolution rate (OER), as oxygen is the direct product of photosynthesis. I also determined the OER of PCC6803 without or with TiO_2. As shown in Fig. 6, addition of 0.5 mg/mL of TiO_2 did not significantly increase the OER, while addition of 2 mg/mL TiO_2 slightly reduced the OER of PCC6803 cells. Together with the inhibitory effect of TiO_2 on the photochemical activities of PSII (Fig. 5a, 5b), it seems likely that TiO_2 would not bring beneficial effect on photosynthesis.

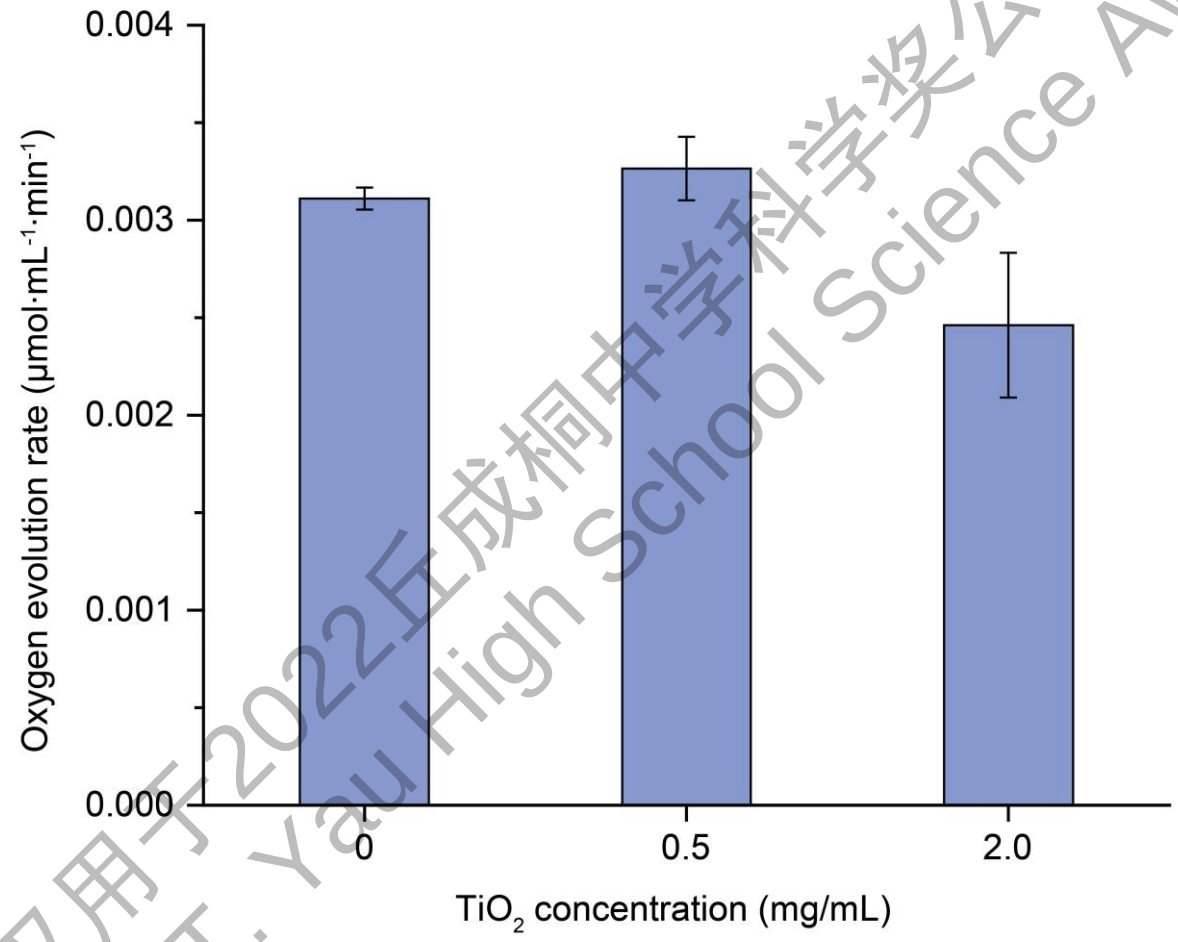

Figure 6. Photosynthetic oxygen evolution rate at different concentrations of TiO_2. Different volume of TiO_2 (100 mg/mL) was mixed with 2.5 OD_{730} of cyanobacterial cells. The final concentrations of TiO_2 were labeled in the figure.

In order to demonstrate the data obtained were valid, I further conducted a negative control experiment. Firstly, I used a heat-treated cyanobacteria cells, and no photocurrent was

observed in the presence or absence of TiO_2 (Fig. 7a). This suggest if cyanobacteria cells were dead, no photocurrent could be generated. Secondly, a natural electricity-generating microbial strain, *Shewanella oneidensis*, which is capable of generating electricity from organic compounds, was placed in BG11 medium with or without TiO_2. No photocurrent was observed (Fig. 7b), suggesting *Shewanella oneidensis* is not able to generating electricity from light, while TiO_2 itself could not generate electricity from light. These control experiments further demonstrated TiO_2 enhances the photocurrent generation of cyanobacteria.

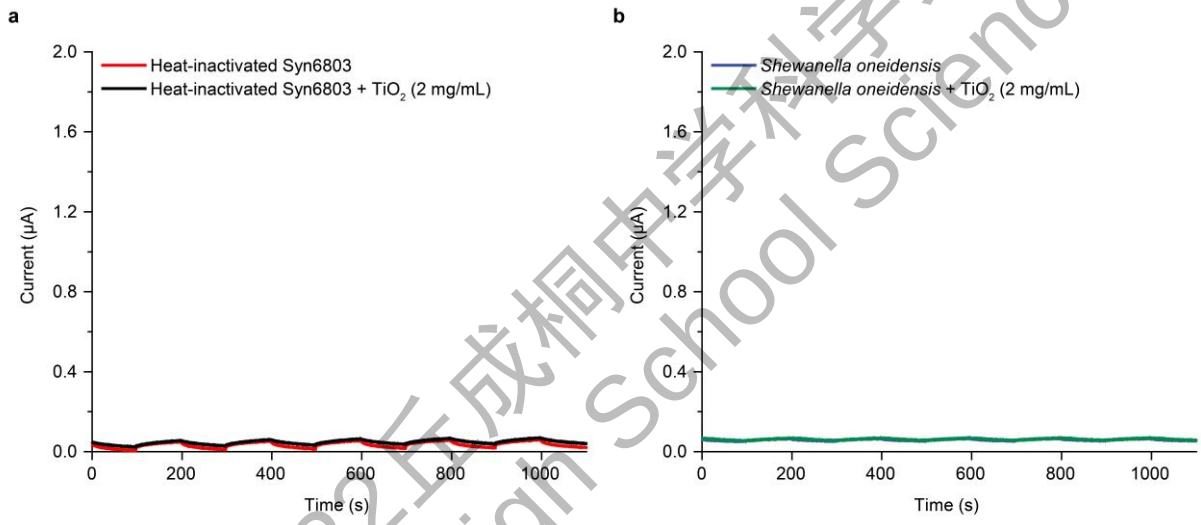

Figure 7. Photocurrent negative control. PCC6803 cells were treated at 100ºC for 15 minutes. *Shewanella oneidensis* was precultured in LB medium at 30ºC for 12 hours.

Lastly, I tested whether TiO_2 is toxic to the cyanobacterial cells. The cyanobacterial cells were firstly incubated with different concentration of TiO_2 for 6 hours, and then serially diluted and dropped onto the BG11 agar plate. After routine cultivation for two weeks, the growth of the cyanobacteria treated with TiO_2 was found to be same as the control, even when TiO_2 concentration was increased to 20 mg/mL (Fig. 8), indicating TiO_2 is not toxic and does not affect the growth of cyanobacteria.

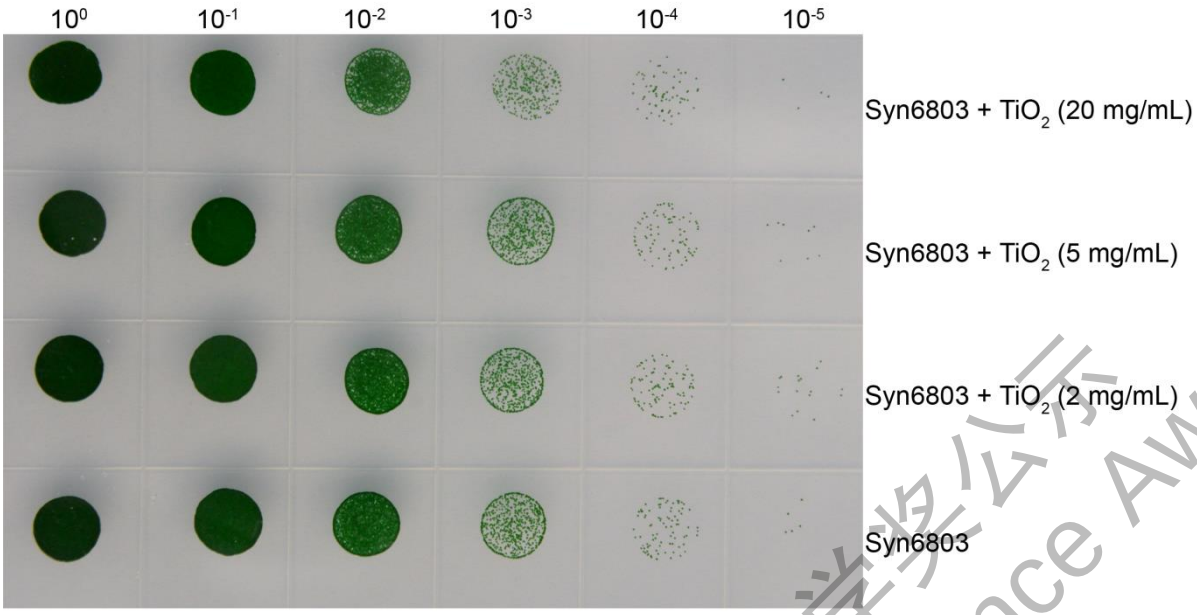

Figure 8. Effect of titanium dioxide on the growth of cyanobacteria. Cyanobacterial cells were incubated with different concentration (0, 2, 5, 20 mg/mL) of TiO_2 for 6 hours. Then the cells were serially diluted with 10-fold gradient, and 10 μL of diluted cells were dropped onto BG11 agar plate. The plates were placed in light incubator to allow cyanobacteria grow for two weeks.

4 Discussion

In this study, I investigated the effect of six nanomaterials on the photocurrent generation of cyanobacterium *Synechocystis* sp. PCC6803 cells, where TiO_2 and ITO were found to enhance the photocurrent generation by 2-4 folds. The role of ITO might be an electron carrier as ITO is often used as a conductive material [10]. Nevertheless, the positive role of TiO_2 on boosting photocurrent generation has not been reported before, therefore it is a new discovery found in this study.

TiO_2 is often used as the main ingredient in sunscreens, and its function is to prevent UV lights from entering the body. The addition of TiO_2 may thus reduce the light absorption, which is consistent with the observation that the photochemical activities of PSII was reduced, and the photosynthetic oxygen evolution rate was decreased. Since the activity of

PSII is usually associated with the oxygen evolution rate, the decrease activity of PSII and oxygen evolution rate at high concentration of TiO_2 suggested TiO_2 is not beneficial for photosynthesis. However, the photochemical activities of PSI increased when I added TiO_2. Moreover, I observed that TiO2 was bound to the cell, apparently crossing the cell wall and cell membrane. How this phenomenon is correlated with the increased photocurrent generation requires further investigation.

Moreover, although the photocurrent of cyanobacteria can be improved to some extent by adding 2 mg/mL TiO_2, the total amount of photocurrent that the cyanobacterium *Synechocystis* sp. PCC6803 can produce is very low - the unit used to measure photocurrent is still at the level of microamps. Therefore, the next step based on this study is to find a better way to direct or transport the electrons outside the cells, and see if it could significantly improve the photocurrent. Once the photocurrent directly generated by cyanobacteria could reach a much higher level, cyanobacteria might be developed into a biophotovoltaics to power ultralow-power electronic facilities, such as environmental sensors.

5 Conclusion and perspectives

Among the six nanomaterials that I tested in this study, incubation cyanobacterium *Synechocystis* sp. PCC6803 with 2 mg/mL titanium dioxide (TiO_2) can increase the photocurrent generation up to four-fold, demonstrating that nanomaterials can be used to boost the photocurrent generation of cyanobacteria. For future studies, I would like to explore the mechanism and understand why TiO_2 shows such a boosting effect.

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致谢

1、感谢中国科学院微生物研究所朱华伟博士与北京大学附属中学唐灵芳老师对本论文的指导。本论文研究在中国科学院微生物研究所朱华伟博士所在实验室完成，所使用的电化学分析设备、透射电镜、培养箱、分光光度计、光合生理分析仪和光合放氧分析仪，均为中国科学院微生物研究所设备。透射电镜实验具体操作得到了中国科学院微生物研究所王海云老师的指导和帮助，特此一并致谢。

2、本论文选题思想最早来源于北京大学附属中学唐灵芳老师讲授的高一生物学课程中的光合作用部分，当时作者了解到已经有科学家利用光合生物发电，但效率比较低，因此对生物光电转化产生了兴趣，联系了在国内较早开展生物光电转化研究的朱华伟博士进行学习。在高一下学期北大附中道尔顿学院的 Science Fair 活动中，作者在朱华伟博士指导下，开展了利用蓝藻类囊体膜进行光电转化的小研究并获得北大附中道尔顿学院老师好评。由于关于纳米材料促进蓝藻光电转化的研究非常少，在朱华伟博士指导下，作者利用高一下学期课外时间和暑假时间，围绕这一方向开展了研究并取得了阶段性进展。

3、本研究的实验部分是在朱华伟博士指导下完成的。实验设计、试剂订购、仪器使用、数据获取、图表制作和论文写作，均得到朱华伟博士的悉心指导，也特别感谢朱华伟博士用他的基金项目经费支持本研究。唐灵芳老师阅读了论文全文并对论文进行了指导和修改。朱华伟博士是作者的课外科研指导老师，唐灵芳老师是作者在北大附中的生物学任课教师。朱华伟博士和唐灵芳老师对本论文的指导都是无偿的。