Effects on Neutral Carbon Source Microbial Solar Cells Using Cyanobacteria as Exoelectrogens

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Abstract*— Microbial solar cells (MSCs) have drawn significant research interests in recent years. The principle of MSCs is different from of the common microbial fuel cells (MFCs), which use organic compounds as fuel to produce electricity. MSCs convert sunlight into electricity through oxygenic photosynthesis by phototrophic microorganisms such as cyanobacteria or algae without providing organic compounds. However, even though it had been proved that cyanobacteria have ability of power generation, yet the mechanism of producing electricity is still ambiguous. The purpose of this study is to investigate several factors that possibly affect MSCs performance. Results show that pH, light intensity, and ion concentration affected MSCs performance. Besides these, oxygen concentration affected system performance significantly but can be controlled by chemical addition. For future studies, these factors should be optimized to maintain system performance.

***Index Terms*—Cyanobacteria, electricity, microbial solar cells, phototrophic microorganisms**

I. Introduction

Cyanobacteria have existed on the earth for more than 3.5 billion years, which could be found in rivers, ocean, ponds, and lakes, etc. Cyanobacteria cell contain carbohydrates, proteins, and lipids, representing an important biomass for use as bioenergy production source. Previous studies on cyanobacteria applications are widespread including bioindustrial processes, agriculture, organism production (e.g. sugars and isoprene), biofuels (e.g. alcohols, bio-oil, methane and hydrogen), carbon sequestration, and other commodity chemicals [1-3]. Cyanobacteria are photosynthetic bacteria and capable of exploiting solar energy to synthesis organic compounds from carbon dioxide. Photosynthetic reaction center at the thylakoid membrane possess two photosystems: photosystem II (PSII) utilizes light energy for splitting hydrogen oxide to hydrogen ion, oxygen, and electron; photosystem I (PS I) utilizes light energy to mediate electron transfer from plastocyanin to ferredoxin, which result in the reduced molecular NADPH and ATP. Both ATP and NADPH are used in the Calvin cycle to convert carbon dioxide into carbohydrates. Therefore, recent researches had focused on using cyanobacteria as exoelectrogenic bacteria to produce electricity.

Microbial Solar Cells (MSCs) is an innovating application of microbial fuel cells, which have been attracting research interests in recent years. MSCs convert sunlight into electricity through oxygenic photosynthesis by phototrophic microorganisms without providing organic compounds. It has been proved that MSCs could produce electricity by using green algae or cyanobacteria as exoelectrogens for at least 20 days [4]. Some cyanobacteria species including freshwater and ocean type were studied for power generation ability as well. Among them, *Lyngbya* sp. produced electricity for up to 35 days [5]. Through coating chemical compounds on anode, *Synechosystis* PCC-6803 strain had a maximum power density about 1.3 mW/m^2 [6]. These applications show the possibility to use cyanobacteria for electricity production without adding organics. However, the possible operating factors that affect system performance have not yet been detailed studied. Therefore, the aim of this study is using selected cyanobacteria to investigate possible factors that affect MSCs performance.

II. Material and Methodes

A. Culture

Pure cyanobacteria were isolated from an ecological pond in Taiwan. The isolates were cultured with BG13 medium under 30±2°C with a 10W LED white bulb, which described by [7]. The BG13 medium contained 1.5 g/L $NaNO_3$, 1.7 g/L $NaHCO_3$, 31 mg/L K_2HPO_4, 75 mg/L $MgSO_4 \cdot 7H_2O$, 36 mg/L $CaCl_2$, 20 mg/L Na_2CO_3, 6 mg/L Citric Acid, 6 mg/L Ferric Ammonium Citrate, 1 mg/L EDTA, 2.86 mg/L H_3BO_3, 1.81 mg/L $MnCl_2 \cdot 4H_2O$, 220 µg/L $ZnSO_4 \cdot 7H_2O$, 390 µg/L $Na_2MoO_4 \cdot 2H_2O$, 80 µg/L $CuSO_4 \cdot 5H_2O$ and 40 µg/L $CoCl_2 \cdot 6H_2O$. The pH was adjusted to 7.5 ± 0.01 after autoclaved under 1.2 atmospheric pressure and 121°C for at least 20 min.

B. MSCs construction and operation

Single-chamber, rectangular reactor modified from the double-chamber reactor [8] was used in this study. The reactor volume was 300 ml. Carbon cloth was used as anode (15 cm × 2 cm, 60 cm^2 projected area) and autoclaved at 121 °C for at least 20 min before use. Air cathode with 3 cm diameter was made from carbon cloth with four PTFE diffusion layers and a 0.5 mg-Pt cm^{-2} catalyst layer [9]. The composition of the solution was the same as that of the culture medium, but $NaHCO_3$ was removed. The reactor was sparged with nitrogen gas to replace headspace gas for anaerobic condition before each batch experiment. The system was operated at fixed external resistance with a

constant temperature of 30±2 °C. The voltage was measured at 10 min intervals using Model 7700 multimeter/digital data acquisition system (Keithley, USA).

C. Chemical analysis

The pH variation was measured with a bench top pH meter SP-2300 (Suntex, Taiwan). Light intensity and wavelength were measured with illuminance spectrometer SRI-2000 (Optimum optoelectronics corp., USA). Dissolved oxygen was measured using HI4421 research grade dissolved oxygen and BOD meter (Hanna instruments, USA).

D. Ethidium monoazide bromide - polymerase chain reactor (EMA - PCR)

Ethidium monoazide bromide (EMA) treatment was used for microorganism activity analysis. EMA was added to samples at a final concentration of 10 µg/ml. Then, samples were incubated in the dark for 5 min and subsequently exposed to light for 20 min. The light source was a 650 W halogen light bulb, which was placed 20 cm from the sample tubes. The microcentrifuge tubes were placed on ice prior to light exposure to minimize elevated temperature in the samples [10]. DNA was isolated with commercial kit DNA Extraction Miniprep System (VIOGENE). PCR amplification was performed as follows: initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min; 72 °C for 7 min; and final cooling at 12 °C for storage [11]. The 25 µl reaction mixture contained 1× TaqMan buffer, 500 U/ml Taq DNA polymerase, 3 mM $MgCl_2$, 400 µM each dNTP, 0.5 µM CYA359F forward primer (5'-GGG GAA TTT TCC GCA ATG GG-3') and CYA781β reverse primer (5'-GAC TAC AGG GGT ATC TAA TCC CTT T-3').

III. Results and Discussion

A. MSCs performance under different initial pH

To investigate the effect of different pH on MSCs system performance, four different pH conditions (pH 5 to 8) were chosen and each examination was performed in duplicate under 1000 lx light intensity, 30±2°C and 1kΩ external resistance for 120 hr. Fig. 1 shows the power generation under different initial pH. When initial pH was 5, MSCs produced the highest voltage (0.002 V) at 70th and 83th hr. MSCs system did not show a good reproducibility under initial pH 6 as the highest voltage output was about 6.5 mV at 23th hr. When system was operated at initial pH 7, it produced a highest voltage at about 28-30th hr and decreased to 0 mV until 60th hr. MSCs had no electricity generation under initial pH 8. By comparing system performance with quantity of electric charge, the performance under different initial pH was pH 5 - 0.072C, pH 6 – 0.2 C, pH 7 – 0.32 C and pH 8 - -0.11C, separately.

Although system had a maximum voltage output under initial pH 6, the quantity of electric charge under initial pH 7 was the best. According to [12], pH range from 7.5 to 10 is appropriate for cyanobacteria growth. However, pH 5 and pH 6 is not suitable for cyanobacteria to growth and could inhibit their activity. In contrast, pH 8 is suitable for cyanobacteria so that they might use most energy for growth but not for power generation.

B. Effect of light intensity on MSCs

System performance affected by light was studied under four different light intensity conditions (1000 lx, 2000 lx, 3000 lx, and 4000 lx). As shown in fig. 2, when provided 1000 lx light to the system, it had a maximum voltage about 100 mV at 30th hr and the electrical charge was 0.645 C. Under 2000 lx light, system produced a maximum voltage about 80 mV at 80th hr, but decreased after 96 hr. The electrical charge was 1.556 C. The maximum voltage and electrical charge under 3000 lx light was 80 mV and 0.248 C. Finally, system produced 60 mV and 0.606 C when provided 4000 lx light to it. Result shows that, in this study, *Synecho*coccus sp. grew more abundantly (data not shown) and had better power generation under lower light intensity because light intensity over 3000 lx could be overexposed to this cyanobacteria. The PS II and PS I would begin to deliver electron when they were excited by visible light. But overexpose to PS II and PS I might cause damage to reactor center. In other words, if reactor center do not be repaired when exposing to high energy light during a long time, photosynthesis would be inhibited and have no power generation.

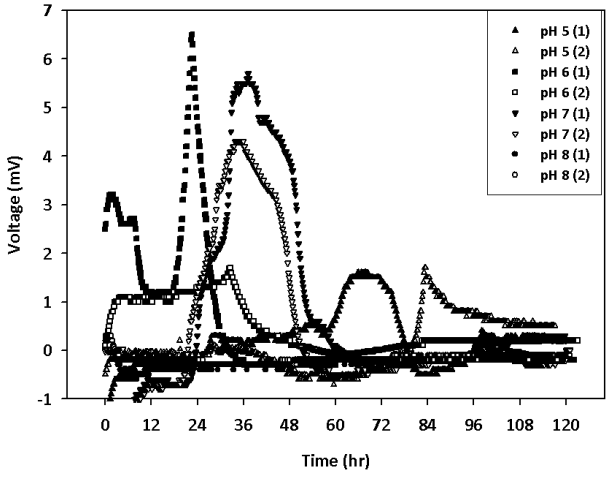


Fig. 1 Power generation under different initial pH conditions

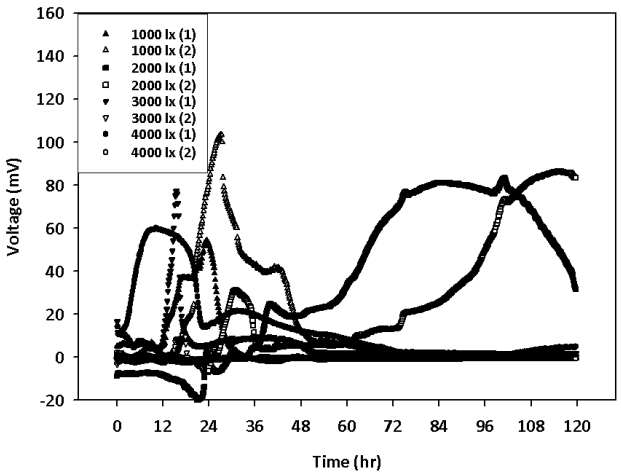


Fig. 2 Power generation under different light intensity

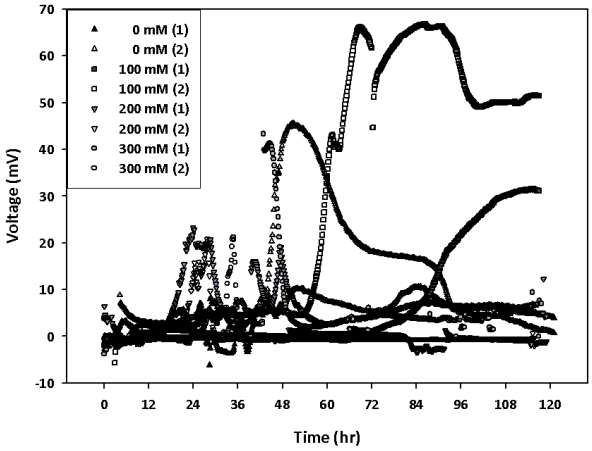


Fig. 3 Power generation under different ion concentration

C. *Effect of ion concentration on MSCs*

Four different ion concentrations were chosen to investigate the most appropriate ion concentration for MSCs system. Each experiment was performed under 1000 lx light intensity, 30±2°C and 10kΩ external resistance for 120 hr. As shown in fig. 3, the maximum voltage under different ion concentrations in ascending order was 23 mV (200 mM), 42 mV (300 mM), 47 mV (0 mM) and 67 mV (100 mM). Furthermore, the electrical charge in ascending order was 0.16 C (300 mM), 0.26 C (200 mM), 0.55 C (0 mM) and 1.26 C (100 mM). Previous researches had shown that increasing ion concentration could enhance bio-electrochemical system performance [13].The result of this study is different from reference. It could be ascribed to that the cyanobacteria *Synechococcus* sp. used in this study belongs to β-cyanobacteria (freshwater type). Because higher ion concentration environment does not fit to freshwater type cyanobacteria, the activity of cyanobacteria was decreased and led to an unfavorable consequence. Another reason for the unfavorable consequence is that cyanobacteria performed photosynthesis and released not only electrons but also oxygen. Oxygen dissolved in solution could intercept electrons from anode and lead to lower power generation.

D. *Dissolved oxygen caused negative performance*

For exploring the possible effect caused by DO, a set of experiments under 100 mM and 300 mM NaCl were conducted. Table 1 shows the initial and final DO of these two conditions. The initial DOs were similar and the average was 4.6±0.14 mg/L. In 100 mM NaCl condition, the final DO concentration was 1.65 and 0.53 mg/L, respectively, which is under micro-aerobic condition. By contrast, unexpectedly,

Table 1 Dissolved oxygen concentrations under 100 mM and 300 mM NaCl

	Initial (mg/L)	Final (mg/L)
100 mM- Number 1	4.75	1.65
100 mM- Number 2	4.37	0.53
300 mM- Number 1	4.58	1.70
300 mM- Number 2	4.64	6.39

Table 2 Chemical analyses w/ and w/o Na_2SO_3 addition

subject	Electrical charge(C)	Final DO(mg/L)	Final pH
w/o Na_2SO_3	< 0	6.47	10.54
w/ Na_2SO_3	0.029	0.33	10.82
w/o Na_2SO_3 + 50 mM PBS	0.016	6.23	7.02
w/ Na_2SO_3 + 50 mM PBS	0.602	0.46	7.02

one of the 300 mM NaCl final DO concentrations was increased to 6.39 mg/L. This explains the lower performance under 300 mM NaCl condition was caused by increasing dissolved oxygen.

E. *Reductant addition for improving MSCs performance*

Various kinds of reductant are capable of reacting and decreasing dissolved oxygen. Here, sodium hydrosulphite (Na_2SO_3) was used to reduce dissolved oxygen in MSCs system and the final sodium hydrosulphite concentration was 3%. Each experiment was performed under sunlight (light/dark cycle), ambient temperature and 10kΩ external resistance. The electrical charge, final DO, and final pH with and without sodium hydrosulphite addition were measured as well (Table 2). Results showed that power generation with sodium hydrosulphite addition was higher and more significantly response to light/dark

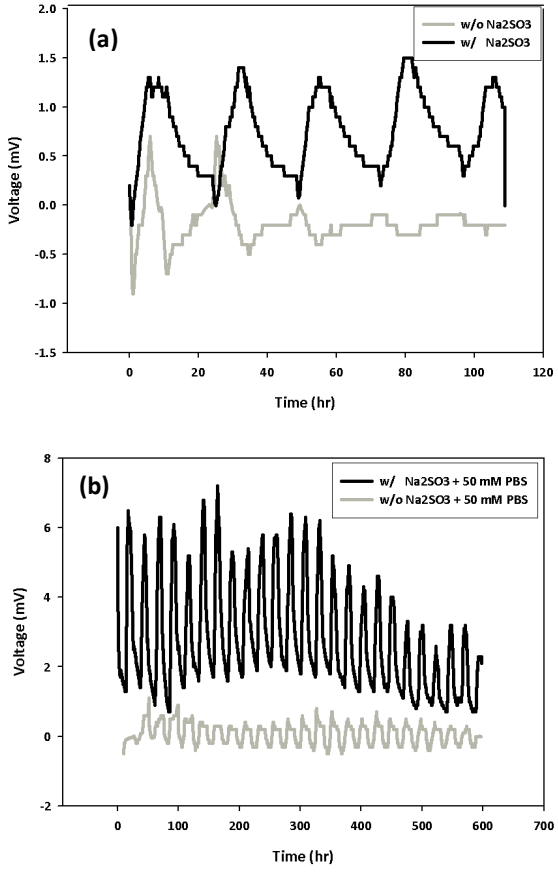
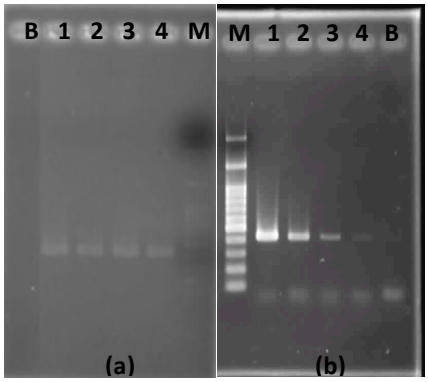


Fig. 4 Power generation w/ and w/o Na_2SO_3 (a) No phosphate buffer addition (b) Phosphate buffer addition



M：Marker
1：EMA (w/o Na_2SO_3)
2：DNA (w/o Na_2SO_3)
3：EMA(w/ Na_2SO_3)
4：DNA (w/ Na_2SO_3)
B：Blank

Fig. 5 Microorganism activity analysis by using EMA-PCR (a) No phosphate buffer addition (b) Phosphate buffer addition

cycle than that with no sodium hydrosulphite addition (Fig. 4(a)). EMA-PCR method was used to confirm whether sodium hydrosulphite addition could potentially damage cyanobacteria cell or inhibit electricity production. This is based on the reaction of EMA entering erupted cell and combining with DNA, making PCR amplification failure. Since results showed that PCR signals were identified from the EMA pretreated sample as well, there is no inhibition by this addition of 3% sodium hydrosulphite. (Fig.5). Although DO was successfully reduced by reductant addition, the pH raised from neutral to alkaline, which could cause lower system performance. Therefore, 50 mM PBS were provided as buffer and electrolyte for enhancing system performance. The maximum voltage with 50 mM PBS was about three times higher than normal condition (Fig. 4(b)). In summary, dissolved oxygen, pH, and ion concentration have significant effects on power generation of MSCs system. This should be noticed for avoiding lower power generation in future relative researches.

IV. Conclusion

In this study, numerous factors that possibly affect MSCs performance were investigated. The activity of cyanobacteria depends on environmental pH value and influences system performance. An appropriate pH should be controlled at neutral for ideal power generation. Phototrophic activity was directly affected by light intensity. Below 2000 lx, the more light energy provided, the more power generation. Over expose to cyanobacteria would decrease system performance. The most important factor is dissolved oxygen, which might intercept electrons and reduce performance obviously. Sodium hydrosulphite could be one of the reductants to avoid dissolved oxygen causing performance reduction. The authors would like to appreciate Taiwan's National Science Council (NSC 101-2221-E-005-045-MY3) for providing research founding.

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