IN-SITU MEASUREMENT OF PHOTOSYNTHESIS USING SINGLE SYNECOCYSTIS SP. PCC 6803 IN A MICROCHAMBER WITH GAS BARRIER WALL

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ABSTRACT

We developed a novel single cell measurement of photosynthesis activity using fluorescent oxygen sensor in a microchamber with gas barrier layer. We used Synechocystis sp. PCC 6803, which is a series of cyanobacteria. The chamber wall contains oxygen sensor and gas barrier layer to measure the dissolved oxygen concentration in the chamber. The oxygen concentration is changed by aerobic respiration and photosynthesis of Synechocystis. The variation of the oxygen concentration was used for evaluation of aerobic respiration and photosynthesis of single Synechocystis. To achieve precise oxygen measurement, we used our proposed compensation of photo-degradation of fluorescence. We demonstrated photosynthesis evaluation of single Synechocystis and calculated the photosynthetic performance of sugar production of single Synechocystis.

KEYWORDS

Single cell measurement, Photosynthesis, Micro chamber, Fluorescence.

INTRODUCTION

Recently, photosynthesis is received high attention in various fields, such as the food industry and environmental purification. Synechocystis sp. PCC 6803 is a unicellular cyanobacterium and is the model microorganism for photosynthesis study [1]. Conventionally, the photosynthesis performance and respiration of Synechocystis was measured using group cell. However, photosynthesis evaluation of single Synechocystis is quite difficult because variation of sugar and oxygen is quite low. Microfluidic chip having microchamber is suitable for this purpose. Moreover, the oxygen electrodes are not applicable for this purpose because the electrodes resolve dissolved oxygen.

Fluorescence measurement using fluorescence intensity and fluorescence lifetime, is one of the most promising methods for on-chip cell analysis [2]. This method acquires the local information at sub μm resolution. Fluorescence information has been revealed a lot of physiological parameters. In fluorescence measurement, measurement using fluorescent intensity is major because a lot of indicator exits for this method and measurement cost is low. However, fluorescent intensity from the indicator decreases by photochemical destruction of the indicator during measurement. Therefore, quantitative measurement of single cell using fluorescence requires fluorescence compensation method.

THEORY

Photosynthesis mechanism of Synechocystis sp. PCC 6803 is represented as calvin cycle. Aerobic respiration and photosynthesis are represented in equations 1 and 2.

$$C_6H_{12}O_6 + 6H_2O + 6O_2 \rightarrow 6CO_2 + 12H_2O \quad (1)$$

$$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2 \quad (2)$$

From these equations, we can evaluate the photosynthesis activity of single cell. We used Synechocystis, which is a series of cyanobacterium for this experiment. These two reactions are performed in the microorganism simultaneously. Therefore, the activity of photosynthesis is evaluated by comparing these reactions. First, rate of oxygen reduction by aerobic respiration is measured in the dark condition. Then, rate of oxygen increase is measured during irradiation of visible light. The difference between increase rate and reduction rate of oxygen is the rate of the oxygen production in the microorganism. From this result, sugar production rate is calcurated because both production rate of sugar and oxygen is same.

EXPERIMENT

We propose functional micro chamber with oxygen sensor and gas barrier layer to measure the photosynthetic performance by oxygen measurement. Figure 1(a) shows a concept of microchamber for measurement of photosynthesis activity. The micro chamber consists of polydimethylsiloxane (PDMS) as main body, Pt(II) tetrakis (pentafluorophenyl) porphyrin (PtTFPP) as oxygen sensor [3], parylene is used as gas barrier layer, and glass substrate. The chamber is surrounded by parylene and glass. Oxygen permeability of parylene is 2.4 cm^3•mil/100in^2/hour. Therefore, oxygen concentration in micro chamber is not affected by outer condition. Measurement of fluorescence from PtTFPP can detect oxygen concentration in microchamber [2]. Figures 1(b) shows the experimental system equipped fluorescent measurement system and multi-beam manipulation system [16]. This system consists of a commercial inverted microscope equipped with a high-numerical-aperture oil-immersion lens, an epi-fluorescent measurement system, and laser confocal scanning system with EM-CCD. A near-infrared laser (Maximum power: 5.6 W; wavelength: 1064 nm, TEM_{00}), is used for the optical manipulation of

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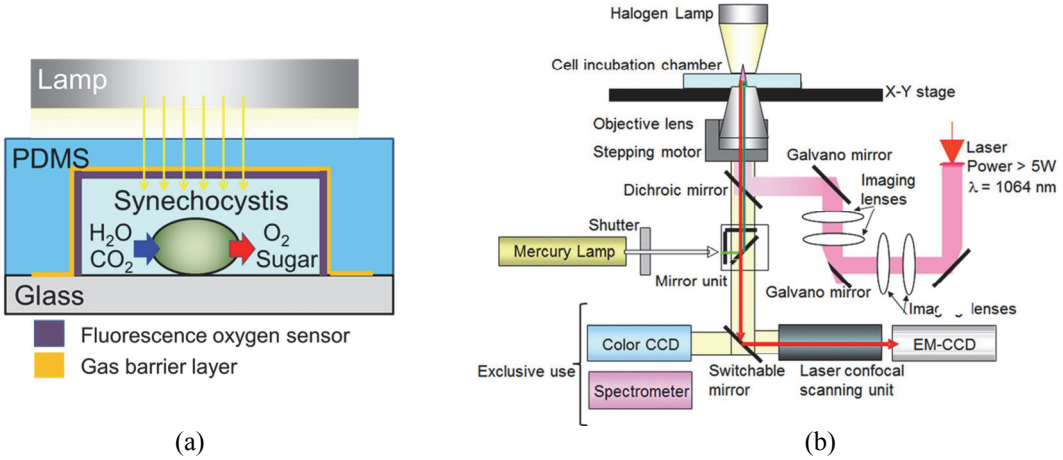
microorganism. A cell incubation chamber is used to control the temperature (accuracy of ±0.3 K). Fluorescent image of the microchamber is acquired using EM-CCD and color CCD camera (WAT-250D2, Watec). Photo spectrometer can be used with color CCD exclusively.

Figure 2 shows the fabrication process of the microchamber. First, nega-resist SU-8 was patterned to the chamber pattern. Then, the sensor layer and gas barrier layer was coated on the mold of SU-8. Fig. 13(a) shows an optical photomicrograph of the microchamber. Chamber volume is 1 pl (10 μm x 10 μm x 10 μm). Fig. 13(b) shows fluorescence image of the chambers acquired by color CCD. Fig. 3(c) shows the calibration result between oxygen concentration and fluorescent intensity of PtTFPP using Stern-Volmer as shown in equation 3. Photo degradation of the fluorescence was shown in Fig. 4(a). We proposed compensation of photo-degradation using equation 4. The photo-degradation is compensated as shown in Fig. 4(b).

$$I_{ref} / I = 7.3 \times 10^{-1} \cdot [O_2]/[O_{2ref}] + 2.9 \times 10^{-1} \tag{3}$$

$$I'_n = I_0 + \sum_1^n \left[(I_n - I_{n-1}) / \exp\{\ln(I_0) - \ln(I_n)\} - (I_1 - I_0) / \exp\{\ln(I_0) - \ln(I_1)\} \right] \tag{4}$$



(a) (b)

Figure 1. Experimental setup. (a) A schematic of photosynthesis analysis using single cell in micro chamber. (b) A schematic of optical system.

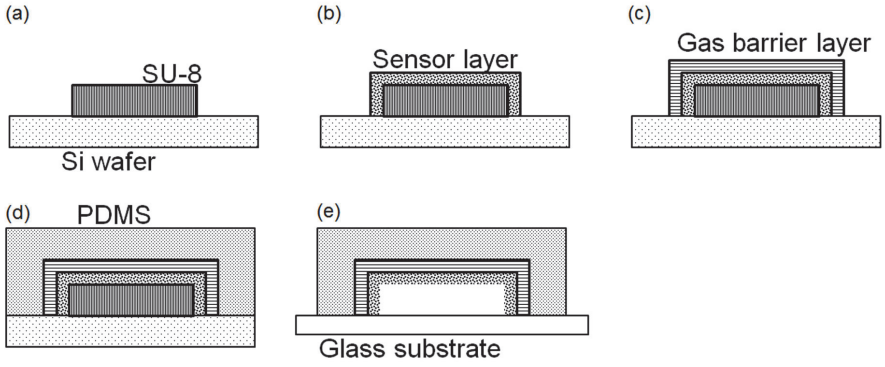
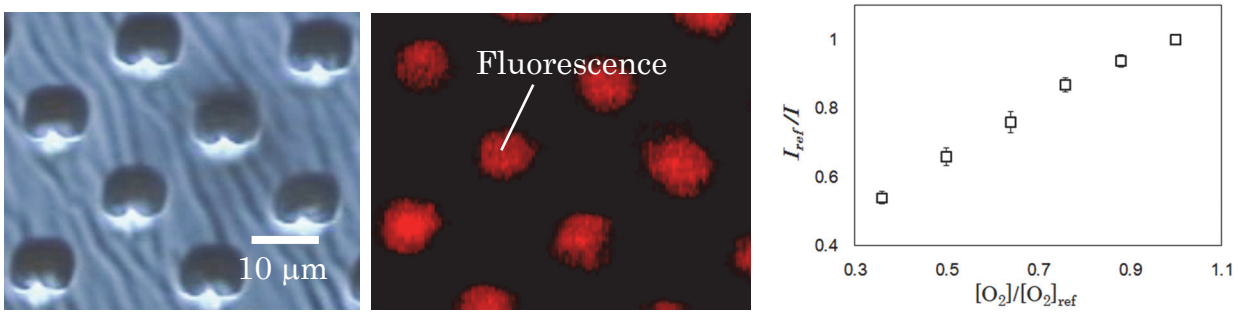
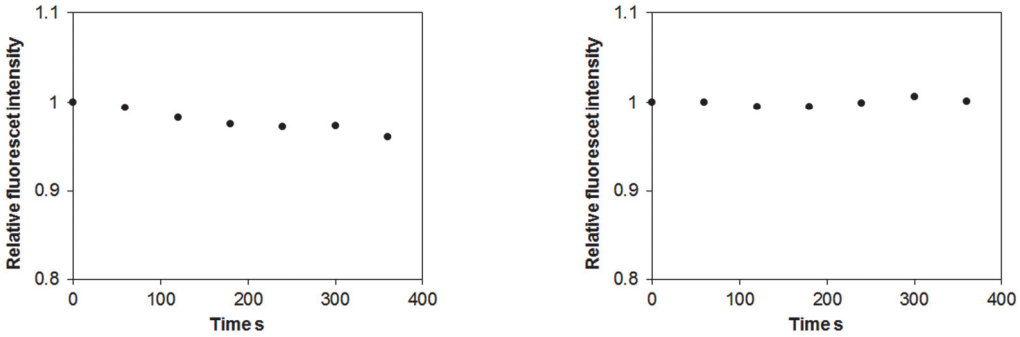


Figure 2. Fabrication process of microchamber with oxygen sensor and gas barrier layer. (a) Spin coat of SU-8 on Si wafer. (b) Coat of sensor layer on the SU-8 mold. (c) Coat of gas barrier layer. (d) Pour of PDMS. (e) Peer off from Si wafer and bond to glass substrate.



(a) (b) (c)

Figure 3. Microchambers with oxygen sensor and gas barrier wall. (a) Photo of microchambers. (b) Fluorescent image of the chambers. (c) Calibration result of oxygen concentration and fluorescent intensity.

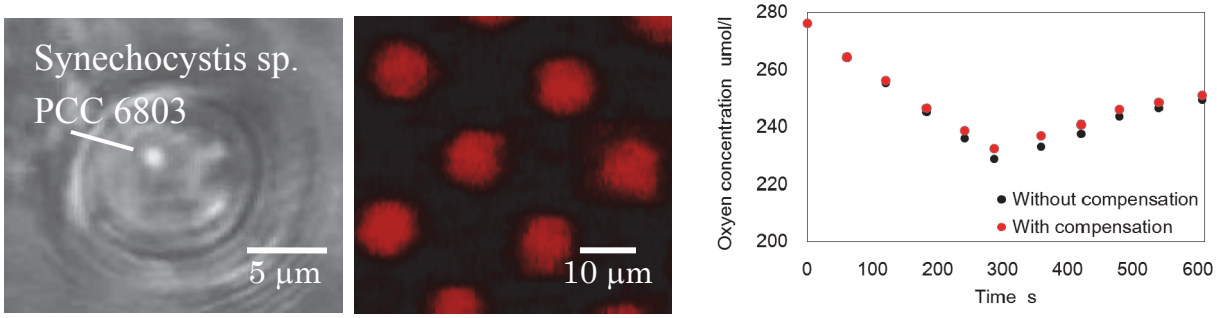


(a) (b)

Figure 4. Compensation of photo-degradation using difference compensation method. (a) Without compensation. (b) With compensation

RESULTS AND DISCUSSIONS

Figure 5 shows the measurement results. The size of the Synechocystis was about 2 μm. The chamber was sealed by the glass substrate after injection of the 20 % fructose solution containing Synechocystis. Chamber was set in darkness until 300s and was set in bright field (2000 lux). Imaging time was 60 s. From experimental results, oxygen consumption ratio was $0.6*10^{-16}$ mol/s (without compensation: $0.7*10^{-16}$ mol/s) and oxygen generation ratio was $2.0*10^{-16}$ mol/s (without compensation: $2.3*10^{-16}$ mol/s). Compensated value is near to the oxygen consumption ratio measured by group cell in previous work ($0.42*10^{-16}$ mol/s). From equation 2, we calculated the sugar production ratio of single Synechocystis by photosynthesis was 25 fmol/s. This method is possible to identification of the gene contributing the photosynthesis using disruptant of Synechocystis sp. PCC 6803 and other microorganism. From this result, we confirmed the effectiveness of our proposed cell analysis approach.



(a) (b) (c)

Figure 5. Measurement of photosynthesis activity of single Synechocystis sp. PCC 6803. (a) Photograph of single Synechocystis sp. PCC 6803 in microchamber. (b) t = 0 s. (c) Measurement result of oxygen concentration in microchamber.

CONCLUSIONS

We measured aerobic respiration and photosynthesis of single Synechocystis sp. PCC 6803 in a microchamber as single cell activity analysis. We also developed difference compensation method for precise fluorescence measurement. This method can be used to measure the physiological properties in a single cell and can be used for analysis of cell to cell communication. This technique will make a great contribution in cell biology.

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