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USING DIELECTROPHORESIS AND ELECTROROTATION TECHNIQUES TO ASSESS THE DIELECTRIC PROPERTIES OF *SCENEDESMUS ABUNDANS* AT DIFFERENT GROWTH STATES

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ABSTRACT

Microalgae cellular contents are affected by various factors such as temperature, carbon dioxide, light intensity and so on. This study finds that microalgae cells were heterogeneous in terms of dielectric properties although their growth curves and nutrient consumption rates were similar. The shifting of dielectrophoresis direction between different culture generations of microalgae showed the gradual change of cell wall structure and compositions along the cultivation. Additionally, electrorotation spectra of microalgae cells behaving the same in dielectrophoresis were different. Two groups of cells were found in the log phase while the spectra of cells in the stationary phase were relatively uniform, indicating cellular contents were diverse in the log phase but similar in the stationary phase among individual cells. These results show that dielectrophoresis and electrorotation are informative and rapid technologies for analyzing microalgae cellular compositions with single cell resolutions.

KEYWORDS: Microalgae, Dielectrophoresis, Electrorotation, Single Cell Analysis

INTRODUCTION

Microalgae are photosynthetic microorganisms that convert carbon dioxide into lipids, high-value proteins, pigments and carbohydrates and they are well known for carbon neutrality. Previously, lots of efforts have been made to study the effects of various cultivation strategies on the enhancement of microalgal products. However, the cultivation strategies are species and strain dependent and conventional analysis are time consuming, resulting in the slow progress of advances. In this study, dielectrophoresis and electrorotation techniques were applied to rapidly monitor the changes in dielectric properties of single microalgae cell at different growth phases. Compared with other electrical characterization techniques such as impedance spectroscopy [1], dielectrophoresis and electrorotation do not require high concentration of cells to perform the measurement. Additionally, they are single cell measurement techniques, being insensitive to the volume factor [2].

THEORY

The dielectrophoresis (DEP) force, which expression is shown in equation (1), drives the cell to the highest (positive DEP) or lowest (negative DEP) electric field regions, depending on the polarizability of the cell compared to the polarizability of the suspending medium. The direction of the DEP force, linked to the real part of the Clausius Mossotti factor, also depends on the frequency of the electrical field that is applied:

$$\vec{F}_{DEP} = 2\pi R_{cell}^3 \epsilon_0 \epsilon_m Re[f_{CM}(\omega)] \vec{\nabla} E_{RMS}^2 \quad (1)$$

where ϵ_0 and ϵ_m respectively stand for the permittivities of the vacuum and the external medium, R_{cell} is the radius of the cell, $Re[f_{CM}(\omega)]$ is the real part of the Clausius-Mossotti factor and E is the electric field.

During electrorotation experiments, the rotational speed, which is shown in equation (2), is proportional to the electric field and the imaginary part of the Clausius Mossotti factor.

$$\Omega_{cell} = \frac{\epsilon_0 \epsilon_{medium} E^2}{2\eta} Im(f_{CM}(\epsilon_{cell}^*, \epsilon_{medium}^*)) \quad (2)$$

where η is the dynamic viscosity of the medium, $Im(f_{CM}(\epsilon_{cell}^*, \epsilon_{medium}^*))$ is the imaginary part of the Clausius-Mossotti factor which is affected by cellular compositions such as lipids, carbohydrates, proteins.

EXPERIMENTAL SETUP

Microalgae cells at different growth states were monitored using dielectrophoresis, electrorotation, and optical density. Growth curve of *S. abundans* and nitrate concentration were measured by UV/Visible spectrophotometer (Biochrom Ltd.) with different wavelengths. The amount of chlorophyll was measured at 682 nm which was also proportional to the amount of cell amount in the log phase. For the nitrate quantification, the supernatant of sample was collected by centrifugation (9000 rpm, 3 minutes) and measured at 220 nm. The sample was diluted to an OD less than one and the actual OD value was obtained by multiplying the measured value by the dilution factor. All OD measurement were carried out in duplicates.

Four planar parabolic electrodes with a 75 μm gap (Figure 1a) on the quartz wafer were fabricated based on conventional photolithography technology. Microalgae samples were rinsed three times and resuspended in BG-11 medium with 0.02 S/m conductivity.

Microalgae sample of 15 μl with a cell concentration of 5×10^5 cells/ml was infused between a glass slide and the quartz wafer separated by the 3M tape with a thickness about 50 μm . The electrodes were then connected with a Printed Circuit Board (PCB) which synthesized electric signals for single cell trapping (conventional dielectrophoresis, DEP), and electrorotation experiment (ROT) [3]. The

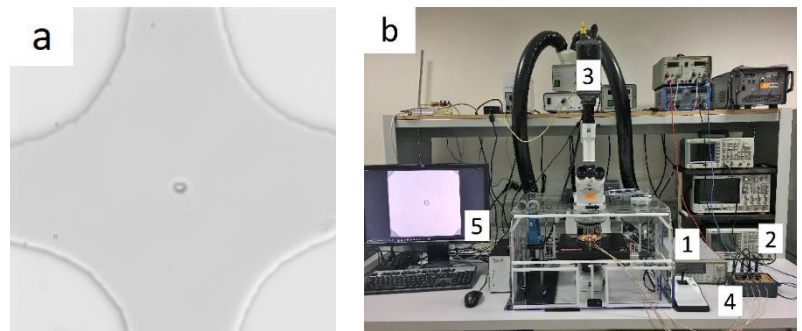


Figure 1 (a) Single microalgae trapped at the center of the four-electrode set; (b) Experimental setup; 1. DEP generator; 2. ROT generator; 3. Fast camera; 4. PCB; 5. Computer.

setup is shown in Figure 1b. The conventional dielectrophoresis signals (3 Vpp, 35 kHz) were applied to drive the cell to the highest and lowest electric field regions which was the positive and negative dielectrophoresis, respectively. The behavior of dielectrophoresis depended on the polarizability of the cell component compared to the polarizability of external medium. If microalgae were less polarizable than the medium (negative dielectrophoresis) which was trapped at the center of the four-electrodes set (Figure 1a). After single cell was trapped, the rotating electric field (3 Vpp, 37 kHz to 25 MHz) was then applied to rotate the microalgae to obtain the electrorotation spectrum.

RESULTS AND DISCUSSION

To understand the growth of microalgae, their growth curve and nitrate amount were monitored by spectrophotometer as shown in Figure 2. Growth curve of *S. abundans* were in the log phase in the first 4 days and entered the stationary phase between day 4 and 6. The optical density was doubled in 0.5 day ~ 0.75 day for all batches and an optical density higher than 10 can be reached in 3 days. The rapidly decreasing amounts in nitrate (OD_{220}) also indicates the highly thriving culture. Although the growth curve and nitrate consumption rate were similar between all batches, the dielectrophoretic properties were various as shown in Table 1. In batch 1 generation 2, 100 % of cells experienced positive dielectrophoresis; however, in batch 1 generation 5, 100 % of cells experienced negative dielectrophoresis. As the generation increased from 2 to 5, the percentage of *S. abundans* cells experienced negative dielectrophoresis increased proportionally. The change of cell wall composition and its surface properties is the probable cause of this phenomenon since the dielectrophoresis was conducted under relatively low frequency, making cell wall the most sensible cellular part toward the electric field.

Additionally, even though all cells experienced negative dielectrophoresis in one batch, their electrorotation

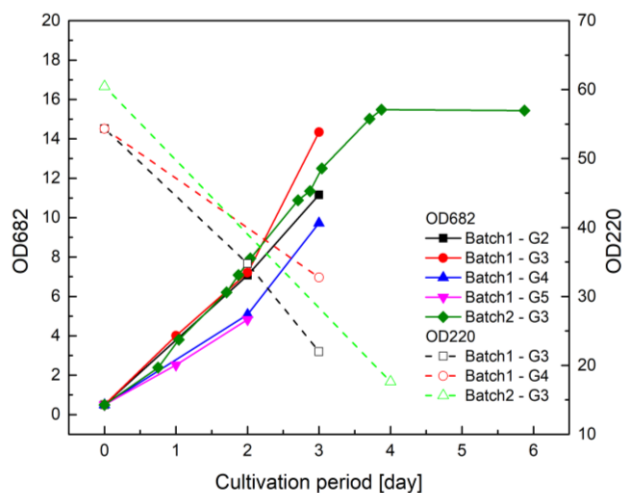


Figure 2. Growth curve of *S. abundans* and the nitrate consumption rate. OD_{682} indicates the amount of Chlorophyll and is usually proportional to the cell number before entering stationary phase. OD_{220} indicates the amount of nitrate in the medium.

spectra were different. In batch 2 generation 3, all cells experienced negative dielectrophoresis and their electrorotation spectra were shown in Figure 3. In log phase, 80 % of the cells had a maximum rotational of 40 rps while 20 % of cells had 10 rps. As the duration of incubation increased, the percentage of cells having maximum rotational speed over 30 rps increased from 20 % to 50 %. Unlike dielectrophoresis, the electrorotation was conducted under a wide range of alternating frequency of electric field and the spectrum not only shows the change of cell wall but also other cellular compositions such as proteins, carbohydrates, and lipids. Therefore, these results indicate that not only the composition of cell wall but also other cell

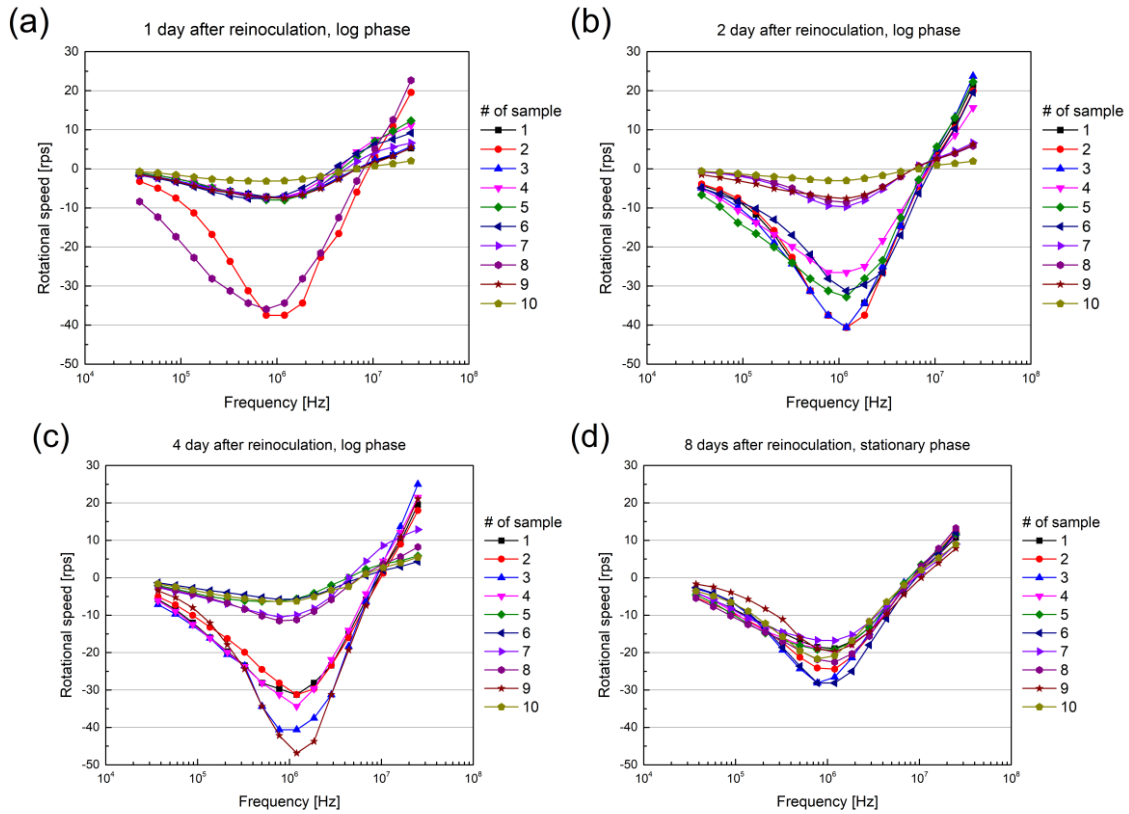


Figure 3. Electrorotation spectra of *S. abundans* at different growth phases. (a) 1 day after reinoculation, log phase; (b) 2 days after reinoculation, log phase; (c) 4 days after reinoculation, log phase; (d) 8 days after reinoculation, stationary phase.

compositions changed during the cultivation. After reaching the stationary phase, the difference of cellular compositions between cells diminished and their electrorotation spectra became alike. Interestingly, the maximum rotational speed fell within 15 to 30 rps in the stationary phase, which was slightly lower than the maximum speed of 40 rps in the log phase. Further investigations for the change of cellular compositions are required to reveal the reason of this change. We are also working on the mathematical model describing the effects of dielectric parameters of cell wall, cell membrane, and cytoplasm on the electrorotation spectrum. Combining the information of cellular compositions and mathematical model, the change of cellular contents in microalgae can be interpreted from the observed electrorotation spectra.

Table 1. Dielectrophoresis behavior of different batches and generations

| | Positive dielectrophoresis [%] | Negative dielectrophoresis [%] |
|--------------|--------------------------------|--------------------------------|
| Batch1 – G2* | 100 | 0 |
| Batch1 – G3 | 60 | 40 |
| Batch1 – G4 | 42 | 58 |
| Batch1 – G5 | 0 | 100 |
| Batch2 – G3 | 0 | 100 |

*All the data were obtained from ten cells except Batch1 – G2

CONCLUSION

Even though the growth curve and nitrate consumption were similar in different batches and generations, microalgae cells still had differences in dielectric properties, which depends on cellular structure and compositions. The change of cell wall structure and compositions of *S. abundans* cells was successfully observed using the dielectrophoresis at relatively low alternating frequency of the electric field. Additionally, the variation of electrorotation spectra of *S. abundans* cells in the log phase show the change of cellular compositions including not only cell wall but also lipids, protein,...etc. The similar electrorotation spectra of different cells in the stationary phase indicate these cells had similar cellular compositions. Therefore, dielectrophoresis and electrorotation are innovative and informative technologies for monitoring the change of cellular compositions of microalgae during the cultivation.

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