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MONITORING THE GROWTH PHASES OF MICROALGAE USING STRAIGHTFORWARD DIELECTROPHORESIS MEASUREMENTS

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ABSTRACT

This study demonstrates a straightforward method for differentiating single microalgal cells in different growth phases based on dielectrophoresis (DEP). The balance of DEP force and drag force leads to an easy-to-use linear relation between DEP velocity and the real-part of the Clausius Mossotti factor, which determines the velocity of DEP. The DEP velocity of microalgae in the stationary phase is slower than microalgae in the exponential phase, owing to the cellular compositions changed during growth. Microalgal cells in the stationary phase also showed a higher transition frequency than cells in the exponential phase.

KEYWORDS: Microalgae, Growth Phases, Dielectrophoresis Measurement

INTRODUCTION

Photosynthetic microalgae convert carbon dioxide into high-value metabolic products such as pigments, proteins, and lipids. Microalgae produce metabolic products such as pigments, proteins, lipids in different growth phases and the changes of cellular compositions can be considerable in a short period of time [1]. However, conventional analyses either are time-consuming or require fluorescent labels. Our previous study showed that microalgae cells have apparent differences in dielectrophoresis and electrorotation between generations and growth phases [2]. In this study, a straightforward method for identifying cellular status based on dielectrophoretic movements is developed and applied to the monitor of microalgae in different growth phases.

THEORY

Dielectrophoresis (DEP) force, as shown in equation (1), drives a cell to the highest (positive DEP) or lowest (negative DEP) electric field regions, depending on the polarizability of the cell compared to its medium. The direction of DEP force (\vec{F}_{DEP}) is determined by the real part of the Clausius Mossotti factor ($Re[f_{CM}(\omega)]$).

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

where ϵ_0 and ϵ_m respectively stand for the permittivities of the vacuum and the medium, R_{cell} is the radius of the cell and ω is the angular frequency of applied electric field E .

When the movement of a cell is at steady state, dielectrophoresis force is balanced with the viscous drag as shown in equation (2) [3]. In equation (2), v is the velocity of the cell and δ is the distance between the wall and the microalgae cell. The velocity v , shown in equation (3), thus can be obtained by balancing equation (1) and (2).

$$F_{drag} = 6\pi\eta R_{cell} v (-(8/15) \ln(\delta/R_{cell}) + 0.9588) \quad (2)$$

$$v = \frac{R_{cell}^2 \epsilon_0 \epsilon_m Re[f_{CM}(\omega)] \vec{\nabla} E_{RMS}^2}{2\eta((8/15) \ln(R_{cell}/\delta) + 0.9588)} \quad (3)$$

EXPERIMENTAL

The microalgae *Scenedesmus abundans* was cultured in BG-11 medium. The growth was monitored by UV/Visible spectrometry and dielectrophoretic movements. The growth of *S. abundans* was measured by a spectrophotometer at wavelengths of 682 nm (OD682) and 220 nm (OD220), which represented the total amount of chlorophylls and nitrate concentration, respectively. The dielectrophoretic movements were measured between 4 planar parabolic electrodes (Figure 1a) with a 75 μ m gap on the quartz wafer. The electrodes were fabricated by conventional photolithography technology and the fluidic chamber was constructed by 3M tapes. The conductivity of the medium was adjusted to 0.02 S/m for DEP measurement. The electrodes were then connected to two-channel function generators (AFG3102, Tektronix, USA) with electric signals of 3 Vpp, 180° phase shift,

and 5 frequencies ranging from 37 kHz to 38.6 MHz. At each frequency, at least 10 movements of microalgal cells induced by DEP forces were recorded during 5 seconds by a high-speed camera with a frame rate of 100 fps.

RESULT AND DISCUSSION

The amount of chlorophylls and nitrate consumption of *S. abundans* were monitored daily to assess the growth conditions of microalgae as shown in Figure 1b. The OD682 (total chlorophylls) doubled and OD220 (nitrate) decreased by 68 % in the first fifth days. The rapid rates of chlorophylls increase and nitrate consumption are a characteristic of the exponential phase of microalgae. The much slower increase in OD682 and decrease in OD220 after the 7th day of the reinoculation indicated the stationary phase had been reached. *S. abundans* cells were subjected to dielectrophoresis at the second, fourth and eighth days after the reinoculation.

The DEP velocities of microalgae shown in Figure 1c indicated that DEP velocity of microalgae in the stationary phase is slower than the one of microalgae in the exponential phase. Moreover, the movement of microalgae in the exponential phase changed from negative DEP velocity to positive DEP velocity (i. e. the transition frequency) at much lower frequency (253 kHz) compared with microalgae in the stationary phase (436 kHz). The differences in the above dielectric behavior were the results of changing cellular compositions during growth and can be applied to separate cells with different physiological status.

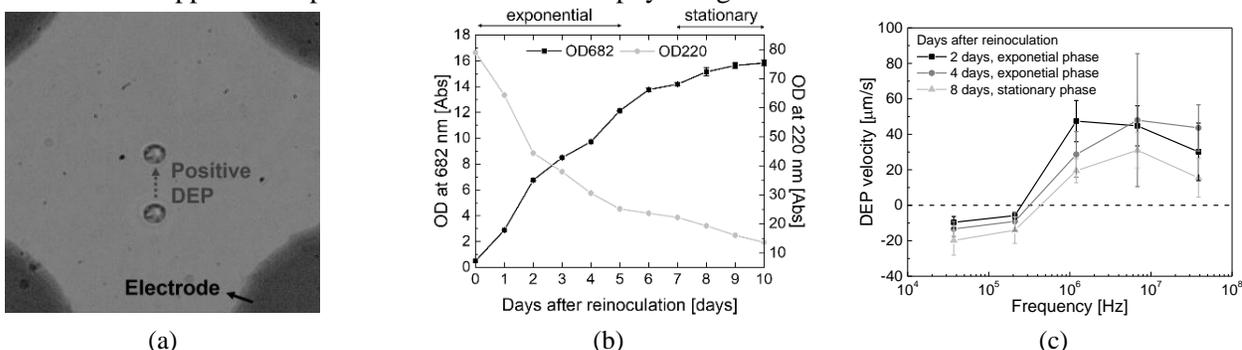


Figure 1: (a) Single microalgal cell trapped and moved to the center of the electrode set by positive DEP force. (b) Growth curve of *S. abundans* (OD682) and the nitrate concentration in BG-11 medium (OD220). (c) DEP velocity of *S. abundans* at different days after reinoculation.

CONCLUSION

This study demonstrated a straightforward method for discriminating microalgal cells in different growth phases using dielectrophoresis. When DEP force is balanced by the drag force, the variation in dielectric properties of microalgal cells at different physiological states lead to different DEP velocities. The DEP velocity of microalgal cells in the stationary phase are slower than those in the exponential phase, making DEP a straightforward method for separating microalgal cells in different growth phases.

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