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Separation of microalgae with different lipid contents by dielectrophoresis

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HIGHLIGHTS

▶ We propose a new strategy to separate microalgae by utilizing electrokinetic force.
▶ Effect of solution conductivity on the crossover frequency for the microalgae was examined.
▶ Separation of microalgae with different lipid contents by dielectrophoresis was demonstrated.

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ABSTRACT

In this study, the effect of the solution conductivity on the behavior of microalgal cells (*Chlorella*) with different lipid contents under a non-uniform electric field was investigated. It was found that, for the algal cells with 11 wt% lipid content, the crossover frequency is between 2 and 10 MHz when the solution conductivity is within 1.4 and 2.95 mS/cm, and increases as the solution conductivity increases. As to the microalgal cells with 45 wt% lipid content, they experienced negative DEP at frequency below 20 MHz when the solution conductivity is within 2.06 and 2.95 mS/cm. However, positive DEP was observed when the solution conductivity becomes 1.4 mS/cm. In a mixture of the algal cells, those with different lipid contents were successfully separated by DEP at solution conductivity of 2.95 mS/cm and frequency of 20 MHz.

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1. Introduction

In recent years, substitute for petroleum fuel has become a frequently discussed issue in the territory of energy and been gaining more and more attention. Among different types of renewable energy sources such as sunlight, wind, hydropower, biofuel, geothermal heat, and so on, biofuel is considered as one of the most promising energy sources, which is acquired from plant or microalgae to meet global consumption of fuel. There are several advantages of using lipid inside microalgae as the feedstock for biofuels since microalgae are highly efficient in transferring solar energy into energy-rich compounds, mitigating global warming, and their cultivation has a low requirement in arable land and freshwater, especially for marine microalgal species (Chisti, 2007; Williams, 2007). Since extracting lipid fraction from the lipid-rich microalgae will facilitate in acquiring biomass more efficiently, various cultivation methods have been developed to increase lipid content inside microalgae in short cultivation cycles (Chen et al., 2010, 2011; Satyanarayana et al., 2011; Singh and Olsen, 2011). Once the microalgae are cultivated, the algae biomass is harvested by various methods such as centrifugation, flocculation, sedimentation, floatation, and electrophoresis (Mutanda et al., 2011). Some issues were addressed on improvement of harvesting techniques or system. For example, poor performance of floatation response of marine microalgae was found due to their lower degree of hydrophobicity and the use of cationic surfactant can improve its floatation recovery (Garg et al., 2012). The continuous electrolytic microalgae (CEM) harvest system using polarity exchange was investigated and the performance of CEM can be greatly enhanced by choosing the suitable electrodes (e.g. Al-DSA) and optimizing the timing of polarity exchange (Kim et al., 2012). After recovery of microalgae, lipid extraction is performed and various techniques for cell disruption such as bead beating, microwaves, sonication, autoclaving, osmotic shock have been evaluated to increase lipid extraction efficiency (Lee et al., 2010). It was reported that the osmotic shock treatment can be employed directly to the wet algae biomass and the lipid recovery could be increased approximately two times (Yoo et al., 2012). Meanwhile, selection of lipid-rich microalgae can be achieved via using the conventional methods

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such as extraction, fluorescence measurement and gram measurement to first determine the lipid content inside microalgae, followed by separation. However, they are time consuming, complicated in process (Bligh, 1978; Montero et al., 2011; Priscu et al., 1990) and cannot be achieved in a timely manner. Therefore, it is of great demand to develop faster, more efficient and high-throughput tools for the screening and selection of lipid-rich microalgae species from the environment.

Dielectrophoresis (DEP) is one of the mechanisms occurred in AC electrokinetics and commonly applied to manipulate microscopic particles in microfluidic devices. The fundamental principle of DEP was first presented by Phol in 1970s and has subsequently been utilized to separate many kinds of microparticles (Falokun et al., 2003; Green and Morgan, 1997; Morgan and Green, 1997; Pohl et al., 1978; Ramos et al., 1998). Briefly speaking, there are two types of DEP motion induced by non-uniform electric field. When particles move to the region with the strongest electric field gradient (i.e. the edge of the electrodes), they experience so called positive DEP (pDEP). When particles move to the region with the lowest electric field gradient (i.e. the center of the electrodes), they experience so called negative DEP (nDEP). For a spherical neutral particle, the time-averaged DEP force is given by the following equation

$$F_{\mathrm{DEP}} = 2\pi a^3 \varepsilon_{\mathrm{m}} \mathrm{Re}(f_{\mathrm{CM}}) \nabla |E|^2$$

where a is radius of the particle, ε_{m} is the permittivity of the medium, $\bigtriangledown|E|^2$ is the gradient of the square of the applied electric field strength, and $\mathrm{Re}(f_{\mathrm{CM}})$ is the real partition of the complex Clausius–Mossotti (CM) factor. The CM factor is given by the following:

$$f_{\mathrm{CM}} = (\varepsilon_{\mathrm{p}}^* - \varepsilon_{\mathrm{m}}^*)/(\varepsilon_{\mathrm{p}}^* + 2\varepsilon_{\mathrm{m}}^*)$$

where $\varepsilon^* = \varepsilon - i(\sigma/2\pi\omega)$ is the complex permittivity, and the subscripts p and m stand for particle and medium, respectively. σ is conductivity, ω is the frequency of the applied alternating current, and $i = \sqrt{-1}$. The value of $\mathrm{Re}(f_{\mathrm{CM}})$ ranges from −0.5 to 1., The particle experiences pDEP force when $\mathrm{Re}(f_{\mathrm{CM}})$ is greater than 0 and nDEP when $\mathrm{Re}(f_{\mathrm{CM}})$ is less than 0. The particle does not experience any DEP when $\mathrm{Re}(f_{\mathrm{CM}})$ is equal to 0 and, under this circumstance, the operating frequency is called the crossover frequency. Determination of particle motion becomes less straightforward when the particle has multi-shell structure where there might be many crossover frequencies and the $\mathrm{Re}(f_{\mathrm{CM}})$ becomes complicated.

DEP as the driving force has been demonstrated to separate or trap bio-particles. For example, Green et al. used interdigitated castle electrode to separate latex beads with different sizes and trap various types of viruses (Morgan et al., 1999). Doh and Cho (2005) combined DEP with fluid flow to separate dead and live yeast cells in the microfluidic device. Hu et al. utilized dielectrophoretically responsive label to discriminate labeled *Escherichia coli* from those without being labeled in the DEP-activated cell sorting device (Hu et al., 2005). Cheng et al. (2010) applied DEP to separate and collect pathogen at the roughened Au surface for SERS detection. Sabuncu et al. (2010) used DEP to separate mouse melanoma clones with different melanin contents.

Although DEP has been demonstrated to be an effective tool in the separation of various microorganisms and bio-particles, there is still no example in the literature showing the use of DEP in separating microalgal cells. Thus, in this study, the first attempt to use DEP to separate microalgae (*Chlorella vulgaris*) with different lipid contents was made. In addition, the effect of solution conductivity on crossover frequency will be examined and discussed.

2. Methods

2.1. Sample preparation

Cultivation procedures of *C. vulgaris* with different lipid contents were described in our recent work (Yeh and Chang, 2011; Yeh et al., 2010). To distinguish microalgae with 45 wt% lipid content from those with 11 wt% lipid content, *Nile Red* fluorescence dye was used to label the microalgae with 45 wt% lipid content. One millilitre of original microalgae solution with 10^6 cells/ml was concentrated to 10^7 cells/ml via centrifugation. The algal solution was then mixed with 1 μl *Nile Red* stock solution (Sigma–Aldrich, Inc.) for 10 min using vortex machine, followed by being washed three times with KH_2PO_4 buffer solution. The conductivity of the algal solution was adjusted by proper amount of KH_2PO_4 buffer solution with conductivity 14.3 mS/cm.

2.2. Fabrication of DEP microfluidic device

To construct a DEP device, the glass slide was first cleaned by basic washing solution (NH_4OH:H_2O_2:H_2O = 1:1:5) at 75 °C for 1 h. The Ti adhesion layer was then deposited on the cleaned slide, followed by deposition of Au conductive layer using electron beam evaporator. After the photolithographic process, the parallel lines with 30 μm width and spacing were defined on the substrate and the line electrode array was obtained through wet etching process. The 30 μm thick PET tape was cut through by CO_2 laser machine and placed between the cover slide and parallel line electrode array to form the channel with 30 μm in depth. The copper leading wires were then glued to the parallel line electrode array and connected to the AC function generator.

2.3. Separation of microalgae by DEP

The crossover frequencies of each type of algal cells with different lipid contents need to be found prior to separating the microalgae with different lipid contents.

These crossover frequencies were determined through frequency sweep using functional generator which can provide sine wave from 20 MHz to 1 mHz and voltage from 0.1 to 10 Vp-p. The frequency sweep was performed by first injecting 0.5 ml solution with algae cells into the DEP microfluidic device with line electrode array. The AC power with 10Vp-p was then turned on and the frequency was tuned from 20 to 1 MHz or lower frequencies. The movement of the algal cells was observed by the inverted fluorescent microscope (Nikon TE2000-S) and recorded by the CCD camera (Photometrics, Cool Snap-HQ2) for analysis. The KH_2PO_4 buffer was used to adjust the conductivity of the solution.

After frequency sweeping for both algal cells with 11 and 45 wt% lipid contents, the suitable AC frequency and electrical conductivity of buffer solution were determined and the mixture solution of these two types of microalgae (1:1 v/v) was loaded into our device to perform DEP separation. Note that the fluorescence from chlorophyll inside microalgae overlaps with that from *Nile Red* used to stained the lipid content inside algal cells. Therefore, to show the fluorescence from *Nile Red* only, the filter with appropriate range of wave length (515–555 nm) was used to screen the fluorescence from chlorophyll.

3. Results and discussion

3.1. Effect of solution conductivity on crossover frequency

Fig. 1 shows the frequency sweep for the microalgae with 11 wt% lipid content at different solution conductivities. The

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