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Synergistic effect of multiple stress conditions for improving microalgal lipid production

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ABSTRACT

This increasing consumption of fossil fuels and emission of greenhouse gases causes climate change. Thus, the development of alternative energy sources such as biofuel is necessary. Microalgae have been spotlighted as renewable energy resources because the potential for producing biofuels from CO_2. However, the unit cost for the production of algae-based biofuel must be reduced for commercialization compared to the petroleum. The optimization of culture conditions of microalgal strains is crucial to increase lipid production for economically viable biofuels. We rapidly analyzed the combined effect of various stress conditions (nitrogen starvation, temperature, pH, and salt concentration) on the lipid production in various strains using a multiplex microfluidic system, enabling multiple operations from cell culture to lipid extraction of different strains. We found the lipid productivity was enhanced by 25 to 54% under combinations of two stress condition compared to the single stress condition. However, the combination of more than three stress conditions reduced the lipid productivities of all microalgal strains because of more stressful environment to the cells compared to the combinations of two stress conditions. We further validated the synergistic effect of combined stress conditions in flask culture with the increases in lipid productivities by up to 106% compared to the single stress condition. We also observed that fatty acids composition, which influences the quality of algal biofuels, was changed according to the combination of stress conditions. In particular, *C. protothecoides* can be good candidate for production of high quality of biodiesel, because it has high CN, IV and low CFPP which is suitable for high quality of biodiesel. These results indicate that combination of multiple stress conditions can be efficient strategy for the optimization of microalgal cultivation to produce algal biofuels with high quality and economic feasibility.

1. Introduction

The global CO_2 emissions from fossil fuel use were almost 9.795 gigatonnes (Gt) in 2014 [1]. Currently, the CO_2 concentration in the atmosphere has increased from approximately 277 ppm (ppm) in 1750 [2] to 395.31 ppm in 2013 [1]. In addition, the CO_2 concentration in the atmosphere is about 60% above 1990 levels [3]. The CO_2 emission should be reduced by 50% for limiting the rise of global average temperature to 2 °C by 2050. Therefore, carbon capture and utilization (CCU) recently has received worldwide attention because it enables conversion of CO_2 into valuable products such as fuels and polymers [3]. In particular, biological CCU by photosynthetic organisms including microalgae has the advantage of negative CO_2 emission [4]. However, low biomass and lipid productivity remain barriers to economic viability of algae-based biodiesel. Therefore, it is important to find strains with fast growth rate and high lipid productivity. In addition, it is necessary to optimize the environmental condition for improving lipid productivity of selected strains.

To date, there has been a wide range of studies for lipid accumulation in various microalgal strains using nutrient starvation [5], high salt concentration [6], high temperature condition [7] and pH shift [8] due to the ability of microalgae to synthesize neutral lipid such as triacylglyceride (TAG) under stress conditions as defense mechanisms [9]. In addition, several studies reported the synergistic effect of several stress conditions on the lipid productivity in a selected strain [10,11].

Abbreviations: CCU, carbon capture and utilization; TAG, triacylglyceride; PDMS, polydimethylsiloxane; FA, fatty acid; FAME, fatty acid methyl ester; SV, saponification value; CFPP, cold filter plug point; DU, degree of unsaturation; IV, iodine value; CN, cetane number; PUFAs, polyunsaturated fatty acids; MUFAs, monounsaturated fatty acids; TP medium, tris-phosphate medium; LCSF, long chain saturation factor.

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Therefore, comparative analysis of the combined effect of multiple stresses on lipid productivity in diverse algal strains can provide more useful information for optimization of culture conditions to improve algal lipid production. However, the quantitative analysis of microalgal productivity and optimization of culture condition rely on conventional benchtop methods, which require time-consuming and laborious tasks including cell harvesting, medium changing, resuspension, dewatering, lipid extraction and phase separation [12]. To overcome this obstacle, it is necessary to develop a new platform for rapid and efficient evaluation of the cell performances such as biomass and lipid productivity.

Microfluidic approaches are very useful for biochemical analysis [13]. Microfluidic systems have been applied to various biological research fields including DNA microarrays [14], manipulation of single cells [15] and point-of-care diagnostics [16]. Microfluidics also enables rapid evaluation of the diverse conditions at the same time on a single device because microfluidic systems can reduce analysis volume and space restriction associated with macroscale methods. Polydimethylsiloxane (PDMS), a widely used material in microfluidic systems, has a strong advantage for cultivation of cells due to its high gas-permeability and transparency that allow sufficient aeration for cell cultivation and efficient monitoring of cell physiology such as cell behavior and growth at the cellular level which were not possible through conventional methods [12,17]. These properties are essential for the culture of microalgae, which require light and CO_2 for their growth. Thus, various PDMS-based microfluidic systems have been applied to the microalgae, particularly, for high-throughput screening of growth and oil contents of diverse strains using micro-droplet [18,19] or microfluidic devices with well-defined design [20–23].

In a previous study, we developed a novel PDMS-based microfluidic analytical system, which is able to perform multiplex lipid sample preparation processes and simplify the complex downstream processes by integrating the sequential operations from cell culture to lipid extraction [12]. In addition, the lipid extraction efficiency using aqueous alcohol at microfluidic device was improved up to 102.6% compared to the Bligh-Dyer lipid extraction method, because of the large surface area, mass transfer, stable maintenance of temperature and proper pressure for cell lysis and lipid extraction. Here, we applied the microfluidic system for rapid analysis of various stress conditions including nitrogen starvation, pH shift, high salt concentration, and high temperature for improving lipid productivity in diverse microalgal strains. Using this multiplex microfluidic device, we rapidly investigated all combination of four different stress conditions: two by two, three by three, and all four. Then, we validated the results from the microfluidic approach in flask culture with 200-fold larger volume than the microfluidic chamber. We focused on the lipid production of different microalgal strains under diverse stress condition. Thus, we calculated the lipid productivity and lipid contents of each microalgal strains in this study. Finally, we also observed changes of fatty acids (FAs) composition by different environmental stress conditions, which can affect the quality of algae-derived biodiesel. Our approach can provide useful solutions to optimization of microalgal cultivation process for improving lipid productivity as well as the quality of algal biofuels.

2. Material and methods

2.1. Microalgal strains and preculture condition

Eight different microalgal strains, which have been frequently studied for lipid production [24–27], were used in this study. Three different *Chlamydomonas reinhardtii* strains were obtained from the Chlamydomonas Resource Center at University of Minnesota; CC124 (*mt − agg-1 nit1 nit2*), CC125 (*mt + nit1 nit2*), CC4348 (*BAF-J5*; *cw15* arg*7-7 nit1 nit2 sta6-1::ARG7*). *Chlorella vulgaris* (UTEX2714) and *Chlorella protothecoides* (UTEX256) and *Neochloris oleoabundans* (UTEX1185) were obtained from the Culture Collection of Algae at University of Texas. *Chlorella zofingiensis* (AG10067) and *Scenedesmus* sp. (AG10266) were obtained from the Korean Collection for Type Cultures. All cells were grown in 100 ml flask containing tris-phosphate medium (TP, TAP medium without acetate) at 23 °C at 120 rpm under the light intensity of 50 μmol photons m^{-2} s^{-1} with 5% CO_2-enriched air.

2.2. Fabrication of multiplex microfluidic device

The multiplex microfluidic device was fabricated using standard soft photolithography [28]. The microfluidic device consists of two PDMS layers [12]. The bottom layer has eight different culture chambers (500 μl) and inlets. The top layer contains the eight microchannels (4 mm in length, 2 mm in width, 3 μm in height) filled with square micropillar array structures (10 μm by 10 μm in cross-section) that prevent the cell leakage and filter cells and cell debris during medium change and solvent extraction. The bottom layer was fabricated by pouring PDMS prepolymer (10:1 mixture of 184 sylgard base and curing agent, Dow Corning) onto SU-8 silicon mold and was cured at 80 °C in an oven. The bottom layer and the glass (70 mm in length and width) were bonded after oxygen plasma treatment. The top layer was fabricated using more flexible PDMS prepolymer (20:1 mixture of 184 sylgard base and curing agent) onto SU-8 masters and was cured at 80 °C in an oven for 10 min. After the top layer has been attached to the bottom layer, the device was cured at 80 °C in an oven for perfect combination of two layers. Then, chambers and microchannels in device were immediately filled with TP medium.

2.3. Microalgal culture and lipid accumulation in multiplex microfluidic device

Cells precultured in TP medium were diluted to an OD_{800} of 0.1. The diluted cell cultures were inoculated into the chambers of the microfluidic device using a syringe pump (PHD 2000, Harvard Apparatus) at a flow rate of 200 μl min^{-1}. The cells in the microfluidic device were cultivated for 4 days at 23 °C with 5% CO_2-enriched air. To avoid evaporation of the moisture in the microfluidic device, the cultures were conducted under high humidity condition. The cell growth was measured by optical density at 800 nm using a microplate reader (BioTek, USA). The specific growth rate was calculated using the Gompertz function [29]. For lipid induction, the medium was changed with TP-N medium (nitrogen deficient TP medium) for N-starvation condition using a syringe pump at a flow rate of 200 μl min^{-1}. To avoid cell leakage in each chamber of the microfluidic device during the medium change, the cell inlets were blocked with 3 M tape. Keeping all variables constant, another cell culture was performed at 30 °C for the analysis of high temperature effect. For analysis of salt effect, cells were cultivated in fresh TP medium containing sodium chloride (200 mM). For analysis of pH effect, cells were cultivated in fresh TP medium at pH 4.0 (acidic condition) and pH 10.0 (alkaline condition). In addition, various combinations of stress conditions were used to analyze the combined effect of stress conditions on the lipid productivities in microalgal strains. All cell cultures were cultivated for 4 days to induce lipid accumulation after changing medium.

2.4. Evaluation of lipid productivity using microfluidic device

Alcohol is more suitable for the extraction of microalgal lipids in PDMS-based microfluidic device and can be alternative solvent for chloroform-based extraction method, because it can extract lipids from wet biomass and reduce the deformation of PDMS [30]. Lipids were extracted by flowing 10 ml of 70% isopropanol (w/w) at a flow rate of 200 μl min^{-1} into a central inlet of microfluidic device placed on a hot plate to maintain the temperature at 60 °C during the lipid extraction. During the lipid extraction, the width and height of the microchannel which is connected to the central inlet were designed by 3 and 5 μm, respectively, to prevent mixing the different microalgal strains in each chamber. After the lipid extraction, the aqueous alcohol was evaporated