EVALUATION OF ETHANOL TOXICITY TO OIL PRODUCING ALGAE USING A MICROFLUIDIC DEVICE

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ABSTRACT

Effects of ethanol toxicity to microalgae were assessed by using a microfluidic device. The developed device has a function to sample a small amount of the individual microalgae and line them up in the channel. The target microalga is *Botryococcus braunii* (*B. braunii*) which yields hydrocarbons. The hydrocarbons are able to be separated from the *B. braunii* by exposing to ethanol. To confirm the time-dependent effect of the ethanol toxicity to microalgae, ethanol and DAPI were injected into the device after sampling of *B. braunii*. We could continuously observe the damaged cells of microalgae by the fluorescence of DAPI.

KEYWORDS

Microalgae, Botryococcus braunii, Toxicity assay, Vernier wall.

INTRODUCTION

Botryococcus braunii (*B. braunii*) yield some kind of hydrocarbons of which main component is similar to heavy oil fuel (Figure 1). *B. braunii* discharge hydrocarbons to outside of the cells, while the other algae stock oils inside of them. Hence it is expected that the heavy oil fuel can be continuously acquired from *B. braunii* without cell disruption. However, the acquirable amount of the produced hydrocarbon is not yet enough to meet the production cost. One of the reasons of the problem is depressed cellular function due to the damage caused by the toxicity of alcohols or organic solvents used for deoiling of *B. braunii* [1]. To understand the effects of those chemicals on *B. braunii* and to improve the deoiling process, a continuous assay of individuals is one of the most desired approaches for floating microorganism such as *B. braunii*.

On the other hand, we had developed a microfluidic device which has a function to sample a small amount of the individual microorganisms and line them up in the channel [2]. After sampling of them, some reagents can be injected into the channel.

In this research, small amount of the cells of *B. braunii* were sampled in the device. Then ethanol and DAPI were injected into the device for exposing *B. braunii*. We concurrently observed the effects of ethanol toxicity to individual cells of *B. braunii* by confirming the fluorescence of the DAPI.

MICROFLUIDIC DEVICE

The microfluidic device made of PDMS has a capability to sample few amount of microorganisms. The function is realized by a specific channel structure named as vernier walls which are standing on the ceiling and on the bottom of the channel [2]. The closest vernier walls make the channel narrow periodically as shown in Figure 2. Hence, microorganisms of uniform size are captured between the closest vernier walls. Additionally, to avoid clogging, the microfluidic device has a bypass channel which almost microorganisms are going through.

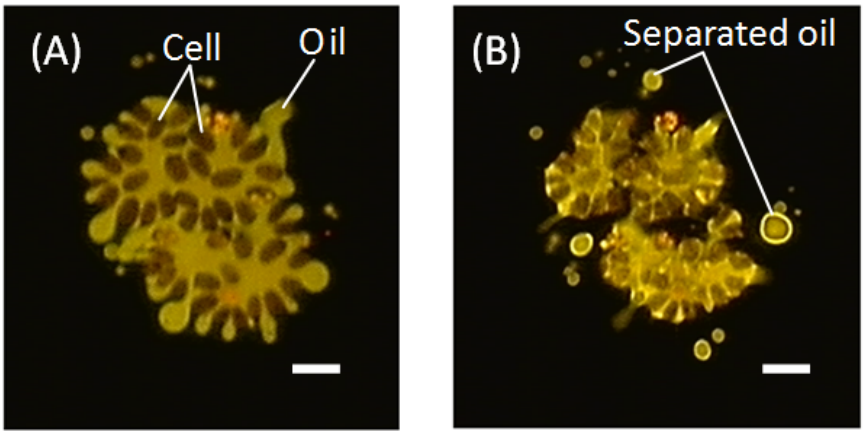

Figure 1: Botryococcus braunii (NIES-836). (A) Aggregated B. braunii with their oil. Single cell size is 7μm-11μm. (B) B. braunii after exposing to ethanol. Scale bar is 15μm.

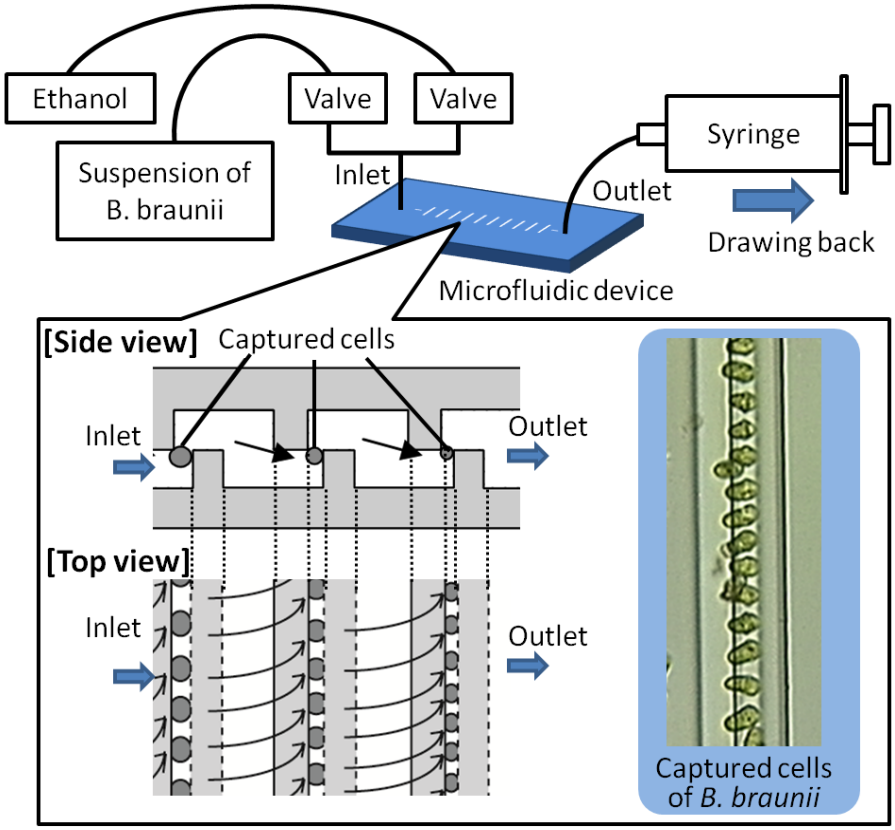

Figure 2: Schematic of microfluidic device.

EXPERIMENTS

First, we had confirmed the device utility as a system for toxicity assay by an experiment of exposing budding yeast: Saccharomyces cerevisiae (BY2779, The National Bio-Resource Project (NBRP), Japan) to lithium acetate solution (Figure 3). The yeast is widely used for basic research of microorganisms because of the easy handling and the short doubling time. The lithium acetate is well known as a reagent for gene transfer of the yeast.

Secondary, based on the yeast experiment, we applied the device to an experiment of exposing ethanol to *B. braunii* (NIES-836, National Institute for Environmental Studies (NIES), Japan). Suspension of *B. braunii* was injected into the device for the capturing of them. We could confirm the individual cells of *B. braunii* had been captured on the corresponding line in the channel (Figure 2). Following the capturing, ethanol with DAPI (Dojin Chem, Japan) 0.5% (v/v) was injected into the channel at 10μL/min for 40minutes.

RESULT AND DISCUSSION

The result of the first experiment show the lithium acetate solution damaged the yeast gradually. We could decide the effective amount of the injected solution for gene transfer.

The result of the microalgae experiment shows most of the same tendency of the yeast experiment. Damaged cells are confirmed by their fluorescence of DAPI. The strong fluorescence of DAPI was confirmed after 20 minutes. Additionally, the fluorescence of the chloroplast pigment became weak in contrast to the fluorescence of DAPI as shown Figure 4. On the other hand, we could not see any changes of the cells in the case of injection culture medium with DAPI 0.5% for 40 minutes.

CONCLUSION

As the result of the demonstration experiments, it is concluded that the time-dependent effect of the ethanol toxicity to individual microalgae is successfully confirmed by the present device.

To protect microalgae from the toxicity of ethanol in the micro channel, the exposing time must be less than 10 minutes.

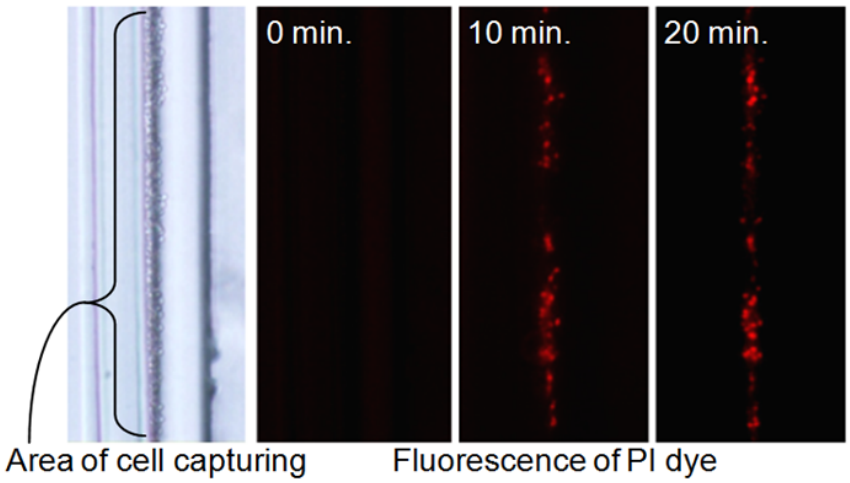

Figure 3: Experiment result of exposing budding yeast to lithium acetate for 20 minutes. Damaged cells are confirmed by fluorescence of PI dye (Dojin Chem, Japan).

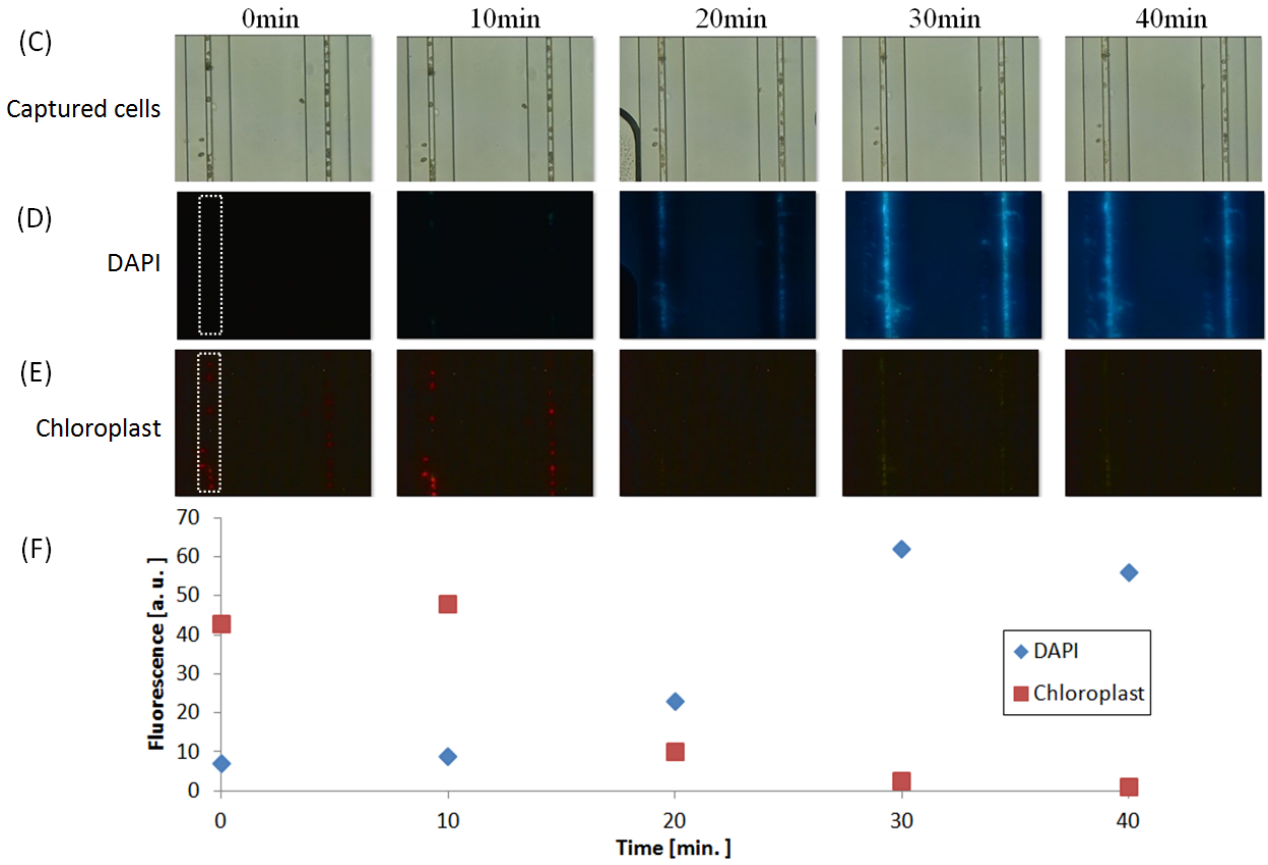

Figure 4: Result of exposing B. braunii to ethanol for 40 minutes. (C) Captured cells deformed caused by toxicity of ethanol. (D) DAPI (Dojin Chem, Japan) is used for confirmation of damaged cells. (E) Ethanol deprives auto-fluorescence of chloroplast. (F) Fluorescence of DAPI and Chloroplast is contrastive. Measurement region is rectangle of white dash line at (D) and (E).

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