CONTINOUS-FLOW LIPID EXTRACTION FROM WET ALGAL CELL SUSPENSION BY INTEGRATED MICROFLUIDIC PROCESS

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ABSTRACT

The lipid extraction procedure of microalgae based on switchable solvent in a microreactor is attractive, because of the fast flow and high throughput. Additionally, using CO₂ can be utilized sequentially to remove solvent from the extracted mixture in tube in tube system without solvent evaporation. Here we present an integrated microfluidic process of lipid extraction. Lipid extraction with switchable solvent is optimized by controlling the residence time in the microreactor to 125 sec under sonication treatment. The total extracted lipids was almost 2 times higher than that chloroform method or non-sonication condition.

KEYWORDS: Microalgae, Lipid extraction, Switchable solvent

INTRODUCTION

Algal bio-fuels have been considered as a renewable, potentially carbon-neutral and scalable alternative energy resource since they can be produced without usage of external fields and have relatively high oil yield [1]. The emerging technologies on algal harvesting and oil extraction are investigated to improve algal biomass and biofuels conversion efficiencies.

As one of these technologies, microfluidic systems provide opportunities due to the special characteristics such as fast and effective mass/heat transform and high throughput [2]. In general, chloroform or hexane and the mixture are used for the separation of extracted lipids, which results in long and complicated steps for the entire process. Also, evaporation is needed which is energy-consuming and harmful for the environment. On the other hand, a switchable polarity solvent was reported for extracting and recovering lipid of wet algal sample [3]. This fascinating solvent of extraction source is capable of turning from non-polar form to polar liquid by simple addition of carbon dioxide, which is possible to recover both of extracted material and solvents after extraction process. Using such solvent also utilizes CO₂ gas, and therefore this process is highly eco-friendly and can contribute to alleviating the greenhouse effect when done in large scale.

In this study, we propose a novel continuous-flow lipid extraction and recover process from wet algal samples using a behavior of switchable solvents in a tube-in-tube microfluidic system. Chlamydomonas reinhardtii was used as a model species for algal harvesting. Wet algal biomass was used as the raw material. The influences of the solvent, flowrate, treatment and CO₂ recover of the microfluidic process were examined.

EXPERIMENTAL

Chlamydomonas reinhardtii strains cc125 was grown photoheterotrophically at 25°C in 500 mL flasks containing 50 mL of standard tris/acetate/phosphate (TAP) medium [4]. Dry weight of the algal biomass was determined filtering aliquots of 10 mL of algal suspension using gravimetric method of dry weight to measure total suspended solid.

50 mL of algal biomass, 50 mL of extraction solvent (DMCHA) were taken in separate glass syringes, and introduced two units of capillary microreactors. For the first step of extraction, the T-junction was connected to PTFE capillary tube ( id = 100 μm, length = 200 cm), and then, reaction mixture was passed through ultrasonication. The second capillary reactor was introduced CO₂ gas reaction in the mixture of extracts using tube-in-tube microfluidic system as a gas/liquid phase microfluidic reactor by positioning a gas permeable inner Teflon tube (id = 310 μm, length = 100 cm) into the PTFE tube.

The extracted and separated lipids from microfluidic device were measured gravimetrically and reported as percentage on algae dry weight basis.
RESULTS AND DISCUSSION

Lipids were extracted by applying N, N-dimethylcyclohexyamine (DMCHA) that can be reversibly recovered by simply bubbling CO$_2$ which turned switchable solvent from non-polar form to polar form into hydrogen carbonate ammonium salt (Fig. 1).

![Figure 1: The switching of DMCHA in aqueous solution. The CO$_2$ triggers change phase behavior.](image)

In this work, we present an integrated one-flow microfluidic process for the efficient lipid extraction of algal biomass, which increase high surface to volume ratio contact area between algal biomass to solvent (Fig. 2). The efficiency of DMCHA solvent to extraction of algal biomass in microfluidic device was expressed as TLs on algal dry weight basis, was proved to achieve significantly higher total lipid yield, compared to convention CHCl$_3$-MeOH (Fig. 3). Furthermore, the superior lipid extraction yield (TLs = 18.75 wt%) was obtained by additional sonication treatment for 125 seconds, and compatible TLs even at shorter time 94 seconds (Table 1). We also examined the ultrasound treatment efficiency of the system. The temperature of the sonication process for microfluidic system was maintained using an ice bath. The sonication treatment caused an increase in the extracted lipids fraction, which might be due to disintegration of the microalgae cell structure and improvement of mass transfer between biomass and solvent. The extracted lipids rate in the sonicated and in the retention time was compared (Table 1). The sonication treatment increased the lipid fraction by 2 fold compared with the control from algal biomass in microfluidic process. However, short term retention time (47 seconds) had an insignificant influence (less than 10%) on the release of lipid from the microfluidic device in both sonication treatment and control, indicating that sufficient retention time is required for effective lysis of cell wall and extraction of lipids.

With the optimized extraction conditions, we also examined the lipid recovery efficiency upon converting the solvent via phase separation. The introduction of CO$_2$ adopts a tube-in-tube system to efficiently dissolve CO$_2$ into organic solvent, resulting in higher recovered fraction of lipid than that from the batch system. Thus, this simplified system was obtained avoiding organic solvent evaporation step and for the lipid recovery.

![Figure 2: Scheme of algal biomass extraction based on DMCHA](image)

![Figure 3: Comparison of total lipids (TLs) from DMCHA and CHCl$_3$-MeOH extraction of algal biomass in microfluidic reactors.](image)
Table 1. Effect of retention time and sonication treatment on total lipids (TLs) composition, expressed algal dry weight basis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Solvent flow-rate (mL/min)</th>
<th>Algal biomass flow-rate (mL/min)</th>
<th>Retention time (sec)</th>
<th>TLs (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sonication</td>
<td>0.5</td>
<td>0.5</td>
<td>94</td>
<td>9.25</td>
</tr>
<tr>
<td>Sonication</td>
<td>1</td>
<td>1</td>
<td>47</td>
<td>8</td>
</tr>
<tr>
<td>Sonication</td>
<td>0.5</td>
<td>0.5</td>
<td>94</td>
<td>18.25</td>
</tr>
<tr>
<td>Sonication</td>
<td>0.5</td>
<td>0.25</td>
<td>125</td>
<td>18.75</td>
</tr>
</tbody>
</table>

CONCLUSION

In conclusion, we have demonstrated the continuous-flow lipid extraction from wet algal cell integrated microfluidic process. The switchable solvent, DMCHA can be applied to the extraction of algal biomass. The lipids were extracted in yields ranging from 8 to 18.75% within 125s by capillary microreactor flow system. The microfluidic extraction for lipids of microalgal biomass in high productivity that could be extended to recover reaction by addition of CO$_2$ in the tube-in-tube system for the separation of extracted lipids from mixture of extracts. Because most of previous extraction protocols to date have relied on high cost and extreme condition, we believe that our novel method based on simple microfluidic device was potentially valuable and environmentally friendly to achieve continuous processing for biodiesel production.

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REFERENCES


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