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Integrating fermentation of *Chlamydomonas mexicana* by oleaginous *Lipomyces starkeyi* and switchable ionic liquid extraction for enhanced biodiesel production

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A B S T R A C T

The conversion of multi-bioconstituents of microalgal biomass such as carbohydrates and proteins to lipids can substantially reduce the downstream and overall process cost, as the bioprocessing can be aimed at single-desired biofuel product, e.g., biodiesel. The present study developed an integrated process to enhance the lipid yield through oleaginous yeast fermentation of microalgal biomass, and cost-effectively extract the lipids through permeable and switchable ionic liquid (IL). A 60 g/L of microalgal (*Chlamydomonas mexicana*) biomass was pretreated via microwave which showed 37% cell disruption efficiency. The pretreated biomass was fermented using an oleaginous yeast, *L. starkeyi* which effectively consumed the carbohydrates (92%) and proteins (91%) of *C. mexicana* and converted it to excessive lipids (188%) in comparison to the original microalgal lipids (12.6 g/L) before fermentation. The ionic liquid (IL), Dissopropanolamine-Im was used for high-thoughput disruption of the biomass and extraction of total lipids. It significantly improved cell disruption efficiency (95%) and lipid extraction efficiency (99%) than the conventional (Bligh & Dyer) lipid extraction method (87%) and previously reported methods using several ionic liquids (~92%), and required a relatively shorter process time. More than 93% of IL could be recovered from the solution, and its recyclability was tested for >5 times where it could maintain an efficiency of >80% after five cycles. The integration of microalgal biomass and *L. starkeyi* mediated fermentation improved the overall lipid production (23.72 g/L) and yielded 2.2 times higher biodiesel than the conventional process. The outcomes of this study provides a economic and sustainable model to overcome the existing limitations of biodiesel production from microalgal biomass.

1. Introduction

Microalgae are potential resources for biofuel production, owing to their rich organic contents (e.g., carbohydrates 50%, proteins 20%, and lipids 21% for *C. mexicana*) [1]. However, several bottlenecks restrict microalgal biotechnology in delivering cost-effective extractions; for instance, partial conversion of biomass to biofuels, where only lipids (7%–64% of microalgae components) are utilized for biodiesel production and the other components remain unutilized [2,3]. The limited contents of lipids in microalgal cells, and requirement of expensive downstream process for bioconsituents extraction is a hurdle in 3rd generation microalgal biodiesel industry. The improvement of single constituent of microalgae (e.g., lipids) is possible, however complex metabolic engineering and gene editing is required to design such species with high productivity of lipids [4,5]. The rigid and thick cell wall (150–364 nm) of microalgae makes it difficult to extract biocomponents that require harsh pretreatment conditions adding more costs to the bioprocessing [3,6,7]. Moreover, pretreatment efficiency is reported to

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