Saccharification and bioethanol production from microalgal residual biomass

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Introduction. The depletion of fossil fuels has triggered the development of alternative biofuels, such as bioethanol and biodiesel, from various types of biomass. Microalgae have been considered as a 3rd-generation promising feedstock for biodiesel production due to its high lipid content (1). After lipid extraction for the production of biodiesel, the residual biomass needs to be utilized, for example bioethanol fermentation, for the economic feasibility of microalge-based production of biofuels (2).
The objective of this study is to saccharify the residual biomass of microalgae after lipid extraction and then produce bioethanol from the saccharification broth.

Methods. *Dunaliella tertiolecta* and *Chlorella* spp. were cultured in a plate type photobioreactor with fluorescent lighting. The harvested cells were freeze-dried and used for lipid extraction. The total lipids were extracted twice from the freeze-dried cells with chloroform/methanol (1:2 (v/v)) or other organic solvents, respectively. The residual biomass was saccharified using HCl and enzymes (2,3). For chemical saccharification, the residual biomass was autoclaved at 121°C for 15 min in the presence of various concentrations of HCl. Enzymatic saccharification was performed at various temperatures (35-55°C) and pH (3.5-6.5) in the presence of enzymes such as xylanase, cellulase or glucosidase. *Saccharomyces cerevisiae* and *Pichia* spp. were used for bioethanol fermentation of the enzymatic saccharification products (4,5).

Results.

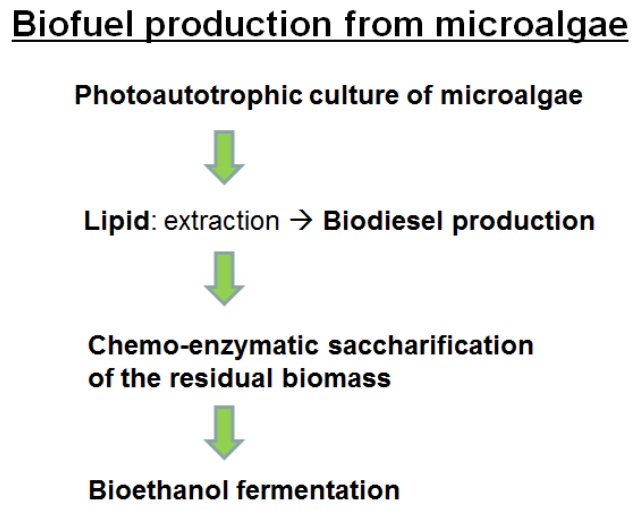

Fig.1 Schematic diagram of the biofuel production from microalgae.

After photoautotrophic culture of *D. tertiolecta* and *Chlorella* spp., the cells were harvested and then the lipids were extracted using by organic solvents. The residual biomass was analyzed to determine the biomass composition, and the contents of carbohydrates were determined to be more than 50%(w/w), which is valuable resource that can be used for bioethanol fermentation.
Diluted acid-mediated saccharification of the residual biomass was conducted. Saccharification yields were 57 and 41%(w/w), based on the total amount of carbohydrates of the residual biomass of *D. tertiolecta* and *Chlorella* spp., respectively.
Enzymatic saccharification of the residual biomass was conducted using various saccharifying enzymes including glucosidase, cellulose, hemicellulose and xylanase. Enzymatic saccharification yield of the residual microalgal biomass treated with methanol and chloroform was up to 80%(w/w), based on the carbohydrate amount. The yield for the *Chlorella* spp. biomass was only 34%(w/w).
The resulting saccharification solution contained glucose as the main monosaccharide and the relative content was dependent on the microalgal strain. The glucose and galactose were successfully used for bioethanol fermentation using *S. cerevisiae* and *Pichia* spp., respectively. The bioethanol yield based on the amount of saccharified monosaccharides was more than 85%, representing that the carbohydrate in the residual microalgal biomass after lipid extraction could be efficiently used for bioethanol fermentation.

Conclusions. The residual biomass of the lipid-extracted microalgae was successfully saccharified using acid and enzyme. The resulting glucose and other monosaccharides were efficiently used for bioethanol fermentation with more than 85% yield.

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