Application of Electroporation for Lipid Extraction from Microalgae

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Summary

Microscopic observations, solvent-based lipid extraction and purification, and GC/MS analysis confirmed that electroporation or electropermeabilization altered the cellular membranes and cell walls of tested algal cells and improved lipid extraction efficiency in terms of time and solvent use without affecting the composition of extracted fatty acids.

Introduction

To commercialize algae-based biofuel, there are some critical issues that must be overcome, including efficient extraction of lipids/oils from the microalgal cells.

Traditional press/expeller methods applied for oil extraction from single-celled microalgae is ineffective due to the small sizes of algal cells and their thick, chemically complex membranes and rigid cell walls. Oil extraction from lipid-containing algal biomass is typically carried out by using organic solvents, such as hexane, chloroform, acetone and methanol. Although it works effectively with some algal strains, but has proven to be very difficult for others. This process is also time- and labor-consuming.

Electroporation, or electropermeabilization, is a membrane phenomenon which involves a significant increase in the electrical conductivity and permeability of the cell wall and cytoplasmic membrane resulting from an externally applied electrical field. This process results in the formation of aqueous pores in the membrane. Electroporation has been used to aid in gene transformation of bacteria, yeast, algae and plant protoplasts.

Methods

Two chlorophycean microalgae (*Scendesmus* sp., and *Pseudochlorococcum* sp.), that differed in cell size, wall composition and structure, were selected for the evaluation. Algal biomass was collected during the logarithmic phase and stationary phase of the cell cycle. Only the data from the stationary phase is described here.

Chemical solvent extraction was used as a control. Experimental design: a. electroporation alone; b. electroporation in combination with chemical solvent extraction. Total lipid, neutral lipid, polar lipid, and fatty acid composition were analyzed.

Preliminary Assumptions and Economic Analysis

Plant Size:	10 MGY of biodiesel crude
Cost of Electricity:	$0.05 per kWh
Lipid Content:	27% (by weight)
Total Operating Cost:	$0.0262 per gallon of biodiesel crude*

Cost includes maintenance and operation costs as well as depreciation.

Fig 1. Bench-scale electroporation apparatus used for this study.

Objective

To determine:
1) if electroporation can alter the primary physical-chemical barriers (cell wall and cell membrane) to enhance lipid extraction and
2) if electroporation affects the composition of the extracted fatty acids.

Results

Electroporation altered the cells, as revealed by the increased distance between the cell wall and cytoplasmic membrane (Fig 2C), and the distortion and fusion of intracellular membranes (Fig 2D), as compared to the control cells (Fig 2A,B).

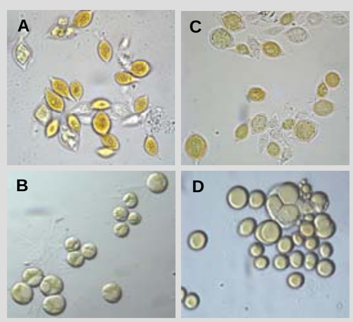

Fig 2. Light microscope images of the cells of the green algae *Scendesmus sp.* and *Pseudochlorococcum* sp. before (A, B) and after electroporation (C, D).

Preliminary results from the lipid analysis (including the total lipid, neutral lipid, and polar lipid content) indicated that electroporation of the green algal cells increased the extraction efficiency by ca. 20% compared to the control (Fig 3).

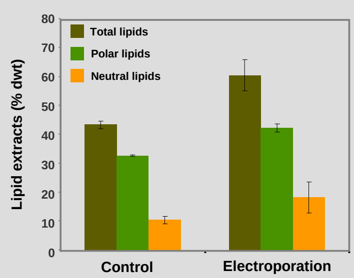

Fig 3. Lipid extracts of *Pseudochlorococcum* biomass before and after electroporation. Total lipids were extracted according to Bligh and Dyer (1959). Crude lipids were further separated by column chromatography for neutral and polar lipid measurements. For each treatment, Duplicate analyses were performed.

In a subsequent analysis to determine the effect of electroporation on lipid extraction from algae, the lipid recovered from each of three sequential treatments were determined. Fig. 4 illustrates that 92% of the total lipid was extracted from the algal biomass after a single electroporation treatment, while only 62% of the total lipid was extracted from the same amount of algal biomass without the electroporation treatment. GC/MS analysis indicated that there was no significant change in the fatty acid composition from the electroporation treatment.

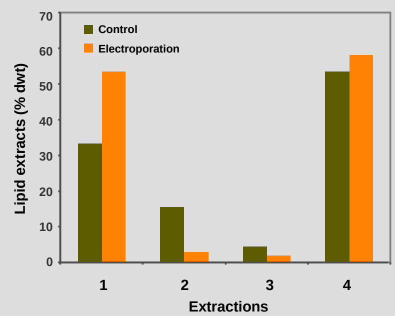

Fig 4. Comparison of lipid recoveries from each of three extractions in *Pseudochlorococcum* biomass before and after electroporation. Total lipids were extracted according to Bligh and Dyer (1959). Treatments: 1 = 1st extraction; 2 = 2nd extraction; 3 = 3rd extraction; 4 = total extracted lipids.

Conclusions

- Electroporation altered the cellular membranes and cell walls of the tested green algal cells.
- One-step solvent extraction of algal biomass after electroporation yielded over 90% of the total lipids from the algae cells. Three repetitive extraction cycles were required to achieve the same level of extraction of intracellular lipids without electroporation.
- Electroporation reduced the extraction time and quantity of solvents used by two-thirds.
- Electroporation did not cause significant changes in the composition or oxidation of fatty acids.

References

Bligh, E.G., Dyer, W.J., 1959. A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37, 911–917.