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Investigation of the effects of microalgal cell concentration and electroporation, microwave and ultrasonication on lipid extraction efficiency

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ABSTRACT

This study investigated the effects of *Chlorella vulgaris (C. vulgaris)* concentrations and pretreatment methods, electroporation, ultrasonication, and microwave, on lipids extraction. The *C. vulgaris* concentrations were varied in the range of 8.4–28.8% for chloroform/methanol/water solvent system and in the range of 7.6–32.0% for n-hexane/methanol/water solvent system. A maximum total lipid yield of 0.248 g/g of dry *C. vulgaris* was achieved at biomass concentration of about 15% for the chloroform/methanol/water system. This is the highest yield reported for lipids extracted without pretreatment. On the other hand, a maximum lipids yield of 0.139 g/g of dry *C. vulgaris* was obtained at about 24% biomass concentration for the n-hexane/methanol/water system. When pretreated with electroporation, ultrasonication, and microwave, the yield for lipid extraction increased by 5.3, 26.4, and 28.9%, respectively. Although electroporation resulted in the least amount of yield, it was the most efficient in terms of energy gain per energy input.

1. Introduction

Microalgae have emerged as the most promising long-term and sustainable feedstock for biofuels production due to their high productivity rate [1], ability to tolerate a wide range of growth conditions [2], and lack of competition for land with food crops [3]. The basic concept of using algal biomass as feedstock for biofuels production has been extensively explored [4]. However, a scalable, sustainable, and commercially viable system has yet to emerge. Microalgae cultivation, harvesting, extraction, and conversion are the main steps involved in the algae-to-biofuels pathway. Of these steps, the processes used for algae cultivation and the conversion of extracted cell contents to biofuels are relatively well-established. However, algal biomass harvesting and extraction of lipids still attract intense interest from researchers around the world.

Multi-phase solvent extraction is the most commonly researched method for extracting algal lipids. The process involves the use of a solvent that matches the polarity of the target compound, non-polar lipids [5]. The solvent must also make contact with the lipids inside of the cell [6], which generally requires a second polar solvent to break the cell wall and membrane. Several studies [7–12] employed the Bligh and Dyer method [13], which uses chloroform, methanol, and water as co-solvents for extracting and purifying lipids. Additionally, other solvent systems have been investigated as possible extracting solvents, including dichloromethane/methanol/water [14], dichloromethane/water [15], n-hexane/water [12], and ethanol/water and hexane/water [16].

Several pretreatment methods have been investigated to improve the efficiency of solvent extraction systems, including ultrasonication [7–12,14,17], microwave [7–11,14,15], electroporation [18], and bead mills, autoclave and osmotic pressure [7]. These studies reported a significant increase in the quantity of lipids extracted due to pretreatment. For ultrasonication, the percent increase ranged from 30% [10] to 400% [8] with a mean and median of 136% and 99%, respectively. The percent increase for microwaves ranged from 38% [14] to 606% [15] with a mean and median of 288% and 150%, respectively. However, a majority of these studies included additional sample processing steps before pretreatment and extraction, including freeze drying [8–11,14,15] and treating the sample with saline solution [17], making very difficult the quantification of the effects of the pretreatments on lipids extraction. Freeze drying and treatment with saline solution have both been shown to be potential pretreatment techniques [7,19] and

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could affect the results.

Moreover, comparing the effectiveness of the different solvent systems investigated in prior studies would not be easy since different solvent ratios and solids concentration were used. Even in the studies that used the same solvents, the ratios of the non-polar to polar solvents were varied widely. For instance, of the studies that used Bligh and Dyer method [13], the chloroform (non-polar) to methanol and water (polar) ratios varied from 2:1 [10] to 2:6 [7,12] compared to a chloroform to methanol and water ratio of 2:3.8 prescribed by the method. Additionally, the solids concentration varied from as low as 0.25% [8] to as high as 100% [11,17], while a 20% solids concentration was recommended by the method.

Considering these limitations, the present study: (1) investigated the effects of algal biomass concentration on lipid extraction with chloroform/methanol/water and n-hexane/methanol/water solvent systems, (2) compared the lipid extraction yields of chloroform and n-hexane as an extracting solvent, and (3) evaluated the impacts of microalgae pretreatment with ultrasonication, microwaves, and electroporation for potential improvements in solvent extraction yield. *C. vulgaris* was chosen as representative microalgae because it has relatively high lipid content and biomass productivity [20], and it is one of the most widely researched microalgal species [21].

2. Materials and methods

2.1. Experimental approach

2.1.1. Experimental conditions

To achieve the objectives of the study, five sets of experiments (Table 1) were conducted. The first set of experiments, exp. run 1 through 4, evaluated the effects of varying *C. vulgaris* concentrations on lipid extractions using the Bligh and Dyer method [13]. In the second set of experiments (exp. run 5 through 8), the effects of varying biomass concentration on lipid extraction using n-hexane/methanol/water solvent system were investigated. Lipid extractions with the Bligh and Dyer method (exp. run 9) and n-hexane/methanol/water system (exp. run 10) were compared in third set of experiments. In the fourth set of experiments, samples prepared at equal concentration of *C. vulgaris* were pretreated with electroporation (exp. run 12), ultrasonication (exp. run 13), or microwaves (exp. run 14) and lipids were extracted using the Bligh and Dyer method. In the final set of experiments, lipids were extracted from samples prepared with equal concentration of *C. vulgaris* using a modified method that replaced the addition of methanol with either electroporation (exp. run 16) or ultrasonication (exp. run 17) pretreatment. Exp. runs 11 and 15 were controls for the fourth and fifth experimental sets, respectively. All experimental conditions, including controls, were conducted in triplicate.

In the presented study, non-polar to polar solvent ratios in the range of 2:3.7 and 2:3.9 were used, closely matching the 2:3.8 ratio prescribed by the Bligh and Dyer method [13]. This has allowed us to compare the effectiveness of the chloroform/methanol/water and n-hexane/methanol/water solvent systems.

2.1.2. Pretreatment conditions

Samples were electroporated using a bench-scale electroporation unit built in the authors' laboratory for a prior study [22]. The sample for the fourth set of experiments was electroporated at 25 kWh/m^3 treatment intensity (TI), while the TI for the samples prepared for lipid extraction with the modified extraction method was 26 kWh/m^3. For sonication, a Qsonica Q55 ultrasonic (Newtown, CT) rated at 20 kHz and 55 W was used. The horn was placed midway into the depth of the sample and the sample were ultrasonicated for 20 min. For microwave, samples were poured in a 3-inch covered, microwave safe, glass petri dish and microwaved with an Emerson 700 W microwave oven (Moonachie, NJ) on high power for 10 s on and 30 s off cycle. This was done to avoid overheating and boiling of the mixture. The total microwave time was 50 s.

In the present study, the TIs were not optimized since the main purpose was to quantify the effects of the pretreatment methods by minimizing the influence of culture processing before pretreatment (refer to Section 2.2.3). Therefore, the TIs used above were selected based on results reported in prior studies for electroporation [22], ultrasonication [10,12], and microwave [14,15].

2.1.3. Lipid extraction

The lipid extraction process was performed using a clean VWR 50-mL centrifuge tubes as a reactor. During a typical lipid extraction process, 5 g of microalgae paste was transferred to the centrifuge tubes. For the Bligh and Dryer method, 10 mL of methanol and 5 mL of chloroform were added to the sample in the centrifuge tube. Then the content of the tube was mixed for 2 min using a Thermolyne Maxi Mix Plus™ vortex (Dubuque, IA). An additional 5 mL of chloroform was added to the sample and the tube was mixed for 30 s using the vortex. Finally, 5 mL of distilled water was added to the sample and then mixed for 30 s using the vortex. The lipid

Table 1
Experimental conditions.

Lipid extraction method	Exp. run	*C. vulgaris* concentration (%)	Solvent system	Solvent ratio (v:v:v)	Non-polar/polar solvents ratio
Bligh and Dyer	1	8.4	Chloroform:Methanol:Water	2:2:1.9	2:3.9
	2	15.2	Chloroform:Methanol:Water	2:2:1.8	2:3.8
	3	23.0	Chloroform:Methanol:Water	2:2:1.8	2:3.8
	4	28.8	Chloroform:Methanol:Water	2:2:1.7	2:3.7
n-hexane/methanol/water	5	7.6	n-hexane:Methanol:Water	2:2:1.9	2:3.9
	6	13.9	n-hexane:Methanol:Water	2:2:1.9	2:3.9
	7	24.1	n-hexane:Methanol:Water	2:2:1.8	2:3.8
	8	32.0	n-hexane:Methanol:Water	2:2:1.7	2:3.7
Bligh and Dyer and n-hexane/methanol/water	9	13.9	Chloroform:Methanol:Water	2:2:1.9	2:3.9
	10	13.9	n-hexane:Methanol:Water	2:2:1.9	2:3.9
Bligh and Dyer with pretreatment	11	14.7	Chloroform:Methanol:Water	2:2:1.9	2:3.9
	12	14.7	Chloroform:Methanol:Water	2:2:1.9	2:3.9
	13	14.7	Chloroform:Methanol:Water	2:2:1.9	2:3.9
	14	14.7	Chloroform:Methanol:Water	2:2:1.9	2:3.9
Modified Bligh and Dyer using pretreatment in the place of methanol	15	18.4	Chloroform:Methanol:Water	2:2:1.8	2:3.8
	16	18.4	Chloroform:Water	2:3.8	2:3.8
	17	18.4	Chloroform:Water	2:3.8	2:3.8