OIL EXTRACTION FROM *NANNOCHLOROPSIS OCULATA* MICROALGAE FOR BIODIESEL PRODUCTION

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ABSTRACT

Microalgae, the third generation biodiesel feedstock, have emerged as one of the most promising alternative sources of lipids that can be used in the production of biodiesel due to their advantages over conventional crops. Oil extraction is particularly important because this process is one of the more costly processes which can determine the sustainability of algae-based biodiesel. In this paper several methods have been tested for oil extraction from *Nannochloropsis oculata* microalgae: static hexane extraction, static hexane-isopropanol extraction, dynamic hexane extraction (Soxhlet). The lipid yield for each method was compared with the total lipid content determined using the combination of microwaves with chloroform-methanol extraction.

INTRODUCTION

Biodiesel is a mixture of monoalkyl esters of long-chain fatty acids obtained in the transesterification reaction of vegetable oils or animal fats with short chain alcohols.
The cost of biodiesel production remains the major obstacle to its use at industrial scale, primarily because of the high cost of vegetable oils used as feedstocks [1]. Another important reason is the inefficiency and unsustainability of first and second generation biodiesel [2]. Microalgae, the third generation biodiesel feedstock, have emerged as one of the most promising alternative sources of lipids that can be used in the production of biodiesel due to their advantages: photosynthetic efficiency, high biomass production, higher growth rates and productivity when compared to conventional crops [3].
Microalgal biomass processing for biodiesel production consists in: oil extraction, oil transesterification, biodiesel separation and biodiesel purification. First microalgae are dried and grinded and then subjected to the extraction process to obtain algal oil. After extraction, the oil is converted into biodiesel using the transesterification process. The final stages are the separation and purification of biodiesel product.
Although all these steps are essential, the lipid extraction is particularly important because this process is one of the more costly processes which can determine the sustainability of algae-based biodiesel. Different procedures can be applied for extracting oil from microalgae, those being mechanical pressing, homogenization, milling, solvent extraction, supercritical fluid extraction, enzymatic extractions, ultrasonic-assisted extraction and osmotic shock. Solvent extraction involves extracting oil from microalgae by repeated washing or percolation with an organic solvent. Hexane is a popular choice due to its relatively low cost and high extraction efficiency.

An increased efficiency of lipid extraction can be achieved by using appropriate methods of cell disruption to release cellular contents into the extraction medium. Numerous methods have been used for cell disruption, such as microwaves, ultrasonic, mechanical crushing [4]. For example, the use of microwaves proved to be an effective method for the extraction of vegetable oils [5], while ultrasonic disruption is widely used for microbial cells [6].

In this paper several methods have been tested for oil extraction from *Nannochloropsis oculata* microalgae: static hexane extraction, static hexane-isopropanol extraction, dynamic hexane extraction (Soxhlet). The lipids were gravimetrically quantified for each method and compared with the total lipids that were extracted using the combination of microwaves with solvent extraction.

MATERIALS and METHODS

Materials

Nannochloropsis oculata microalgae were purchased from Astaxa GmbH (Germany Milz Gerbergrasse). Hexane, isopropanol, chloroform and methanol were purchased from Merck (Darmstadt, Germany) and were of highest purity.

Static hexane lipid extraction

For static hexane lipid extraction, a quantity of 4 g microalgal powder was used. A volume of 300 mL n-hexane was added to the microalgal powder in a 500 mL Erlenmeyer flask. In order to reduce solvent evaporation, the flask was sealed with a ground joint. The extraction mixture was agitated at 800 rpm at ambient conditions for 8 h. After extraction cell residues were removed by filtering through Whatman GF/C paper. The hexane phase was collected in a pre-weighed flask and then submitted to vacuum evaporation using a rotational evaporator to enable gravimetric quantification of the lipid extract.

Static hexane-isopropanol lipid extraction

For static hexane-isopropanol extraction a volume of 300 mL mixture of hexane/isopropanol 3:2 v/v was added to 4 g of microalgal powder into a 500 mL Erlenmeyer flask. The flask was sealed with ground joint and the mixture was agitated at 800 rpm at ambient conditions for 8 h. After extraction cell residues were removed by filtering through Whatman GF/C paper. The filtrate was transferred into a separating funnel and a volume of 50 mL hexane and 50 mL water were added to induce biphasic layering. After settling, two distinct phases were formed: a dark-green top layer of hexane that contains most of the extracted lipids and a light-green bottom layer of isopropanol-water mixture containing most of the co-extracted non-lipid contaminants. The hexane phase was collected in a pre-weighed flask and then submitted to vacuum evaporation using a rotational evaporator to enable gravimetric quantification of the lipid extract.

Dynamic hexane lipid extraction

The performance of static hexane extraction with dynamic hexane extraction was compared using a Soxhlet apparatus. A quantity of 4 g microalgal powder was packed in a cellulose thimble inside the extraction chamber of the Soxhlet unit. A volume of 300 mL *n*-hexane was used to extract the lipid and the extraction was performed for 8 h at the rate of approximately 10 refluxes per hour. The extracted lipid collected in a pre-weighed flask and then submitted to vacuum evaporation using a rotational evaporator to enable gravimetric quantification of the lipid extract.

Microwave lipid extraction

A quantity of 0.5 g dry microalgae biomass was mixed with 20 ml distilled water. The mixture was further subjected to cell disintegration using a Speedwave MWS Berghof microwave

digester (2450MHz) at 100 °C for 5 min. Total lipids were extracted from microalgae biomass using a modified version of Bligh and Dyer method [7]. Lipids were extracted with a mixture of chloroform-methanol 2:1 (v/v). The volume ratio of biomass subjected to the extraction and the organic solvent mixture is 1:1. The mixture was subjected to stirring for 5 minutes in a separating funnel. After extraction the mixture was introduced over a further 10 ml of methanol to separate the two phases: aqueous and organic. The chloroform organic phase was washed with 10 ml 5% sodium chloride and then submitted to vacuum evaporation using a rotational evaporator to enable gravimetric quantification of the lipid extract.

RESULTS

In order to compare the efficiency of lipid extraction methods used for *Nannochloropsis oculata* microalgae, the lipid yields obtained for each method were compared to total lipids extracted using the combination of microwaves with chloroform-methanol extraction.
A summary of the results is given in table 1.

Table 1. Lipid yield using several methods for lipid extraction from *Nannochloropsis oculata* microalgae

	Static hexane extraction	Static hexane-isopropanol extraction	Dynamic extraction (Soxhlet)	Microwave cell disruption – solvent extraction (total lipids)
Lipid yield (g lipid /g dried microalgae	0.136	0.219	0.190	0.328

It can be seen that the combination of microwaves with chloroform-methanol extraction gave the higher yield of 0.328 g/g dried microalgae, which is in fact the total lipid content. But for microalgal biodiesel production the ideal lipid extraction method should not only be lipid-specific – in order to minimize co-extraction of non-lipid contaminants, but also selective – towards only a few lipid fractions, e.g. neutral lipids like triacylglycerols [8].
Even though the classic chloroform-based lipid extraction protocol is particularly suitable for most microalgal lipid analyses, alternative organic solvents that are less toxic are preferred prior to scale-up. Hexane is less efficient than chloroform for extraction of oils from microalgae, but is also less toxic – and has a marginal affinity for non-lipid contaminants, and an apparently higher selectivity for neutral lipid fractions [8]. This is why hexane was chosen for oil extraction from *Nannochloropsis oculata* microalgae, together with the effect of isopropanol addition.
Soxhlet extraction with hexane was found to be significantly more efficient than static hexane extraction, with a lipid yield of 0.190 g/g dried microalgae comparatively to only 0.136 g lipid/g dried microalgae for static hexane extraction. This improvement was expected since Soxhlet operation, through solvent refluxing, constantly exposed a fresh batch of hexane to the microalgae biomass and enabled continuous re-establishment of mass transfer equilibrium [9].
In the same time Soxhlet extraction was a little less efficient than the hexane-isopropanol extraction in static mode that gave a lipid yield of 0.219 g/g dried microalgae.

With the addition of isopropanol as co-solvent the final lipid yield substantially increased. The reason is that polar lipids (phospholipids, glycolipids, and cholesterols) that are bound to protein molecules in the cell membrane via hydrogen or strong electrostatic bonds, in the presence of a polar solvent (such as isopropanol) are detached from the complex lipid–protein interactions before they are extracted out by hexane. Additionally, even though non-polar hexane readily interacts with neutral lipid molecules, the micelle-type structures that triacylglycerols frequently form in their natural state prevent quantitative lipid extraction and often require the assistance of an alcohol for their rapid destruction [10]

CONCLUSIONS

- From the methods that have been tested for oil extraction from *Nannochloropsis oculata* microalgae, Soxhlet extraction with hexane was found to be significantly more efficient than static hexane extraction.
- The addition of isopropanol as co-solvent for the lipid extraction in static mode determined a substantially increase of lipid yield when compared to static hexane extraction and gave the highest lipid yield.

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