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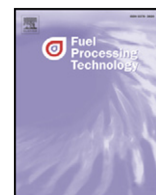

Steam explosion as a fractionation step in biofuel production from microalgae

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ABSTRACT

In this study, various pretreatment methods (autoclaving, ultrasound, microwave and steam explosion) have been compared to determine the most efficient method for the extraction of lipids from three different samples of microalgae (*Nannochloropsis gaditana*, *Chlorella sorokiniana* and *Phaeodactylum tricornutum*). Among the studied methods, steam explosion gave the highest lipid extraction yields for the three microalgae species. Therefore, the method was further studied and the application of acid catalysed steam explosion pretreatment was investigated for simultaneous lipid extraction and sugar release from microalgae. The effect of different variables, including temperature and acid concentration, was analysed. The experimental results demonstrate the efficacy and feasibility of the acid catalysed steam explosion pretreatment, followed by n-hexane lipid extraction. Remarkable sugar yields up to 96% were achieved under the pretreatment conditions of 1.7% sulphuric acid concentration and a temperature of 150 °C during steam explosion. Besides, this study verified high efficiencies in the extraction of lipid of exploded microalgae using n-hexane against the low efficiencies obtained for the untreated microalgae.

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1. Introduction

The potential use of microalgae as an alternative renewable source of biomass for biofuel production and biorefineries has received significant attention in recent years [1,2]. Microalgae are photosynthetic microorganisms capable of converting CO_2 and water into organic macromolecules such as lipids, polysaccharides and proteins, under light conditions. Compared with traditional terrestrial biomass feedstock, microalgae possess many advantageous characteristics including a fast growth rate, high areal productivity, efficient carbon dioxide fixation, possibility of exploitation in different climates, with a wide variety of water sources (fresh, saline, wastewater) and on nonarable land, and ability to accumulate relatively high amounts of lipids and carbohydrates inside their cells for biodiesel and bioethanol production, respectively [3]. However, despite these promising benefits, a considerable amount of research and development efforts is still to be carried out because of several remaining hurdles to the development of the microalgae to biofuel technology, mainly related to harvesting and feedstock extraction [4].

One of the main challenges that have attracted much interest from the industrial and research communities is the extraction of lipids from algal biomass for biodiesel production [5]. Organic solvent extraction is a widely used method for lipid extraction from traditional oilseed plants, and it has also been tested with algae cultures. Significant research efforts are being devoted to screening effective solvents (including toluene, hexane, butanol, and ionic liquids) or combinations of solvents, capable of recovering maximum amounts of lipids from algal biomass [6,7]. Lipid extraction from microalgae is particularly problematic due to the chemically complex and structurally robust nature of algal cell walls. Therefore, it is often necessary to disrupt the cell walls and other cellular structures in order to make lipids sufficiently accessible to solvent extraction. Several pretreatment methods such as bead-beating, microwave and sonication, have been proposed for microalgae cell disruption, in order to promote better penetration of the solvent into the cells and increase the lipid yield. However, the most efficient pretreatment method for microalgae has not yet been unequivocally confirmed [8–10].

Besides their interest for lipid production, microalgae rich in carbohydrates can also be a suitable feedstock for production of biofuels (such as bioethanol, biobutanol, and biohydrogen) by several biomass conversion technologies [11]. It is known that complex carbohydrates are entrapped in the cell wall of microalgae [12]. Therefore, a pretreatment stage for disruption of microalgae cell walls is necessary to release and convert the carbohydrates into simple sugars prior to its posterior use as carbon source. Acid/alkaline hydrolysis has been successfully applied to sugar production from microalgae [13,14]. In particular, since microalgal biomass should be in principle easier to hydrolyse in comparison to conventional lignocellulosic biomass, chemical hydrolysis under relatively mild conditions is of great interest.

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The search for appropriate pretreatment cell disruption methods for microalgae is essential in order to increase the lipid extraction efficiency and/or to release the carbohydrates and sugars present in the cell. In this context, the application of acid-catalysed steam explosion pretreatment has the potential to facilitate both lipid extraction and post-extraction use of the microalgal biomass.

Steam explosion is an efficient and economical pretreatment method which has long been used for fractionating and modifying lignocellulosic materials in order to improve the biomass feedstock quality for downstream processing [15,16]. This treatment exposes biomass to steam at temperatures between 180 and 240 °C (1.03–3.45 MPa) for several minutes, followed by sudden depressurization to ambient condition. The use of catalysts such as sulphuric acid or sodium hydroxide enhances the pretreatment efficiency [17]. With autohydrolysis and explosive depressurization particle size distribution, shapes and chemical composition of the biomass feedstock are altered. The current potential applications of steam exploded biomass to produce chemicals and fuels include ethanol from lignocellulosic feedstock [18,19] and hydrogen by fermentation [20]. Preliminary results on the use of steam explosion for lipid recovery from microalgae obtained in a pilot plant have been recently presented [21].

In the present study, various pretreatment methods (autoclaving, ultrasound, microwave and steam explosion) will be compared to determine the most efficient method for the extraction of lipids from three different samples of microalgae (*Nannochloropsis gaditana*, *Chlorella sorokiniana* and *Phaeodactylum tricornutum*). Furthermore, we explore the feasibility of applying the acid-catalysed steam explosion technique for simultaneous lipid and sugar extraction from microalgae as a preliminary step on the production of biofuels.

2. Materials and methods

2.1. Microalgae biomass

Three different microalgae strains were used for this study: two marine species, *N. gaditana* and *P. tricornutum*, and a freshwater species, *C. sorokiniana*. These species are potentially good candidates for biofuel production since they have high photoautotrophic biomass and lipid production rates and can grow to high densities (>10 g L^{-1}) while tolerating a wide range of conditions with regard to pH and temperature [22,23].

The microalga *N. gaditana* was obtained as a paste, containing 78% (w/w, wet basis) moisture, whereas the microalgae *C. sorokiniana* and *P. tricornutum* were in lyophilized state. For the microalgae powders, distilled water was added before its use, to reproduce the harvested and concentrated microalgae with water content of 80–90%.

Table 1 shows the elemental analysis (C, H, S, N and O contents) of the microalgae, which was determined using a Leco 628 Series Analyser.

Table 1
Elemental analysis of microalgae samples (w/w, DAF basis).

	Nannochloropsis gaditana	*Chlorella sorokiniana*	*Phaeodactylum tricornutum*
C	54.21%	52.30%	52.01%
H	7.32%	7.41%	7.13%
N	11.03%	9.10%	9.22%
S	1.30%	0.77%	2.39%
O	26.15%	30.43%	29.25%

2.2. Pretreatment methods for cell disruption

The microalgal samples were disrupted using four different methods: (1) steam explosion at 120 °C/150 °C for 5 min (further experimental details are given in Section 2.3. Steam explosion pretreatment); (2) ultrasound using an ultrasonic bath (Fisher Scientific, UK) at a resonance of 37 kHz and at ambient temperature for 5 min; (3) microwave using a microwave oven (Berghof, Germany) at 2450 MHz and at a temperature of 150 °C for 5 min and (4) autoclaving at 120 °C for 5 min.

2.3. Steam explosion pretreatment

Steam explosion of microalgae was carried out in a batch unit, equipped with a 4 L steam generator, 2 L reactor and a collection vessel. A flash valve at the bottom of the reactor allows a sudden decompression to the atmospheric pressure of the collecting tank. A schematic diagram of the steam explosion setup is shown in Fig. 1.

In each experiment, 100 g of microalgal sample was introduced into the reactor, which had been preheated. Some samples were previously impregnated with sulphuric acid at a concentration of 5% or 10% (w/w, wet sample basis) by mixing for 10 min at 30 °C. The steam explosion pretreatments were conducted at two different temperatures, 120 °C and 150 °C (corresponding to saturated steam pressures of 2 bar and 4.7 bar, respectively) with a fixed retention time of 5 min. Only one reaction time was checked. Due to the small size of microalgae, the selected reaction time (5 min) is enough to reach homogeneity in temperature. This homogeneity is assumed to provide a proper and smooth explosion. After reaction, the exploded samples were collected for further analysis. Table 2 shows the specific experimental conditions, i.e. temperature, time and acid concentration at impregnation and at reaction, used for each steam explosion experiment. It must be noted that the concentration of sulphuric acid during reaction differs from the initial concentration at impregnation due to the dilution effect of the added steam. Since the amount of added steam varied for each experimental condition, the values of acid concentration were experimentally determined considering the amount of acid at impregnation and the total amount of sample collected after the steam explosion treatment.

The mass balance before and after the steam explosion treatment was checked by measuring the dry matter content and ash content of the original and exploded samples by means of a thermogravimetric analysis (TGA). For all the experiments, good balance closures (>98%) were obtained.

2.4. Lipid extraction

Lipids were extracted from microalgal biomass using two methods: the Bligh and Dyer method [24] which uses a ternary system of chloroform/methanol/water and is the most commonly used method for the quantitative extraction of lipids from microalgae at analytical level [25]; secondly, n-hexane extraction was performed to evaluate the extraction performance compared to the Bligh and Dyer method.

For the Bligh and Dyer method, 20 mL of the microalgal sample was mixed with a 75 mL mixture of chloroform–methanol (1:2 v/v) using a magnetic stirrer at 300 rpm for 10 min. Then 25 mL of chloroform and 25 mL of distilled water were added to form a two phase system. The phases were separated by 5 min centrifugation at 3500 rpm. The chloroform phase was then separated (after carefully transferring the mixture to a separatory funnel) and the solvent was evaporated using a rotary evaporator. Finally, the amount of lipid obtained from each sample was measured after further drying overnight in an oven at 70 °C.

The extraction of lipids with n-hexane was performed by mixing 20 mL of sample and 20 mL of n-hexane. The mixture was kept at 40 °C/60 °C and 200 rpm for 4 h, and then centrifuged at 4000 rpm

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