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"Solvent-free" ultrasound-assisted extraction of lipids from fresh microalgae cells: A green, clean and scalable process

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ABSTRACT

In order to comply with criteria of green chemistry concepts and sustainability, a new procedure has been performed for solvent-free ultrasound-assisted extraction (UAE) to extract lipids from fresh *Nannochloropsis oculata* biomass. Through response surface methodology (RSM) parameters affecting the oil recovery were optimized. Optimum conditions for oil extraction were estimated as follows: (i) 1000 W ultrasonic power, (ii) 30 min extraction time and (iii) biomass dry weight content at 5%. Yields were calculated by the total fatty acids methyl esters amounts analyzed by GC–FID–MS. The maximum oil recovery was around 0.21%. This value was compared with the one obtained with the conventional extraction method (Bligh and Dyer). Furthermore, effect of temperature on the yield was also investigated. The overall results show an innovative and effective extraction method adapted for microalgae oil recovery, without using solvent and with an enable scaling up.

1. Introduction

In recent past years, stimulated by that international energy crisis, research projects on alternative and renewable energies including the concept of producing biofuels from the intensive culture of microalgae, as a third-generation biofuel, have been proposed. This research is specially aimed at facilitating the transition to a low-carbon economy with an emphasis to more diversified energy resources and encouraging investments in sustainable sources. The concept to harness energy released by microalgae seems to be increasingly less marginalized for microalgae have been estimated as very good candidates for fuel production (algaefuel). Their main advantages are their higher photosynthetic efficiency, higher biomass production and faster growth, compared to other energy crops (Mata et al., 2010). Microalgae can be produced in large scale on non-arable lands and do not need potable water to grow. Consequently, there is no competition with food production for a growing human population (Singh and Gu, 2010).

A few microalgae strains are known to contain high level of lipids, and they represent a great interest in the research of sustainable sources for biodiesel production. The lipid and fatty acid amounts of microalgae differ according to the culture conditions. For instance, in conditions of nitrogen starvation, some species can accumulate high amounts of triacylglycerides (TAGs), the major feedstock for biodiesel production (Scott et al., 2010).

Lipids extraction process from dry or wet microalgae biomass and its efficiency represent an important key step in the process of biodiesel production. So it is essential to find an extraction one with an efficient device to increase the lipid extraction yield (Lee et al., 2010; Mercer and Armenta, 2011). Various methods have already been used for this purpose (Ranjan et al., 2010), most of them being assisted with solvent, such as Soxhlet extraction with *n*-hexane (Halim et al., 2011), Bligh and Dyer method with a chloroform/methanol solvent mixture (Bligh and Dyer, 1959), supercritical fluid extraction with CO_2 (Andrich et al., 2005) or methanol (Patil et al., 2010). However, for other authors, processes do not necessary require this solvent assistance, they use bead mills (Richmond, 2004), expeller press procedure, extraction with enzyme (Sander and Murthy, 2009), microwave assisted pyrolysis extraction (Du et al., 2011), ultrasound and microwave assisted extractions, pulsed electric field and hydrothermal liquefaction (Brown et al., 2010). Compared to traditional chemical methods involving solvent addition (usually toxic chemicals such as benzene, ether and hexane), solvent-free extraction is often a more ecologic (in terms of sustainable development) and a more economic process, indeed, it need no supplementary energy to separate phases and to eliminate the solvent if no final product recirculation system exists. At last it avoids the risk of medium contamination.

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In some studies dedicated to microalgae oil recovery, a combination between mechanical and chemical extractions was found, such as an ultrasonic-assisted extraction with ethanol (Wiyarno et al., in press), an ultrasound-mediated Bligh and Dyer method (Ranjan et al., 2010), an ultrasound-mediated extraction in *n*-hexane (Ranjan et al., 2010; Cravotto et al., 2008), an ultrasonic water bath device with chloroform/methanol (Krienitz and Wirth, 2006), a microwave extraction with hexane (Balasubramanian et al., 2011; Cravotto et al., 2008), a pulsed electric field prior to a solid/liquid extraction using ethanol (Gottel et al., 2011).

While these processes differ in their environmental (solvent volume and toxicity) and economic (extraction time, presence of solvent) impacts, an important issue to consider is the scale of the process. In most cases, extraction realized is at the laboratory scale, and therefore the challenge is to upscale the process without losing performance and quality in oil recovery, cost-efficiency and environmental impact.

The current work aims at optimize the oil extraction from microalgae cells by using an innovative ultrasound technology. In the light of earlier publications, it has been decided to develop an eco-extraction process (in a context of sustainable development: solvent-free extraction is a preferred option) on fresh microalgae biomass at the pre-industrial scale with a powerful and adaptable ultrasonic device for lab testing and industrial processing of liquids.

Applications of ultrasounds have been widely developed in the industry and are still an active research field, probably because it is a key technology in achieving the objective of sustainable "green" chemistry. Ultrasounds were preliminarily used as a control system in the food industry by using their high frequency (also called diagnostic ultrasounds), but more and more interest has been given to high power (or low frequency) ultrasounds as an innovative and alternative process. The effect of ultrasounds on extraction yields is attributed to the microstreaming and heightened mass transfer produced by cavitation and bubbles collapse, resulting in cells destruction.

Extraction is a strategic processing step in recovering oils from microalgae cells before transesterification stage (in a two-stage procedure). As outlined above, various conventional methods are available for oil extraction, but they generally require long extraction times, rely on toxic petroleum-based solvents and require high energy inputs. Ultrasound-assisted extraction is an alternative technique able to solve problems associated with the conventional methods, as the process simplifies handling and work-up conditions, gives higher purity of the final product, eliminates post-treatment of waste-water. It has also been shown to be more economical and eco-friendly: the process can be completed in a few minutes instead of a few hours with high reproducibility. It also reduces the amount of solvent (sometimes no solvent is used) as well as the energy required compared to conventional methods, by working at lower temperatures or by refraining from the expensive elimination of the solvent (Chemat et al., in press).

The aim of this work is to demonstrate the potential of ultrasonic assistance as an eco-process for lipids extraction from a fresh matrix of just harvested microalgae biomass. Influence of operating parameters on the extraction yields will be studied and evaluated. For this purpose, lipid classes' contents and fatty acids methyl esters profiles have been analyzed using HP-TLC and GC–FID–MS as fine analytical methods. Experimental studies in batch mode are applied on the high power ultrasonic device 1000 W, 20 kHz, which represents a good interface between lab and industrial processing. A response surface methodology obtained from a multivariate study (central composite design) was used to investigate the performance of the extraction procedure, to study the relevance of the variables required in extraction and to determinate the final optimal settings.

Nannochloropsis oculata was chosen as a microalgae model. This strain is prized for its capacities to remove oils (22–29% dry weight biomass) (Mata et al., 2010) suitable for biodiesel production. Oil extracted from this strain consisted of saturated and unsaturated fatty acids like, such as palmitic acid, oleic acid and linoleic acid, which are common fatty acids used for biodiesel production, but also good candidates to improve the quality of the biodiesel (Gerhard, 2008). *Nannochloropsis* sp. is also considered as one of the most promising EPA (eicosapentaenoic acid) producers (Hanhua and Kunshan, 2003). This compound is widely used in mariculture as a very good source of omega-3 polyunsaturated fatty acids.

2. Methods

2.1. Strain, culture and harvesting conditions

N. oculata was supplied by Alpha Biotech Company (Asserac, Loire Atlantique), France. The cultivation system is a mixture of photobioreactor and outdoor raceways of up to 10 m^3. The culture media is made of sea water with an addition of a nitrogen source (+N). Harvesting is being performed by centrifugation at around 5000 rpm, resulting in a 30% dry weight paste that is directly frozen (−25 °C).

2.2. Conventional lipids extraction methods

Lipids content of *N. oculata* cells was determined using a modified procedure described in (Bligh and Dyer, 1959). Typically, a solution of EDTA (1 ml, 1 mM in 0.15 M acid acetic) was added to 10 mg of fresh microalgae at about 20% dry weight (DW). The mixture was transferred to a glass tube with a Teflon-lined screw cap and, after addition of 3 ml of a mixture methanol–chloroform (2:1, v/v), was vortexed (VX-2500, VWR). Then, 1 ml of chloroform and 0.8 ml of KCl (0.88% w/v) were added before vortexing and centrifuging at 4000 rpm for 2 min. The lower chloroform phase was transferred to a new glass tube. Cells were then extracted again with hexane, centrifuged and the supernatant was combined with the previous chloroform extracts. Lipid extracts were dried under a stream of N_2 and re-suspended in solvent for HP-TLC or GC–FID analysis.

2.3. Ultrasonic assisted extraction in batch of aqueous medium

Ultrasound-assisted extraction was carried out using an ultrasonic device operating at low frequencies (20 kHz) with a 1000 W ultrasonic processor (UIP1000hd, Hielscher Ultrasonics, GmbH, Germany). The transducer was customized with a B2–1.8 booster as booster horn and a BS2d34 standard titanium sonotrode (frontal area: 9 cm^2). The computer-supported control of ultrasonic processor was operated by the UPCCTRL V3.2 WIN software. This program allows the pre-adjustment of the control parameters pulse and amplitude of ultrasonic processor. As process parameters, the gross and net power and the energy input into the medium to be treated are acquired. A temperature probe is also added in the liquid. Data collection is made and registered 10 times per second. The global ultrasonic system works as a self-contained unit, including transducer, sonotrode, flow cell and closed loop refrigeration. These modules are located in a double-wall stainless steel cabinet that comes with a very effective sound insulation (Rittal GmbH & Co., Germany). Ultrasound-assisted extraction was processed in a double glass reactor thermostated by a recirculated chiller module system (Buchi).

100 g of fresh *N. oculata* (30% DW) was extracted at each experiment; an antioxidant, ButylHydroToluene (BHT) was added in the