Algal Research 21 (2017) 27–34

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

# Wet lipid extraction from the microalga *Nannochloropsis* sp.: Disruption, physiological effects and solvent screening

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ARTICLE INFO

*Article history:*
Received 13 November 2015
Received in revised form 28 October 2016
Accepted 7 November 2016
Available online xxxx

*Keywords:*
Microalgae
*Nannochloropsis*
Biodiesel
Wet extraction
Disruption
Solvent screening

ABSTRACT

For biodiesel applications, microalgae and especially *Nannochloropsis* sp. are considered as a promising feedstock for lipid production. Main issues are high lipid productivity, but also robust and energy efficient downstream processes. In the biorefinery process, cellular mechanical pretreatment and solvent extraction are investigated nowadays to allow wet route lipid recovery. In this study, a method to screen solvents according their performance to extract lipids in wet condition was proposed. It consisted in short liquid/liquid extraction on cells suspension of microalgae partially disrupted. In one test, it allowed identifying for each solvent: (i) the ability to solubilize lipids compounds; (ii) the limitation by the cell wall/membrane and (iii) kinetics. The cell disruption appeared to be the main controlling step if low water soluble solvents were used. Extraction of lipids from suspension of disrupted microalgae was more efficient than extraction from dried biomass (same solvent, same energy and time) and the water presence enhanced the selectivity for saturated fatty acids recovery. 50% extraction yield was achieved with 84% disruption rate in 10 min and saturated fatty acids (SFA) content was enriched to 72% of the extracted lipids. The 11 solvents screening showed a significant impact of the solvent choice on a 10 min batch extraction yield. Based on the criteria considered in this study, the best solvents were Methyl tert-butyl ether (MTBE) and cyclopentylmethyl ether (CPME). They were those with Hansen solubility parameters close to the target fatty acids, low solubility in water and low heat of vaporization. They represent alternatives to chlorinated solvents or alkanes.



## 1. Introduction

Microalgae appeared in the last decades as a promising alternative feedstock for biodiesel production [1–3]. By strain screening, metabolomics, processing conditions and photobioreactors intensification the lipid accumulation as oleosomes inside the cells has been significantly enhanced [4–6]. For biodiesel applications, the lipid fraction of major interest is triacylglycerides of saturated fatty acids. Main issues are obviously high lipid productivity at the photobioreactor step, but robust and energy efficient downstream processes are also necessary. For example the energy balance in the case of dry route lipid extraction is not positive. According to K. Sander and G. Murthy [7], the minimum net energy input is −3982 MJ for 24 kg of biomass with a lipid content between 30 and 40% (w/w), necessary for the production of 1000 MJ algae biodiesel. In the dry route, the microalgae biomass is first dewatered by filter press or centrifugation to obtain 80–90% moisture and then by a natural gas dryer to finish at less than 10% moisture. However, a natural gas dryer requires 3556 kJ/kg water removed which represents 89% of the total energy input. Generally life-cycle assessment (LCA) studies of biodiesel from microalgae pointed out that the step which requires the most energy input is the biomass drying operation. If the energy input is reduced with an improvement or removal of drying operation, the net energy balance would be positive [7–9]. Consequently the lipid recovery by wet extraction is of particular interest to reduce the energy demand [10–12]. As well, the biorefinery concepts try to valorize the whole biomass as a strategy to decrease the overall cost of the production. It must not exceed 0.25 $/kg to compete the petroleum, according Y. Chisti [13].

Wet solvent extraction of lipids is a unit operation based on a mass exchange between an aqueous feed and an organic solvent phase. As recently described [14–15], biphasic extraction needs a preliminary cell disruption for solvent accessibility to the lipids. Cell rupture can be obtained by chemical or enzymatic treatments, by electrical treatments especially for hydrophilic molecules release [16] or by mechanical treatments (French press, high pressure homogenizer, bead milling [17–21]). Mechanical disruption has the advantage to be continuous, with no matter addition (acids, enzymes, solvents…) that simplify downstream processing and scalability to industrial level. Another point is the possibility to treat the biomass with variable dry matter concentrations corresponding to the concentration obtained at the harvesting step outlet (around 10–30%$_{dw}$) or the concentration considered for

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http://dx.doi.org/10.1016/j.algal.2016.11.005
2211-9264/

the extraction step (0.1–1%$_{dw}$). It must be denoted that energy consumption for mechanical processing is important and correlated to the volume to treat – the biomass concentration has then to be as concentrated as possible [15]. Main limitation is then the tremendous increase of the suspension viscosity with the dry matter content of the culture, starting from fluid suspension like water to a compact paste. For high pressure processes, matter needs to be fluid for efficient disruption, limiting the biomass concentration at 1%$_{dw}$. Otherwise for bead milling, the limitation is only the pumpability of the product to feed the unit, pushing the limit to 5–15%$_{dw}$ but the efficiency at these concentrations has not been clearly described for the moment. Mechanical processes seem to be especially convenient for lipophilic fraction release without altering the biochemical integrity of the other fractions (proteins, pigments, polysaccharides). In this study, high pressure disruption will be considered at lab-scale as it is efficient and especially convenient for the treatment of small volumes of culture (10 mL of culture at 1 g/L).

Microalgae have very different size, topology, composition and thus mechanical robustness. Strain and physiological impact on robustness might be important parameters that should be studied and taken into account for process development [20,22–23].

The solvent choice is also a critical step for extraction process development, but in fact few data are available in this field on solvent screening, including green solvents, and their rationalized comparison. Solvent choice for extraction requires almost 12–15 criteria that must be compared and integrated for technical and economical optimization. These criteria can be organized in 3 groups: “extraction capability”, “solvent recycling” and “health and safety”. For liquid-liquid extraction, the solvent has to be poorly soluble in water. A solvent of interest allows an efficient and rapid recovery of the lipid fraction. The best solvent should be easy to recycle (mainly by vaporization; low boiling temperature and low heat of vaporization) and with minimum risk for human and environment (chlorinated solvents are not candidates for large scale use for example). Most encountered solvents in the literature are alkanes (hexane and heptane), alcohols (methanol, ethanol, propanol), chlorinated (dichloromethane and chloroform) [24–25] and some alternative solvents like supercritical $CO_2$ [26–27], ionic liquids [28–29] or some terpenes [30–33]. Chloroform ($CHCl_3$) is the most encountered in lipid extraction standard analytical methods. Alkanes like hexane are usual in plant oil industry. Heptane and the cyclohexane, as aliphatic and cyclic hydrocarbons, have a higher number of carbons than hexane and then a lower volatility and toxicity. Others conventional solvents are toluene as aromatic hydrocarbon, methyl isobutyl ketone (MIBK) [34] from ketone class and ethyl acetate (EtOAc), as an ester. Toluene and MIBK are mostly used in paints and lacquers industry. EtOAc is a green solvent with reduced human impact, considered as a substitute to chlorinated solvents in industry [31]. Unconventional solvents from green chemistry can also be referenced: dimethyl carbonate (DMC) [25,35] is produced from renewable resources as well as cyclopentylmethyl ether (CPME), methyl tert butyl ether (MTBE) [34, 36–37] and the 2-methyl tetrahydofuran (MeTHF). R-limonene [31–33] completes the list as a terpene from essential oil.

This article proposes a methodological development for pretreatment and solvent choice, applied to wet (diluted) extraction of lipids from microalgal biomass. Extraction process evaluation is based on the solvent price, availability, distillation cost that depend on the solvent nature, but also on the solvent extracting power (extraction efficiency) and the extraction technology (mixer-settler, columns, centrifuge). This experimental work focused on the solvent lipids extracting efficiency criteria, in the case of diluted biomass.

*Nannochloropsis* sp. was used a microalga model for lipid production, mechanical robustness and variability with physiological state. The method development started with a microalgal mechanical disruption study as a pretreatment. The high pressure cell disruption was chosen as an easily tunable and efficient technique to modulate the cellular integrity at laboratory scale. Then, the effect of the water presence on the extraction yield and selectivity was considered for two different solvents chloroform:methanol (2:1) and heptane. At last, 11 solvents were tested on a short duration lipid extraction, with the same energy input and solvent-to-feed ratio (S/F) of 0.5. The solvents were chosen within various chemical classes to test their extracting efficiency. They were compared according to the Hansen solubility parameters, the total fatty acid (TFA) extraction yield, their solubility in water and the required energy for recycling by vaporization, to propose an objective test before process development. Necessary solvent quantity minimization and biomass concentration increase will be discussed considering large-scale application.

## 2. Material and methods

### 2.1. Biomass

*Nannochloropsis* sp. strain was obtained from Alphabiotech collection (Asserac, France). Microalgae were cultivated in a modified Conway medium (3N3P) using artificial seawater at 25 $g \cdot L^{-1}$ of salt (ASW) [6,38–39]. The medium was filtered at 0.2 μm to remove any microbial contamination (Sartolab P20, Sartorius SAS, Germany). The biomass production was performed in 1 L airlift-type flat panel photobioreactor (PBR) [5]. The pH was regulated at 8 by $CO_2$ injection and the temperature at 22 °C was monitored by a pH sensor (Mettler Toledo SG 3253). Two different physiological states were obtained. The first one was obtained for a continuous biomass production with replete nitrogen (N-replete) with a continuous exposition to 150 $\mu mol_{hv} \cdot m^{-2} \cdot s^{-1}$ of white light. The second one was obtained for a batch reactor under progressive nitrogen starvation (N-starved) and higher photons flux to 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ inducing lipid and especially triacylglycerides (TAG) accumulation [6,38]. The nitrogen concentration was followed by ion-exchange chromatography; biomass and TFA content were followed every day by dry matter and GC-FID. Maximum TFA content was obtained 96–120 h after fully N-depleted conditions. Each batch was harvested from the PBR to a sterile glass bottle and directly used for further studies.

### 2.2. High pressure disruption

Disruption of biomass was performed using a high pressure disrupter Cell-D 2.2 kW (series B, Constant systems Ltd., Warwick, (UK)). Samples were treated by 10 mL shots at pressures ranging from 100 to 270 MPa [23]. The energy consumption of the cell disrupter is scaled linearly with the pressure as calculated with Eq. (1)

$$P_H = F_x \cdot \Delta P \cdot \eta_c \quad (1)$$

The hydraulic power ($P_H$) depends on the flow rate ($F_x$), the pressure applied ($\Delta P$) and the rate of the energy delivered on the input energy ($\eta_c$). Cell disruption rate $\%\tau d$ (*cells*) can be calculated with Eq. (2)

$$\%\tau d\ (cells) = \frac{[X]_0 - [X]_f}{[X]_0} \times 100 \quad (2)$$

With $[X]_0$ the initial cell concentration and $[X]_f$ the cell concentration after treatment.

Cell concentration was determined under microscope using a Malassez cell. Samples from the initial batch of algal suspension has an average cell concentration of $10^8$ cells$\cdot mL^{-1}$ and after disruption was diluted with an appropriate factor (DF) to obtain a cell concentration between 30 and 100 per mesh. 10 μL of Iodine-Potassium iodide (IKI) solution was added to 1 mL to help cells decantation before counting. Numbering was performed on 10 meshes per sample.