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Potential pre-concentration methods for *Nannochloropsis gaditana* and a comparative study of pre-concentrated sample properties

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HIGHLIGHTS

- Pre-concentration methods for high lipid content microalga species were studied.
- Dewatering with autoflocculation is found promising.
- Characteristic properties of pre-concentrated samples were evaluated and compared.
- Viscosity, PSD and Ca/Mg ions of pre-concentrated samples were analysed.

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ABSTRACT

We compared potential pre-concentration techniques for *Nannochloropsis gaditana* (Nng) by testing natural sedimentation; flocculation with aluminium sulphate, polyaluminium chloride and chitosan; and induced pH. Promising flocculation efficiencies and concentration factors were obtained in a short time with alkalinity-induced flocculation at an adjusted pH of 9.7 and with chitosan at an adjusted pH of 9.9 using a concentration of 30 mg L^{-1}. The sedimentation rates of alkalinity-induced flocculation were also evaluated. Additionally, viscosity, particle size distribution and Ca/Mg ions were analysed for pre-concentrated samples of *N. gaditana* (Nng) and the previously studied *Phaeodactylum tricornutum* (Pht) which were obtained by various different harvesting methods under optimal conditions. The rheological properties of the concentrated algae suspensions of two microalgal species showed Newtonian behaviour. The mean diameters of the flocs were between 39 and 48 μm. The Ca/Mg analysis showed that Mg^{+2} is the triggering ion for alkalinity-induced flocculation in the conditions studied.

1. Introduction

Microalgae are photosynthetic organisms that store solar energy in biomass chemical bonds through photosynthesis with the use of inexpensive natural resources such as CO_2 and H_2O. Ever since they were identified as a possible raw material for the production of biodiesel, among other commercial applications, the growth aspects of microalgal cultures have received considerable attention.

Of the various biotechnological processes involved in algae farming, harvesting is especially important in determining the cost and quality of the end product. It is estimated that at least 20–30% of the total cost of producing biomass can be attributed to the recovery process (Christenson and Sims, 2011). Furthermore, microalgal removal has long been used in water treatment, in which the methods applied are similar to those for biofuel production, although with different concerns about the harvested microorganisms. Regardless of the objective of the harvesting process, the small size of the algal cells, the scant difference in density between algae and the growth medium, and the dilute concentrations of algal cultures make the harvesting process a key challenge, especially on an industrial scale. To overcome these challenges, a low-energy based harvesting method needs to be developed. The combination of several separation strategies has been proposed

Abbreviations: *A*, OD_{750} (optical density at 750 nm) of sample; AFDW, ash-free dry weight; AS, aluminium sulphate; *B*, OD_{750} of initial culture; C, algae culture; *C*, OD_{750} of reference blanks; Ca, calcium; CF, concentration factor; D_f, mean diameter of floc; FC, filtered algae culture; FE, flocculation efficiency; GM, growth medium; HCl, hydrochloric acid; h_f, final height of concentrated algae solution; h_o, initial height of examined algae solution; *k*, constant of viscosity; Mg, magnesium; NaOH, sodium hydroxide; Nng, *Nannochloropsis gaditana*; OD, optical density; PAC, polyaluminium chloride; Pht, *Phaeodactylum tricornutum*; SR, sedimentation rate; SSVF, settleable solid volume fraction; τ, shear stress; $\dot{\gamma}$, shear rate.

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in which the selection of strategies depends on the species and the desired final product, among other factors.

The first step in the harvesting process (pre-concentration) should essentially be simple, fast, efficient and cost effective. The aim of the pre-concentration, the primary dewatering operation, is to increase the concentration of algae and to reduce the volume to be processed for further treatment. The simple sedimentation system is suitable for harvesting microalgae which have naturally high sedimentation rates (Danquah et al., 2009). If the species has poor sedimentation properties (Şirin et al., 2011), the major techniques that can be used to harvest microalgae cells are centrifugation, flocculation, filtration and screening, flotation and electrophoresis (Uduman et al., 2010).

Of these techniques, flocculation is one of the most common and its mechanism mostly depends on cell and flocculant charges. The efficiency of flocculation is affected by the concentration, the ionic strength, the zeta potential (ξ), the pH of the culture solution, the dosage of the flocculants and co-flocculants, the pH adjustment before or after the flocculants are added, and the mixing time and speed. The degree of salinity also has an effect. Numerous coagulants or flocculants have been tested, most commonly with metal salts such as aluminium sulphate, ferric chloride and ferric sulphate (Papazi et al., 2010), but also with chitosan (Divakaran and Sivasankara Pillai, 2002), cationic starch (Vandamme et al., 2011), bioflocculant produced from bacteria (Salim et al., 2011), etc.

Autoflocculation is the spontaneous formation of flocs and the subsequent settling of microalgae (Uduman et al., 2010). It occurs as a result of the precipitation of carbonate salts with algal cells at high pH, a consequence of algae photosynthetic CO_2 consumption (Sukenik and Shelef, 1984). Therefore, prolonged cultivation in sunlight with a limited CO_2 supply promotes the autoflocculation of algal cells for harvesting. Some cultures naturally reach high alkaline pHs during the exponential growth phase due to photosynthesis (CO_2 consumption) (Şirin et al., 2011) and the simulation of autoflocculation, alkalinity-induced flocculation, using caustic soda, lime (Nurdogan and Oswald, 1995), and other substances has also been studied on the laboratory scale (Semerjian and Ayoub, 2003; Chen et al., 2011). Autoflocculation is by no means a new harvesting technique in water treatment (Tenney and Verhoff, 1973). However, although autoflocculation might be considered one of the most promising methods because it requires less energy, has a high removal efficiency, is an induced flocculation method that does not need chemicals, and mostly takes advantage of gravity settling, until recently it has not been given much attention by research articles concerning the production of biofuel from microalgae.

Each algal species reacts differently to different processing technologies, and the optimal method for maximising algal biomass recovery may very likely depend on the strain used (Natural Algal Biofuels Technology Roadmap). Among the many species of algae, those belonging to the genus *Nannochloropsis* are particularly interesting because of their ability to accumulate large quantities of lipids, which can reach concentrations of up to 65–70% of their total biomass (Simionato et al., 2011). However, in field studies, the lipid content of dry biomass is much lower than the high values reported on a laboratory scale (Şirin et al., 2011).

Nannochloropsis gaditana is a microalgal species that belongs to the class Eustigmatophyceae and, in addition to its lipid content, is widely recognised as an important source of pigments of great commercial value (Macias-Sanchez et al., 2005). Therefore, this species is also considered a promising candidate for industrial biofuel production applications, and studies on its morphology, ultrastructure and the growth physiology of its system have been conducted by other authors (Lubián, 1982).

The harvesting of high lipid content Nng algae, low-energy harvesting procedures and optimal methods for maximising algae recovery and reducing costs are important research topics. This article focuses on (I) potential pre-concentration methods by natural sedimentation, and flocculation with commercial flocculants (aluminium sulphate, polyaluminium chloride) and chitosan; and (II) analysing the pre-concentrated samples of Nng and the previously studied *Phaeodactylum tricornutum* (Pht) (Şirin et al., 2011). Pre-concentrated sample characteristics such as viscosity, particle size distribution (PSD) and magnesium and/or calcium concentrations that comparatively little research has been published were aimed to provide insight into flocculation behaviours.

2. Methods

2.1. Microalgal cultures

The Nng strain was obtained from the Institut de Recerca i Technologia Agromentaries (IRTA) in Sant Carles de la Rapita (Tarragona, Spain). The composition of culture medium (although here we did not add sodium silicate), the culture preparation and ash-free dry weight (AFDW) protocols were described by earlier studies (Şirin et al., 2011).The cultures (300 L of slurry) were harvested on the first day of the stationary phase after 10 days of growth. Cell density and $pH_{culture}$ were monitored offline by taking daily samples. Cell concentration was estimated by interpolating absorbance in a least squares regression for $y = 14.056 * 10^7x + 928993$ ($R^2 = 0.99$), where y represents the cell density (cells mL^{-1}) and x is the absorbance value at 750 nm (the wavelength for turbidity). Formaldehyde solution (37%) was used as a fixative for turbidity samples (1 mL of sample was fixed with 10 μL of solution).

Microscopic photos were taken using a Zeiss Axio Scope A1 microscope. The culture properties of Nng are detailed in Table 1a.

2.2. Natural sedimentation and flocculation experiments

To determine the effects of light and temperature on the sedimentation of Nng cultures, natural sedimentation experiments were conducted under varying conditions: in daylight and darkness and at different temperatures.

To determine the effect of pH on algal flocculation, the pH of the stock microalgae solution was adjusted to acidic and alkaline pHs ($2 \leqslant pH \leqslant 11$) with HCl or NaOH (0.1 and 1 N) solutions.

The algal cells were also flocculated with metal salts (aluminium sulphate (AS), pre-hydrolyzed metal salt-polyaluminium chloride (PAC)) and with chitin-derived polysaccharide (chitosan) to compare the effects of different flocculants. The flocculants AS and PAC were purchased from Kemira Iberica S.A., Spain, and chitosan was purchased from Sigma Aldrich Co., Spain.

The required concentrations of PAC and AS solutions were prepared by diluting stock solutions of flocculants to a reasonable and effective dilution ratio (e.g. considering algae cell damage and the corrosivity factor). The chitosan stock solution (5 g L^{-1}) was prepared by dissolving chitosan in 1% acetic acid solution under continuous agitation until a clear solution was obtained. Other dilutions of chitosan were made from the stock solution to obtain the required concentrations.

All experiments on sedimentation and flocculation were performed as previously described in Şirin et al. (2011). Flocculation efficiency (FE), the concentration factor (CF) and settleable solid volume fraction (SSVF) were also calculated in the same way described previously (Şirin et al., 2011). Cell concentrations for all flocculation samples were monitored with a UV–visible spectrophotometer (Synergy HT Multi-Mode Microplate Reader (Biotek)) with absorbance set at 750 nm. Fig. 1 shows the sample heights for natural sedimentation and flocculation experiments.