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Research paper

Fatty acid methyl ester production from wet microalgal biomass by lipase-catalyzed direct transesterification

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ABSTRACT

The aim of this work was to optimize the production of fatty acid methyl ester (FAME, biodiesel) from wet *Nannchloropsis gaditana* microalgal biomass by direct enzymatic transesterification. This was done in order to avoid the high cost associated with the prior steps of drying and oil extraction. Saponifiable lipids (SLs) from microalgal biomass were transformed to FAME using the lipase Novozyme 435 (N435) from *Candida antarctica* as the catalyst, and finally the FAME were extracted with hexane. t-Butanol was used as the reaction medium so as to decrease lipase deactivation and increase mass transfer velocity. A FAME conversion of 99.5% was achieved using wet microalgal biomass homogenized at 140 MPa to enhance cell disruption, a N435:oil mass ratio of 0.32, methanol added in 3 stages to achieve a total of 4.6 $cm^3\ g^{-1}$ of oil and 7.1 $cm^3\ g^{-1}$ oil of added t-butanol, with a reaction time of 56 h. The FAME conversion decreased to 57% after catalyzing three reactions with the same lipase batch. This work shows the influence of the polar lipids contained in the microalgal biomass both on the reaction velocity and on lipase activity.

1. Introduction

Microalgae are currently considered one of the most promising alternatives for biodiesel production. They are photosynthetic organisms capable of converting (under light conditions) water and carbon dioxide into macromolecules such as oils, polysaccharides and proteins; some of them can give oil yields (liter/hectare) at least 10 times higher than the best vegetable oil crop and achieve high growth rates compared to agricultural crops [1]. Unlike plant oils, which are mostly neutral lipids (NLs), microalgal oils are rich in polar lipids (glycolipids, GLs, and phospholipids, PLs) and thus have a higher viscosity, which may lead to reduced transesterification velocity and FAME conversion [2,3]. For this and other reasons, the neutral saponifiable lipids (NSLs) (free fatty acids, tri-, di- and monoacylglycerols), which are also found in microalgae [2,4,5], are the most interesting lipids to produce FAME. The lipid composition depends on the microalgal species, culture conditions and time of harvesting. It is known that nitrogen concentration in the culture medium is the main factor that upsets lipid synthesis in microalgae [6]. San Pedro et al. [7] found that the lipid profile and content changed when the microalga *Nannochloropsis gaditana* was cultured under nitrogen starvation conditions – the saponifiable lipids (SLs) greatly increased (21.6% of biomass dry weight) compared to that in continuous culture (12.0%). Moreover, the former is richer in NSLs (17.2% of biomass dry weight) than that cultured without nitrogen limitations (7.9%). However, biomass productivity is lower under nitrogen starvation conditions and such culture conditions are far more laborious and difficult to implement on an industrial scale [7]. Furthermore, algae harvesting in the exponential growth phase (usually when production is in continuous operation mode) will give microalgae with more polar lipids than those harvested in the late stationary growth phase (usually in batch cultures), which contain more NSLs [8].

The production of biodiesel from microalgal biomass has been widely reported in the literature by extraction of lipids from microalgal biomass followed by their conversion to FAME [3,9]. However, these procedures have the inconvenience of the multiple process steps necessary to produce biodiesel, such as: drying, oil extraction and the transesterification reaction. For this reason, direct (in-situ) transesterification has been studied as a promising alternative to other methods since the oil extraction and

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transesterification steps are carried out simultaneously; hence, both energy consumption and process costs can be reduced [5,10,11].

Also, it is possible to apply a prior treatment to the wet microalgal biomass in order to enhance cell disruption, oil extraction and biodiesel conversion. Various cell disruption techniques have been studied by several authors [12–14] such as microwaving, bead beating, sonication and autoclaving. However, the scaling up of some of these is difficult. Consequently, cell disruption by high pressure homogenization (HPH) is one of the most widely used methods since it has proven effective in aqueous environments and is easily scalable [2,15,16].

Microalgal oils must be transesterified using acid or enzymatic catalysts. Alkaline catalysts cannot be used because microalgal oils contain free fatty acids [17]. Lipases work at lower temperatures than acids and the separation and purification of biodiesel and glycerol is easier. Moreover, homogeneous acid catalysts require a large amount of water to be removed [17,18]. However, lipases still have drawbacks that need to be overcome, such as their high cost, low stability and low reaction velocity. Perhaps for these last reasons, almost all of the authors carried out direct transesterification using acid [19,20], basic [21] or heterogeneous catalysts [12]. Only a few authors performed this process using enzymatic catalysis, as was the case with Sivaramakrishnan and Muthukumar [22], who achieved a maximum FAME yield of 82% in the direct transesterification of the macroalga *Oedogonium* sp. oil using a *Bacillus* sp. lipase; and Tran et al. [23], who obtained a saponifiable lipids to FAME conversion of 95.7% by direct transesterification of *Chlorella vulgaris* lipids by direct transesterification of *Chlorella vulgaris* lipids using immobilized *Burkholderia* lipase as the catalyst.

The aim of this work was to study the production of biodiesel by lipase-catalyzed direct transesterification of SLs in wet *Nannochloropsis gaditana* microalgal biomass, taking both the FAME yield and lipase stability into account. t-Butanol was used as the reaction medium to help oil extraction within the microalgal cell, to decrease the reaction mixture viscosity and to preserve lipase stability.

2. Materials and methods

2.1. Microalgal biomass, lipases and chemicals

The strain *Nannochloropsis gaditana* B-3 was used as the oil-rich substrate in this study. It was obtained from the Marine Culture Collection of the Institute of Marine Sciences of Andalucía (CSIC, Cádiz, Spain). It was grown in an outdoor tubular photobioreactor at the "*Las Palmerillas*, *Cajamar*" Research Center (El Ejido, Almería, Spain, latitude and longitude 36° 46′ 23″ N, 2° 48′ 37″ W, respectively). The mass fraction of biomass in the wet biomass was 25%, (31.1 ± 0.1) % of total lipids (TLs or oil) of biomass dry weight and a total fatty acid mass fraction (or saponifiable lipids, SLs, as an equivalent to fatty acids) of (12.1 ± 0.1) % in the dry biomass. The transesterification reactions were catalyzed by the lipase Novozym 435 (N435) from *Candida antarctica* (kindly donated by Novozymes A/S, Bagsvaerd, Denmark), which is supplied immobilized on a macroporous acrylic resin and under the conditions used in this work, the lipase does not show positional specificity.

The chemicals used were analytical grade hexane (95% purity, synthesis quality) (Panreac S.A, Barcelona, Spain), methanol (99.9% purity, Carlo Erba Reagents, Rodano, Italy) and tert-butanol (analytical grade, Fluka, Barcelona, Spain). All reagents used in the analytical determinations were of analytical grade. Standards were obtained from Sigma-Aldrich (St Louis, Mo, USA) and used without further purification.

2.2. Homogenization of the wet microalgal biomass, direct transesterification of the saponifiable lipids (SLs) and the fatty acid methyl ester (FAME) extraction procedure

The homogenization of wet microalgal biomass was carried out in a laboratory homogenizer (PandaPlus 2000 S.N. 8983 model, purchased from Gea Niro Soavi S.p.A, Parma, Italy). This equipment can operate at a feed rate of 9 L/h and up to 200 MPa pressure; although in this case it was not possible to operate above 140 MPa because it proved difficult to pass the wet solid (75% water mass fraction) through the homogenizer. Therefore, wet microalgal biomass was homogenized at 140 MPa and stored at −24 °C until use.

Fig. 1 shows the method proposed for carrying out the "in situ" transesterification. In a typical experiment, 3 g of homogenized biomass (0.23 g oil) was mixed with 0.074 g of lipase N435 (N435:oil mass ratio of 0.32) and 3.3 cm^3 of t-butanol (14.2 $cm^3\ g^{-1}$ oil). Then, 1.07 cm^3 of methanol (4.6 $cm^3\ g^{-1}$ oil) was added in three steps at 0, 10 and 24 h, adding 0.29 cm^3 (1.25 $cm^3\ g^{-1}$ oil) in the first step and 0.39 cm^3 (1.68 $cm^3\ g^{-1}$ oil) in the others. The direct transesterification was carried out in 50 cm^3 Erlenmeyer flasks with silicone-capped stoppers. The mixture was incubated at 40 °C and stirred in an orbital shaking air-bath (Inkubator 1000, Unimax 1010 Heidolph, Klein, Germany) at 21 Hz for 56 h.

After the reaction, 8 cm^3 of hexane was added and the resultant slurry was stirred for 10 min (first extraction). After that, the mixture was centrifuged at 1640 × g for 5 min to remove the residual biomass and separate the lipase; and the biphasic t-butanol-methanol-water-hexane system was separated in a decantation funnel. 5 cm^3 of water and a small amount of NaCl were added in order to facilitate the separation of the extract's hydrophilic components and break the emulsion that had formed. Subsequently, a second extraction was carried out of the FAME remaining in the t-butanol-methanol-water phase by adding 8 cm^3 of hexane.

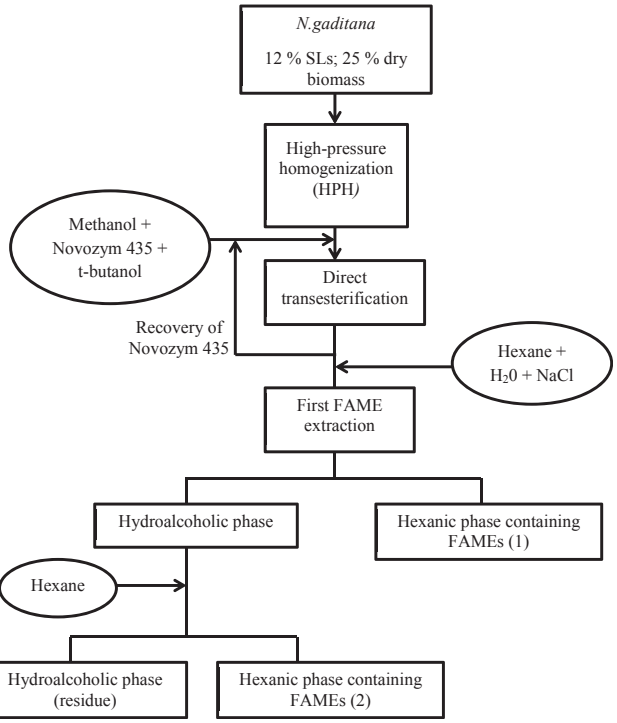

Fig. 1. Direct transesterification of SLs from wet *N. gaditana* biomass and extraction of FAME with hexane.