Biodiesel synthesis by direct transesterification of microalga *Botryococcus braunii* with continuous methanol reflux

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HIGHLIGHTS

- Reflux extraction reactor promotes high fatty acid methyl esters yield (FAME).
- One step process combining lipid extraction and lipid conversion into FAME.
- Biomass is repeatedly contacted with pure solvent enhancing FAME yield.
- Hexane use as co-solvent enhances FAME yield.

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ABSTRACT

Direct transesterification of *Botryococcus braunii* with continuous acyl acceptor reflux was evaluated. This method combines in one step lipid extraction and esterification/transesterification. Fatty acid methyl esters (FAME) synthesis by direct conversion of microalgal biomass was carried out using sulfuric acid as catalyst and methanol as acyl acceptor. In this system, once lipids are extracted, they are contacted with the catalyst and methanol reaching 82% wt of FAME yield. To optimize the reaction conditions, a factorial design using surface response methodology was applied. The effects of catalyst concentration and co-solvent concentration were studied. Hexane was used as co-solvent for increasing lipid extraction performance. The incorporation of hexane in the reaction provoked an increase in FAME yield from 82% (pure methanol) to 95% when a 47% v/v of hexane was incorporated in the reaction. However, the selectivity towards non-saponifiable lipids such as sterols was increased, negatively affecting biodiesel quality.

1. Introduction

Recent interest in the production of microalgae is mainly related to the use of microalgal biomass as renewable source for biofuels and bioproducts development. Microalgae have high lipid content which can be used for aquaculture, human nutrition or biofuel production. A lipid yield between 58,000 L/ha and 136,000 L/ha has been estimated. On the other side, oil from oilseeds such as rapeseed or soybean present oil yields of 1190 L/ha and 446 L/ha, respectively (Chen et al., 2011; Halim et al., 2010).

The lipid extraction process is one of the most limiting steps for the development of biodiesel production industry based in microalgae. In fact, lipid extraction from microalgae is mainly performed by organic solvents and not by conventional physical methods, due to difficulties in breaking the cell wall, making microalgae-based biodiesel production unfeasible at industrial scale (Ehimen et al., 2010; Hidalgo et al., 2013).

Simultaneous lipid extraction and esterification/transesterification is a technique of great value for biodiesel production from microalgae, as it allows extracting and converting fatty acids into fatty acid methyl esters (FAME) in a single step bypassing the use of large quantities of organic solvents used in lipid extraction (Jin et al., 2014; Wahlen et al., 2011).

This process has been traditionally performed by direct contact between the biomass, the catalyst and the acyl-acceptor, where a high quantity of acyl acceptor is necessary to promote lipids diffusion from inside the cell (Griffiths et al., 2010). In this process,

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lipids pass through a polar lipid bilayer by simple diffusion following the concentration gradient.

If the biomass is in direct contact with the acyl acceptor, lipids diffusion from cells to the acyl acceptor could be limited due to a decreasing concentration gradient in time. Thereby, high acyl acceptor volume is necessary to increase lipids extraction. However, a high acyl acceptor volume will decrease the acid strength of the catalyst and thus decrease the reaction yield (Hidalgo et al., 2014). Additionally, the acid catalyst performs a dual role, as catalyst of the reaction and as a cell wall disruptor agent (Ozgul-Yucel and Turkay, 2002). Therefore, a reduction of acid strength of the catalyst due to the increase of acyl acceptor volume could have a negative impact on the total reaction yield.

The traditional configuration used in biomass direct transesterification corresponds to a closed reaction vessel containing the reaction mixture where the acyl acceptor is in direct contact with the biomass (Haas and Wagner, 2011). In this configuration both temperature and agitation are maintained during a specified period of time (Ehimen et al., 2010; El-Shimi et al., 2013; Hidalgo et al., 2013). This system is of easy implementation, but lipids extraction will be limited due to a decreasing concentration gradient of lipids (outside and inside the cell), requiring a subsequent step for separating the biomass from the reaction product (Hidalgo et al., 2014). Normally, high acyl acceptor volume for increasing lipids diffusion and FAME yield has been used in biomass transesterification (Ehimen et al., 2010). Acyl acceptor excess plays also a role as extraction solvent, improving the contact between catalyst and biomass, altering the permeability of the solid substrate (Haas et al., 2007). Besides, acyl acceptor excess is responsible for breaking linkages between glycerin and fatty acids during the reaction (Hidalgo et al., 2013). Siler-Marinkovic and Tomasevic (1998) used a wide range of methanol:lipid molar ratios, ranging between 100:1 and 300:1, for the transesterification of macerated sunflower seeds (Siler-Marinkovic and Tomasevic, 1998). Moreover, Ehimen et al. (2010) used a methanol:lipid molar ratio between 105:1 and 524:1 for the direct transesterification of *Chlorella* microalgae (Ehimen et al., 2010).

Solvent reflux processes have been traditionally carried out for lipid extraction using a continuous or discontinuous (soxhlet) extractor. In this sense, the continuous process could have application in the production of biodiesel from microalgae and until today, only few studies tackle this issue in the literature. In the implementation of this system, microalgal biomass is contacted repeatedly with fresh portions of the acyl acceptor and in so, lipids can be extracted and esterified in the presence of a high acyl acceptor volume, hence favoring product formation. Although in this system no application of shear stress is used to produce microalgae cell wall disruption, highest FAME yields were obtained using this configuration, probably enhanced by high lipids extraction yields due to diffusion (Hidalgo et al., 2014). According to this previous study, the use of solvent reflux increased FAME yield in direct transesterification of microalgal biomass. A FAME yield close to 80% was achieved using this configuration in contrast to the traditional configuration in batch reactor (FAME yield of only 60%) where the biomass is in direct contact with solvent and catalyst. Although better results were obtained in this system, only few studies

related to the use of solvent reflux for the direct transesterification of microalgal biomass have been published yet. Therefore it appears interesting to test this technique and its operational conditions when using *Botryococcus braunii* as a lipid source for microalgae-based biodiesel production.

Hence, the aim of this work was to carry out direct transesterification of microalgal biomass in a reflux extraction reactor (RER), a different configuration compared to the used techniques where lipid diffusion is limited. In RER, the microalgae sample is repeatedly contacted with pure solvent, thus increasing the lipid extraction and conversion yield to FAME. To optimize the operational conditions in this system, an experimental design using surface response methodology was developed to find out the influence of the operational variables and the interaction among them on FAME yield. The variables studied were co-solvent and catalyst concentration. Hexane was used as co-solvent in the reaction.

2. Methods

2.1. Lipid quantification in B. braunii

The microalga used in this study was supplied by Desert Bioenergy S.A., Chile. The moisture content was 7.8% wt and size distribution was <150 µm. The lipids of *B. braunii* were extracted in a soxhlet apparatus for its quantification and characterization using a methanol:chloroform ratio of 2:1 v/v. The extracted lipids (total lipids, TLs) were divided in two main fractions, saponifiable lipids (SLs) and unsaponifiable lipids (USLs). SLs were defined as total fatty acids (TFA) or transesterifiable lipids.

USLs were evaluated according to AOCS method (Cc-6a-53) and SLs by difference between TLs and USLs. The determination of USL profile was performed by gas chromatography coupled to a mass spectrometry detector (GC–MS) using derivatization through silylation with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) in pyridine.

The fatty acid distribution was determined according to AOCS method (Ce 2-66) (AOCS, 2012). The acid value as well as FFA were titrimetrically determined using AOCS method (Cd 3d-63). Phosphatides content was evaluated using AOCS method (Ca 12-55). Acyl-glyceride contents (tri-di and mono-glycerides) were determined by gas chromatography coupled to a flame ionization detector (GC–FID) using tricaprin as internal standard.

2.2. Experimental set up

The experiments were carried out in a reflux extraction reactor (RER). This system has been used for lipids extraction, where the

Table 1
Independent variables and levels of the experimental design.

Independent variable	Levels				
	−1.47	−1	0	1	1.47
Co-solvent (% v/v)	34	40	55	70	76
	(3.4)	(3.1)	(2.3)	(1.5)	(1.2)
Catalyst (% wt)[*]	59.6	75	112.5	150	165.4

[*] On the basis of total fatty acids. Values in parentheses correspond to the polarity index (PI) of the mixture evaluated according to (Snyder, 1978).

Table 2
Lipids composition of *B. braunii* microalgae.

Lipid composition	Content (%)	Content (%) (on the basis of total lipids)
Saponifiable lipids		
Triglycerides	1.7 ± 0.3	1
Diglycerides	2.5 ± 0.5	1.5
Monoglycerides	0.9 ± 0.3	0.5
Free fatty acids	46.7 ± 0.1	27.5
Esters	3.1 ± 0.2	1.8
Phospholipids	16.3 ± 0.2	9.6
Unidentified	28.8	16.9
Unsaponifiable lipids		
Hydrocarbons	32.6 ± 0.5	13.4
Sterols	19.6 ± 1.9	8.1
Alcohols	25.3 ± 1.2	10.4
Ketones	4.6 ± 0.5	1.9
Unidentified	17.9	7.4