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A whole biodiesel conversion process combining isolation, cultivation and *in situ* supercritical methanol transesterification of native microalgae

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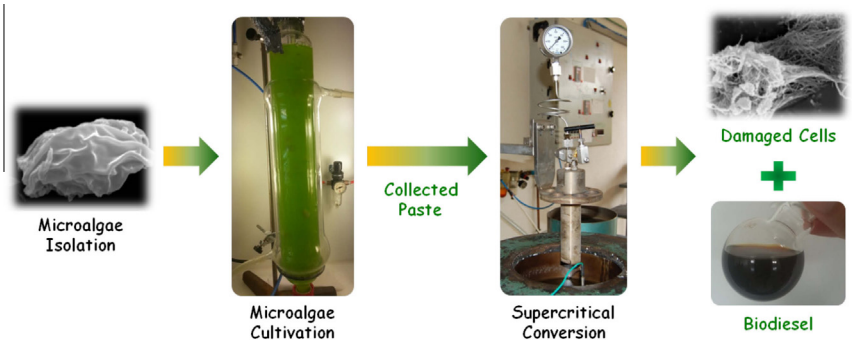
HIGHLIGHTS

- Resistant microalgae isolated from South Mediterranean lagoons were studied.
- Microalgae were successfully cultivated in batch and continuous photobioreactors.
- Photosynthetic performance and nutrient removal efficiency were investigated.
- A high biodiesel yield was obtained by *in situ* supercritical treatment of wet algae.
- All transesterifiable lipids can be converted to biodiesel by supercritical process.

GRAPHICAL ABSTRACT



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ABSTRACT

A coupled process combining microalgae production with direct supercritical biodiesel conversion using a reduced number of operating steps is proposed in this work. Two newly isolated native microalgae strains, identified as *Chlorella* sp. and *Nannochloris* sp., were cultivated in both batch and continuous modes. Maximum productivities were achieved during continuous cultures with 318 mg/l day and 256 mg/l day for *Chlorella* sp. and *Nannochloris* sp., respectively. Microalgae were further characterized by determining their photosynthetic performance and nutrient removal efficiency. Biodiesel was produced by catalyst-free *in situ* supercritical methanol transesterification of wet unwashed algal biomass (75 wt.% of moisture). Maximum biodiesel yields of 45.62 wt.% and 21.79 wt.% were reached for *Chlorella* sp. and *Nannochloris* sp., respectively. The analysis of polyunsaturated fatty acids of *Chlorella* sp. showed a decrease in their proportion when comparing conventional and supercritical transesterification processes (from 37.4% to 13.9%, respectively), thus improving the quality of the biodiesel.

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1. Introduction

With the over-consumption of fossil fuel reserves and their impact on disastrous climate changes, the search for renewable and clean biofuels, such as biodiesel, that can replace petroleum-based fuels is urgently required (Mata et al., 2010; Gao et al., 2013). Microalgae have received particular attention as the most promising alternative for biodiesel production due to their photosynthetic efficiency, high cellular lipid content, CO_2 fixation and growth on non-arable lands unsuitable for food crops in raceway ponds and tubular photobioreactors (Talebi et al., 2013; Taher et al., 2014).

The choice of the best microalgae species is crucial to develop a competitive biodiesel production process. The most important criteria are the ability of microalgae to accumulate lipids, to grow fast and to adapt in local environments. Zhou et al. (2011) reported that native microalgae, isolated from natural water bodies and wastewater treatment plant sites, satisfy all the above criteria.

Microalgae isolation is attracting increasingly attention all around the world. In the context of Mediterranean Sea, microalgae biodiversity has been well exploited in the North Mediterranean area of the European countries, while little attention is paid to the variability and availability of microalgae in the South Mediterranean Sea, especially in the Maghreb countries including Tunisia. Tunisia is a country situated on the south-western part of the Mediterranean Sea and is characterized by abundant sunlight and water resources. Among the aquatic Tunisian environments, there are many semi-enclosed shallow lagoons with high salinity and nutrient levels, allowing the accumulation of phytoplancton to a very high biomass concentration (Armi et al., 2012). Nevertheless, the interest of native microalgae issued from these South Mediterranean waters has not yet been reported to date for biodiesel production. It should be mentioned that the geographical location of microalgae derives from weather, temperature and light intensity variations (Zhu et al., 2013). This geographical distribution leads to variations in the growth rate, biochemical composition and fuel potential of microalgae.

Most of researches on microalgal biodiesel production are focused on only one or a few steps of the whole microalgae-to-biodiesel process: microalgae screening, cultivation, harvesting, drying, lipid extraction and oil to biodiesel conversion. Nevertheless, only a few studies combining all the aforementioned steps are available (Wang et al., 2013). A critical step, accounting for 89% of the energy input required and 70–75% of the total processing cost, is the drying of the algal biomass (Taher et al., 2014). The lipid recovery from microalgae is also a limiting step due to the recalcitrant structure of the algal cell walls, consisting mainly of complex carbohydrate and glycoprotein with strong chemical resistance, which prevents the release of intracellular lipids (Kim et al., 2013). In order to overcome these limitations, it could be interesting to develop a one-step process allowing at the same time the processing of wet algal biomass and the effective disruption of cell walls.

An emerging technology that meets all the above criteria is the *in situ* or direct supercritical methanol transesterification. This technique also permits the conversion of the released lipids into biodiesel at the same step, thus considerably reducing the energy consumption of the process. The main advantages of the supercritical process are: (i) the high used temperature and pressure (240–290 °C and 9.3–30 MPa) successfully disrupted the cell walls (Patil et al., 2011; Jazzar et al., 2015); (ii) no purification steps are required after the process since catalysts are usually not added; (iii) water and free fatty acids, which can be present in large amounts in wet algal biomass, can improve the efficiency of the supercritical conversion process (Olivares-Carrillo and Quesada-Medina, 2011).

The aim of this study is to evaluate the biodiesel potential of new native and robust microalgae strains isolated from South Mediterranean aquatic lagoons and cultivated in bubble column photobioreactors. A whole process is then developed. It combines microalgae isolation, cultivation and direct conversion to biodiesel, which was performed using an innovative technology: the catalyst-free *in situ* supercritical methanol transesterification. This one-step process was directly conducted on wet algal biomass, thus avoiding washing and drying steps.

2. Methods

2.1. Microalgae isolation

Water samples were collected from two different Tunisian saline sites: Tunis lagoon (GPS: 36°49′25.6″N 10°12′36.4″E, salinity: 33.8 psu) and Monastir lagoon (GPS: 35°46′18.0″N 10°46′34.4″E, salinity: 44.4 psu). Once in the laboratory, water samples were filtered through 0.66 μm pore size membrane filters. Aliquots were then directly inoculated into 50 ml Erlenmeyer flasks containing autoclaved natural seawater supplemented with Conway medium (Walne, 1966) containing ampicillin (100 μg/ml), kanamycine (100 μg/ml) and nystatin (50 μg/ml) and maintained for 2 weeks on a rotary shaker (80 rpm, 23 ± 2 °C, photoperiod of 14 h light and 10 h darkness and illumination of 200 $\mu E\ m^{-2}\ s^{-1}$). Then, 30–100 μl from these enrichment cultures were plated in solid Conway medium at the same above conditions. Pure colonies were isolated by successive plating operations as assessed by microscopic observations.

2.2. Molecular identification of isolated microalgae

The molecular identification of two isolated microalgae (SM1 and AM1) was based on 18S rRNA gene sequencing followed by DNA similarity search. Microalgae samples were harvested in the mid-exponential growth phase and genomic DNA was extracted by the conventional method of SDS-phenol chloroform as described by de la Vega et al. (2011). A region of ~700-bp of 18S rRNA gene was amplified by PCR using eukaryotic primers (Fw: 5′-GTCAGAGGTGAAATTCTTGGATTTA-3′, Rev: 5′-AGGGCAGGGACG TAATCAACG-3′) (Rasoul-Amini et al., 2009). PCR reactions were performed in 50 μl reaction mixture containing $MgCl_2$ (2 mM), DNA (50 ng), dNTPs (200 μM), primers (0.6 μM each), and GoTaq DNA polymerase (1.25 U). The following PCR program was conducted: 1 cycle at 94 °C for 5 min followed by 32 cycles at 94 °C for 45 s, 50 °C for 45 s and 72 °C for 1.30 min, and a final cycle at 72 °C for 7 min. The amplified PCR products were automatically sequenced using an Applied Biosystems 3130 DNA sequencer (FST, Tunisia). DNA sequence analysis and editing were performed using the BioEdit program v.7.2.5. Based on multiple alignment and dendrogram construction, phylogenetic analysis was done through MEGA software v.6.0 using the neighbor-joining (NJ) algorithm and 1000 rounds of bootstrap resampling. Sequence identities were generated by the BLASTn algorithm at the NCBI server. Then, the sequenced regions of 18S rRNA gene were submitted to GenBank database under the accession numbers KM401849 (for SM1) and KP119843 (for AM1).

2.3. Photobioreactor cultivation

Microalgae strains were cultivated in progressive increasing volumes from 0.25 l to 1 l flasks with Algal medium (Bionova,

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