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Simultaneous esterification and transesterification of microbial oil from *Chlorella minutissima* by acid catalysis route: A comparison between homogeneous and heterogeneous catalysts

Carla C.A. Loures[a,b], Mateus S. Amaral[b], Patrícia C.M. Da Rós[b], Savienne M.F.E. Zorn[b], Heizir F. de Castro[b,*], Messias B. Silva[b]

[a] Federal Center of Technological Education of Rio de Janeiro, Angra dos Reis, 23953-030 Rio de Janeiro, Brazil
[b] Engineering School of Lorena, University of São Paulo, Estrada Municipal do Campinho s/n, 12602-810 Lorena, São Paulo, Brazil

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ABSTRACT

Using low cost materials, tubular photo-bioreactors type bubble column were built for microalgae cultivation. Six factors/two levels Taguchi orthogonal array (L8) was adopted to identify the input variables with the greatest influence on oil accumulation by *Chlorella minutissima*. The function desirability was used to simultaneously analyze the process variables to attain maximum biomass concentration and lipid content. The microalgal oil was submitted to acid simultaneous esterification and transesterification reactions using homogeneous (H_2SO_4) and heterogeneous (Nb_2O_5/SO_4) catalysts. Both catalysts afforded high biodiesel yield (> 96%), although heterogeneous catalyst showed better yield (98%) and low residual glycerides at 4 h reaction.

1. Introduction

A promising alternative to replace fossil fuels are biofuels from microalgal biomass [1], which comprise third generation of raw materials used to obtain among other liquid fuels, biodiesel. Microalgae, which are photosynthetic microscopic organisms, found in marine and freshwater, have stood out in comparison to other microorganisms that are part of this class due to its potential to accumulate lipid material, that can be converted into biodiesel, presenting also high growth rate and increased photosynthetic efficiency [2,3].

Microalgae can be grown in closed or open systems. The closed systems consist of three most common photo-bioreactors types (PBR): tubular columns, plates and flat, while in open systems, the most commonly used are raceways, which are susceptible to microbial contamination and have limitations for cultivation parameters effective control [3]. On the other hand, closed photo-bioreactors can be optimized to serve as growing system for many microalgae species, allowing for the optimization of cultivation variables for enhancing cell growth and lipid content [4]. Furthermore, this configuration has several advantages towards open systems, such as external contamination protection, high mass transfer rates, lower nutrient loss by evaporation and carbon dioxide gas exhaustion, water economy and controlled energy use (resulting in a process with lower energy consumption) [5].

Bubble-column reactors, a tubular system type, emerge as one of the most promising models for microalgae cultivation [6]. Aeration in this type of equipment is made by bubbling, wherein air is injected into its base resulting in a good mixing and consequent increase in mass transfer gas-liquid, thereby increasing CO_2 supply and O_2 removal from the top [7,8]. Some authors state that this kind of photo-bioreactor configuration provides high mass transfer rates, efficient mixing with low shear rates, low power consumption, high potential for large-scale cultivation, sterilization easiness and photo-inhibition or photo-oxidation reduction [5].

On this basis, a bench scale layout with three photo-bioreactors, bubble-column type, was constructed and operated under different conditions for cultivation of microalgae *Chlorella minutissima*.

C. minutissima species is a microscopic, eukaryotic, spherical, single-cell microalgae with diameter ranging from 5 to 10 μm. It can be found in tanks and lakes, and has a high photosynthetic capacity; it contains green pigments such as chlorophyll *a* and *b* in its chloroplast (≅ 2% DWT) and lipids are one of its main components. It multiplies rapidly and uses carbon dioxide, water, sunlight and small amounts of minerals for reproduction purpose. Thus, this strain was selected for this research due to its high photosynthetic capacity, standing out as a new source of lipid raw material that can be used in the energy segment [9].

Microbial oil, obtained under optimized culture conditions, was characterized and converted into alkyl esters using acid catalysis. In these processes, homogeneous [10] and heterogeneous [11] catalysts

* Corresponding author.
E-mail address: heizir@dequi.eel.usp.br (H.F. de Castro).

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were evaluated in suitable reactor configurations for the processes of simultaneous esterification and transesterification using ethanol as acylating agent.

2. Materials and methods

2.1. Microalgae strain and catalysts

Marine microalgae *C. minutissima* used in this research was obtained from Seaweed Culture Collection (Oceanographic Institute – University of São Paulo) and was provided by the Department of Biological Oceanography (São Paulo, Brazil). All reagents used were of analytical standard. The H_2SO_4 (98%) was acquired from Cromoline (SP-Brazil). The Nb_2O_5/SO_4 catalyst was prepared with a mixture of 5 g Nb_2O_5, 5 mL H_2SO_4 solution (0.5 mol L^{-1}) and 15 mL deionized water in a glass reactor at 90 °C under reflux and constant agitation (500 rpm) for 3 h [11]. The resulted material was dried at 100 °C for 1 h and subsequently calcined at 500 °C for 4 h, and showed the following properties: surface acidity (2.76 mmol H^+ g^{-1}), surface area (63.1 m^2 g^{-1}) and pore volume (0.14 cm^3 g^{-1}) [11].

2.2. Photo-bioreactor construction and parameters estimation

20 L bubble column reactor was constructed using acrylic material according to the following dimensions (internal diameter: 135 mm, height: 1200 mm, and wall thickness: 5.0 mm), 18 L working volume, equipped with external light and pneumatic agitation. A Schematic diagram of the photo-bioreactor experimental apparatus used in this study is shown in Fig. 1.

Airlift was provided by an air compressor Boyu (ACQ-003 model). Aeration system was maintained using airstone diffusers (13.5 cm diameter) which are located at the base in center tubes, connected via silicone tubing to a diaphragm air compressor with flow rate of 2 vvm (volume air per volume medium per minute) for air microbubbles dispersion in the system. CO_2 supply to the medium was 4.0% in relationship to system aeration (2.0 vvm) introduced into system via CO_2 solenoid. Flow was measured using a CO_2 flow meter designed for this application.

CO_2 compressed into cylinders was used concomitantly as industrial airflow to promote aeration, when required as a carbon source, in accordance with experimental design. Experimental apparatus was kept under air-conditioned room and diffused light. Room temperature was kept constant at 25 °C by an air conditioning hot/cold type. Microalgae culture was inoculated at 10% (v/v) under constant white fluorescent illumination (150 klux) for a period of seven days.

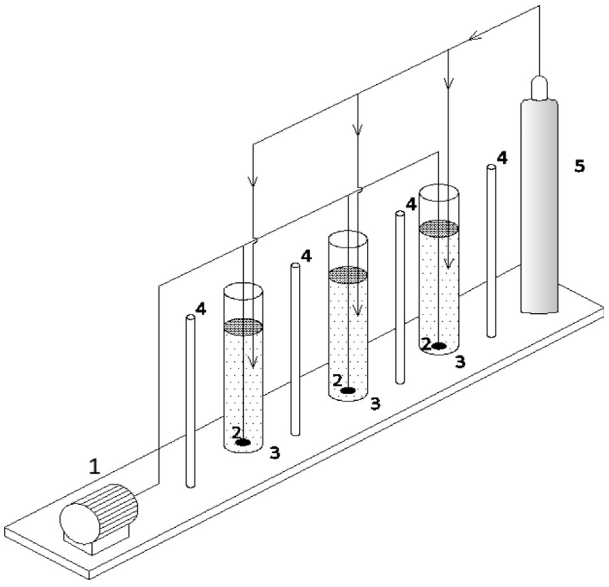

Fig. 1. Schematic diagram to photo-bioreactor experimental apparatus: 1 – compressor, 2 – airstone diffuser, 3 – bubble column reactor, 4 – fluorescent lamps, 5 – CO_2 gas cylinder.

Table 1
Factors evaluated in the experimental design and their respective coding levels.

Code	Factor	Level	
		1	2
A	CO_2 (%)	0.0	2.0
B	$NaNO_3$(g L^{-1})	0.25	0.50
C	$NaH_2PO_4{\cdot}H_2O$ (g L^{-1})	0.35	0.65
D	Supplementation (mL)	1.0	2.0
E	Temperature (°C)	20	30
F	Salinity (g L^{-1})	15	30

Nutrients supplementation: vitamins (Thiamine, Cyanocobalamin Biotin) and metals ($FeCl_3{\cdot}6H_2O$, $ZnSO_4{\cdot}7H_2O$, $MnCl_2{\cdot}4H_2O$, $Na_2MoO_4{\cdot}2H_2O$, $CoCl_2{\cdot}6H_2O$; $CuSO_4{\cdot}5H_2O$).

2.3. Growing conditions for microalgae C. minutissima in photo-bioreactor

A Taguchi orthogonal array experimental design (L8) was applied to evaluate photo-bioreactor performance for microalgae growth, according to the factors and levels displayed in Table 1. Biomass concentration and lipid content were taken as response variables. Statistical analysis was performed using Statistica software (version 11.0). In addition, Desirability function was used for simultaneous optimization of the variables, based on Minitab software (version 16.0) responses. Cell inoculation was conducted on a 10% basis (v/v) and culture was maintained in a modified Guillard f/2 medium without silica [12], under a 150 Lux light intensity for a period of 7 days. Runs were performed in triplicates.

2.4. Biomass determination by turbidimetry and harvesting

Samples were taken during cultivation in order to measure biomass concentration by spectrophotometry, and calculated by optical density (λ = 690 nm) in spectrophotometer UV–Vis (model Bel Photonics), with the aid of a predetermined calibration curve. Cell count was performed using a microscope (Bioval model) with the aid of a Neuberger Chamber, 0.1 mm deep [13]. Upon finishing the cultivation phase, biomass was recovered by coagulation with aluminum sulfate solution (1 mol L^{-1}) subjected to a filtration step and subsequently washed with ammonium formate solution (0.6 mol L^{-1}) for removal of excess salt.

2.5. Microbial oil extraction

Microbial oil was extracted from wet biomass (60% moisture content) according to a modified Bligh's and Dyer's method [14], with chloroform, methanol and water mixture used as extracting solvent at the following respective ratio: 1:2:0.8 (v/v/v). Extracted lipids were dried in a rotatory evaporator to remove solvent residues and subsequently dried at 60 °C until constant weight was attained. The production efficiency of the microbial lipids was calculated on the basis of the biomass concentration (X), lipid concentration (P), lipid yield (% P) and lipid specific yield ($Y_{P/X}$) obtained in the culture cultivations, as shown in Eqs. (1)–(4), respectively. The results were also analyzed by considering the following parameters: volumetric productivity in relation to biomass (Q_X) (Eq. (5)) and volumetric productivity in relation to lipid (Q_P) (Eq. (6)).

$$X = \frac{dry\ weight\ of\ cellular\ biomass\ (g)}{volume\ of\ the\ cultivation\ medium\ (L)} \quad (1)$$

$$P = \frac{lipids\ (g)}{volume\ of\ the\ cultivation\ medium\ (L)} \quad (2)$$