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Cultivation of algae in photobioreactor and obtention of biodiesel

Cristiane B. Hobuss,[1] Pauline F. Rosales,[1] Dalila Venzke,[1] Priscila O. Souza,[1] Priscilla C. Gobbi,[1] Lidiane P. Gouvea,[1] Marco A. Z. Santos,[1] Ernani Pinto,[2] Eduardo Jacob-Lopes,[3] Claudio M. P. Pereira[*,1]

[1] *Laboratório de Heterociclos Bioativos e Química Sustentável, Universidade Federal de Pelotas, Brazil,*
[2] *Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Brazil,*
[3] *Departamento de Tecnologia e Ciência de Alimentos, Universidade Federal de Santa Maria, Brasil.*

Article

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Abstract: In this work we described the cultivation of *Chlorella vulgaris* in a photobioreactor to algal biomass production. The dried biomass was used as feedstock for biodiesel production, it presented 26% lipids and via sonocatalysis stage of the methodology resulted in 60% of fatty acid methyl esters (FAME). The FAME content was confirmed by Gas Chromatography (GC).

Keywords:
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ultrasound irradiation
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Introduction

Chlorella vulgaris is a green algae found in most bodies of fresh water, have industrial uses for producing energy and making processed foods more visually appealing. Also, *C. vulgaris* shows promises as a biomass fuel and as a natural food coloring agent (Lourenço, 2007; Terãn, 1989; Rocha et al., 2007). Because algae grows rapidly in light and dark places with a minimum of nutrients, large amounts of flammable when dried *C. vulgaris* can be produced at low cost (Johnson et al., 2009). Another property of *C. vulgaris* its ability to produce natural colorants and fatty acids (Lourenço, 2007). In this connection, the employ of *Chlorella vulgaris* biomass for the production of biodiesel (fatty acid methyl esters FAME) (Jacob-Lopes et al., 2009) has been described by various authors as one of the most promising biomass feedstocks with the potential to meet fossil diesel replacement targets without encroaching on arable land suitable for food production. In Chisti et al. (2007), particular microalgae as compared to terrestrial plants have high oil content and biomass productivity. In recent years the use of microalgae to biofuel has gained renewed interest with increase in demand for use of renewable energy sources as well as due to rocketing energy prices all over the world (Fulke et al., 2010). Illman et al. (2000) have run a diesel engine with a high proportion of powdered cellulose (85% cellulose/15% diesel) and dried powdered *Chlorella* sp. *Chlorella* sp. was chosen as it has been cultured extensively, does not aggregate and has a mean diameter of 5-10 mm similar to that of powdered coal and cellulose.

The main contribution to the calorific value of cells is from their carbohydrate, protein and lipid content, which it will be necessary for the microalgae to have a high calorific value if they are to be used as a diesel replacement. It is envisaged that the microalgal fuel would be used for the generation of electricity using static diesel engines (Scragg et al., 2002). Thus, due to the environmental benefits, including the carbon dioxide sequestration, and a resource from a renewable source, biodiesel has become increasingly attractive (Francisco et al., 2010).

Material and Methods

Apparatus and analysis

All solvents and chemicals were of research grade and were used as obtained from Aldrich. The reactions were carried out with a microtip probe connected to a 500 W Sonics Vibracell ultrasonic processor operating at 20 kHz at 25% of the maximum power output. The progress of the reactions was monitored on a Shimadzu 2010 Gas Chromatograph equipped with a Rtx-Wax polyethylene glycol capillary column (0.32 mm × 30 m). Nitrogen was used as carrier gas, the initial column temperature was set at 170 ºC, which was later increased to 230 ºC at rate 1 °C min^{-1}. The gun was kept as 250 °C with an injection volume of 2 L in splitless mode. The FID temperature was set at 270 ºC. FAME were identified by chromatographic comparison with authentic standards (Sigma). The amounts of each FAME were calculated from the peak areas of the chromatogram using heptadecanoic acid as internal standard.

Direct transesterification in Ultrasound Irradiation

The dried algal biomass (2 g) was placed in a glass test tube and mixed with 7 mL of methanol, 1 mL of sulfuric acid and 4 mL of chloroform. The reaction mixture was in ultrasound probe for 30 min. After the reaction was completed, the tubes were allowed to cool to room temperature. Then, 10 mL of a solution Na_2SO_4 was added to the tube and mixed for 50 s. The tubes were allowed to separate into two phases. The tubes were centrifuged for 30 min to accelerate phase separation. The solvent layer that contained biodiesel (FAME) was collected and transferred to a preweighed glass vial. The solvent was evaporated using N_2. The FAME determination by Gas Chromatography. Lipid content was determined by the method described by Bligh & Dyer (Bligh & Dyer, 1959).

Microorganism and culture conditions

Chlorella vulgaris (CPCC90), obtained from the Canadian Phycological Culture Centre, was used in the experiments. The stock cultures were propagated and maintained on synthetic BGN medium (Rippka et. al, 1979) with the following composition (g/L): $K_2HPO_4.3H_2O$ (0.040), $MgSO_4.7H_2O$ (0.075), EDTA (0.001), H_3BO_3 (2.860), $MnCl_2.4H_2O$ (1.810), $ZnSO_4.7H_2O$ (0.222), $Na_2MoO_4.2H_2O$ (0.390), $CuSO_4.5H_2O$ (0.079), $CaCl_2.6H_2O$ (0.040), $NaNO_3$ (150) $C_6H_8O_7.H_2O$ (0.006), ammonium iron citrate (0.006), pH 8.0. The incubation conditions used were 25 ºC, photon flux density of 15 $\mu mol.m^{-2}.s^{-1}$ and a photoperiod of 10:14 h (day:night).

Photobioreactor

Measurements were made in a bubble column photobioreactor (Figure 1). The system was built in 4 mm thick glass, had an internal diameter of 7.5 cm, a height of 75 cm, and a nominal working volume of 2 L. The dispersion system for the reactor consisted of a 1.5 cm in diameter air diffuser located in the centre of the column. The reactor was continuously illuminated with sixteen 20 W fluorescent lamps, connected in parallel, located in a photoperiod chamber. The duration of light cycles was controlled by a timer. Airflow into the photobioreactor was provided via filtered air and pure CO_2 cylinders through Teflon tubing. The CO_2/air mixture was adjusted to achieve the desired concentration of carbon dioxide in the airstream through three rotameters that measured the flow rates of the carbon dioxide, the air, and the mixture of gases, respectively.

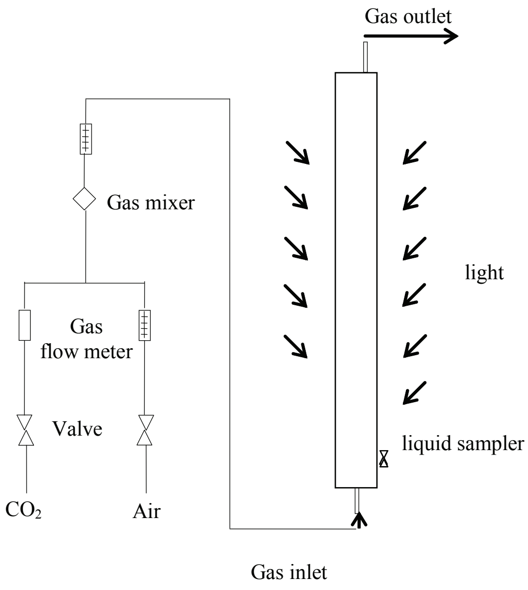

Figure 1. Schematic diagram of the photobioreactor.

Biomass production

The experiments were carried out in bioreactors operating in intermittent regime, fed with 2 L of BGN medium. The experimental conditions were as follows: initial cell concentration of 0.1 $g.L^{-1}$, isothermal reactor operating at a temperature of 25 ºC, photon flux density of 150 $\mu mol.m^{-2}.s^{-1}$, and continuous aeration of 1 VVM with the injection of air enriched with 8% carbon dioxide. The light cycles evaluated were 24:0 (day:night), respectively. The cell density, pH dynamics and lipid content of the biomass were monitored every 12 h during the growth phase of the microorganism. The tests were carried out in duplicate and the kinetic data referred to the mean of four repetitions. Biomass data were used to calculate the biomass productivity (P_x, g/L.day), the maximum specific growth rate (μ_{max}, day^{-1}), the generation time (tg, day^{-1}) and the lipid productivity (P_L, g/L.day).

Harvesting and drying

The biomass was separated from the culture medium by decantation and centrifugation. It was then freeze dried in conditions of temperature of -40 ºC and pressure of 50 µHg.

Results

We have prepared several classes of organic compounds by sonocatalysis (Pizzuti et al., 2009; Pizzuti et al., 2010; Silva et al., 2009; Venzke et al., 2011). In particular, the beneficial effects of ultrasonic irradiation are playing an increasing role in chemical processes, especially in cases where classical methods require drastic conditions or prolonged reaction times, as biodiesel synthesis. Thus, ultrasound is considered to be an important tool for green chemistry in terms of waste minimization and energy conservation (Venzke et al., 2011). Recently it has been reported the preparation of biodiesel from vegetable oils, with good results. Here is reported a preliminary study to obtention of biodiesel from microalgae. The reaction was performed in one pot from biomass dried of *Chlorella vulgaris*, and methanol as solvent at room temperature under ultrasonic irradiation, furnishing the biodiesel in significantly shorter time. The FAME (Table 1) content was confirmed by Gas Chromatography (GC) (Figure 2).

The biomass and lipid production potential of the *Chlorella vulgaris* (CTCC90) were monitored in batch photobioreactors (Table 2). The microalgae growth profile showed a maximum cell density of 2.01 g/L, a maximum specific growth rate of 0.24 day^{-1}, a generation time of 2.88 day, a biomass productivity of 0.19 g/L.day, a lipid content of 26.0% and a lipid productivity of 0.05 g/L.day. A positive growing profile of pH was verified, reaching a maximum value of 10.5. Lipid productivity (P_L) is the main criterion for selection of the operational conditions in bioreactors for microalgae oil production that reflects a combination between biomass productivity and lipid content. Comparatively the PL result obtained in this study is in accordance with the inventory of Griffiths & Harrison (2009), who evaluated lipid productivities for 25 species of microalgae in photosynthetic cultivations and found average values of 0.05 g/L.day.

Table 1. Esters from *Chlorella vulgaris*.

	Ester from *Chlorella vulgaris*	Found (%)
1	Methyl octanoate (C8:0)	0.0544
2	Methyl decanoate (C10:0)	0.01156
3	Methyl dodecanoate (C12:0)	0.0506
4	Methyl myristate (C14:0)	0.26075
5	Methyl palmitate (C16:0)	23.2466
6	Methyl palmitoleate (C16:1)	0.19769
7	Methyl stearate (C18:0)	1.31459
8	Methyl oleate (C18:1n9c)	29.98263
9	Methyl linoleate (C18:2n6c)	3.38901
10	Methyl linolenate (C18:3n3)	5.05722
11	Methyl arachidate (C20:0)	0.16254
12	Methyl behenate (C22:0)	0.21336
13	Methyl *cis*-13-docosenoate (C22:1n9)	0.05382

Table 2. Biomass production parameters.

Parameter	Value
X_{max} (g/L)	2.01
μ_{max} (day^{-1})	0.24
tg (day)	2.88
PX (g/L.day)	0.19
Lipid content (%)	26.0
PL (g/L.day)	0.05
pH_{max}	10.5

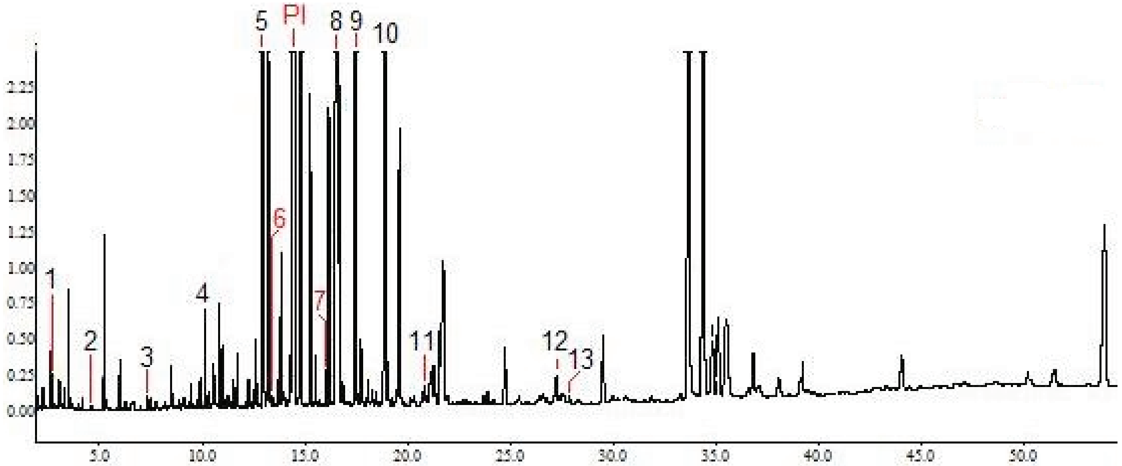

Figure 2. Gas chromatogram of biodiesel (*Chlorella vulgaris*).

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*Correspondence

Claudio M. P. Pereira
Laboratório de Heterociclos Bioativos e Química Sustentável, Universidade Federal de Pelotas
Campus Universitário s/n, Caixa-Postal 354, 96010-900 Pelotas-RS, Brazil
claudio.martin@pq.cnpq.br
Tel. +55 53 3275 7358
Fax: +55 53 3275 7354