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Cultivation of Three Microalgae Strains under Mixotrophic Conditions for Biodiesel Production

Luisa F. Rios*[a], Carla da Silva Soares[a], Marija B. Tasić[b], Maria R. Wolf Maciel[a], Rubens Maciel Filho[a]

[a]University of Campinas, Chemical Engineering Faculty, Laboratory of Optimization, Design and Advanced Process Control-Bioen division, Av. Albert Einstein, 500. Campinas SP – Brazil
[b]Faculty of Technology, University of Niš, Bulevar oslobodjenja 124, 16000 Leskovac, Serbia
luisa.rpinto@yahoo.com

Microalgal biomass have a potential to be used as feedstock for biodiesel production because of high photosynthetic rates, high biomass production, faster growth in comparison to traditional feedstocks and to not be competitive with food production. In this paper, the growth and lipid content of three microalgae strains, namely *Chorella vulgaris*, *Desmodesmus* sp. and *Desmodesmus brasiliensis*, were studied during 9 days of mixotrophic cultivation. The laboratory scale experiments were carried out in BG-11 media enriched with 10 g/L glucose at initial medium pH of 7.5, temperature of 26 ± 4 ºC and light flux of 62 µE m-2 s-1. The highest biomass concentration of 3.82 mg/mL was found in the *Desmodesmus brasiliensis*, followed with 3.64 mg/mL of *Desmodesmus* sp. and 3.32 mg/mL of *Chorella vulgaris* strain. However, the *Desmodesmus brasiliensis* had the lowest lipid content of 6%, followed with 13.2% of *Chorella vulgaris*, whereas the *Desmodesmus* sp. strain produced the highest lipids (19.45%).

1. Introduction

Currently, human society is faced with three global environmental problems: the pollution produced by carbon dioxide release in the air, limited reserves and high costs of fossil fuels. Precisely because of this, sustainable biofuels became very popular field of research. Historically, research began using food crops as feedstock (first generation) for biofuels production. However, first generation biofuels raised a big human concern because of the competition with food industry. The second generation biofuels use forest residues, lignocellulosic agriculture and inedible crops as feedstocks. But, problems with second generation biofuels such as deforestation and expanding the cultivation of agriculture land, limit its commercial application. Microalgae use to produce biofuels belongs to the third generation and is a resource that solves major problems related to first and second biofuel generation (Brennan and Owende, 2010). Microalgae are oleaginous microorganisms that can be used as feedstock for biodiesel production because they are characterized high lipid contents, short and fast growth cycles and high photosynthetic efficiency comparing to other biodiesel feedstock crops. Additionally, microalgae cultivation doesn't need much land as food crops (Huang et al., 2010) and synthetized oil composition can provide biodiesel of very good, stable and standardized quality. The cultivation of microalgae can be done in three different ways: photoautotrophic where cells use carbon source in the form of carbon dioxide and light energy, heterotrophic where cells use only organic carbons as a source of both carbon source and energy, and mixotrophic culture where cells use organic, inorganic carbons and light energy. Although, mixotrophic cultures show faster growth rates and biomass density, photoautotrophic cultivation had higher lipid productivity (Liang et al., 2009). Previously reported studies have mostly being focused on photoautotrophic culture to select the best species of microalgae for biodiesel production (Cheirsilp and Torpee, 2012). Also, there are published papers focused on mixotrophic cultivation with *Chorella vulgaris* (Garcia et al., 2010; Heredia-Arroyoa et al., 2011; Ogawa et al., 2004; Liang et al., 2009), *Desmodesmus* sp (Chenga et al., 2013) but no one with *Desmodesmus brasiliensis*. This objective of this paper is to study three different types of microalgae under mixotrophic conditions to determine which one is most suitable to be optimized for cultivation in biodiesel production. Obtained results

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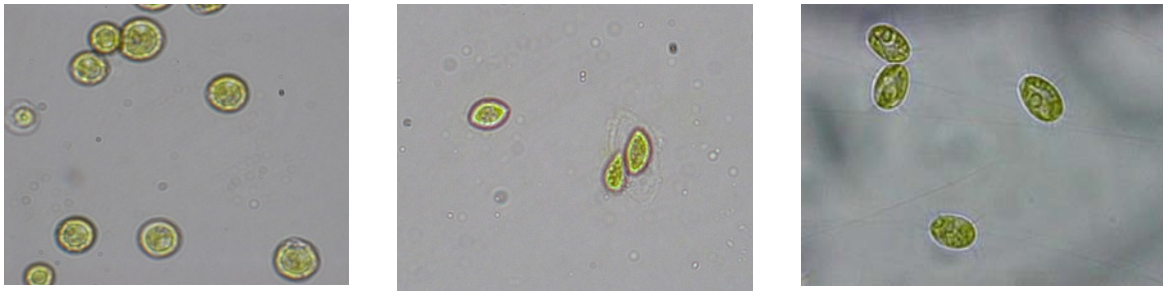
were compared with those obtained under photoautotrophic conditions or under previously published under mixotrophic cultivation.

2. Materials and methods

2.1 Microalgae and culture medium

Three microalgae strain was used in this work: *Chlorella vulgaris* Beijerinck (n. 012, isolated of Reservatório do Lobo in 1979, Itirapina – SP), *Desmodesmus brasiliensis* (Bohlin) E.Hegewald 2000 (n. 262, isolated of lagoa São Miguel - Rio Madeira - Rondônia in 2011 and registried in the World Data Centre of Microorganisms – WDCM n. 835, donated by the Laboratório de Ficologia, Departamento de Botânica da UFSCar) and *Desmodesmus* sp. donated by the Laboratório de Pesquisas com Organismos Aquáticos (LAPOA), Grupo Integrado de Aquicultura e Estudos Ambientais (GIA), Universidade Federal do Paraná (UFPR), Curitiba/Paraná. All three microalga strains are shown in Figure 1. The BG-11 medium (Rippka et al., 1979) with following composition: $NaNO_3$ (1500 mg L^{-1}), K_2HPO_4 (40 mg L^{-1}), $CaCl_2.2H_2O$ (30 mg L^{-1}), Na_2CO_3 (19 mg L^{-1}), $MgSO_4.7H_2O$ (8 mg L^{-1}), $C_6H_8O_7.H_2O$ (7 mg L^{-1}), ammonium ferric citrate (6 mg L^{-1}), H_3BO_3 (3 mg L^{-1}), $MnCl_2.4H_2O$ (2 mg L^{-1}), $Na_2EDTA.2H_2O$ (0.7 mg L^{-1}), $Na_2MoO_4.2H_2O$ (0.4 mg L^{-1}), $ZnSO_4.7H_2O$ (0.2 mg L^{-1}), $CuSO_4.5H_2O$ (0.1 mg L^{-1}) and $Co(NO_3)2.6H_2O$ (0.05 mg L^{-1}); enriched with 10 g/L glucose at initial medium pH of 7.5 was used for cultivation.



(a) (b) (c)

Figure 1: Microalgae strain. (a) Chorella vulgaris, (b) Desmodesmus brasiliensis, (c) Desmodesmus sp.

2.2 Experimental procedure

Microalgae growth was carried out in 250 mL Erlenmeyer flasks with 200 mL of culture medium and 10% microalgae inoculum. As can be seen in Figure 2, the experiments were laboratory scale, carried out under following conditions: light flux of 62 µE m^{-2} s^{-1} for 24 h, shaker rate of 250 rpm, 26 ± 4 ºC for a period of 9 days. All the experiments were performed in duplicate.

Figure 2: Microalgae cultivation system.

2.3 Analytical methods

Cell growth

Cell concentration was measured with spectrophotometer UV (Agilent Tecnologies, model Cary 60) at absorbance of 682, 684 and 680 nm for the *Chlorella vulgaris*, *Desmodesmus* sp and *Desmodesmus*

brasiliensis, respectively. A standard curve was prepared by plotting cell dry weight (dw) values (in g/L) against corresponding absorbance readings.

Microalgae biomass

Biomass was separated from the medium by centrifugation (Eppendorf, model 5810) at 4500 rpm for 20 min. The growth rate of a microalgae population, day^{-1}, is a measure of the increase in biomass over time and it is determined from the exponential phase using following equation:

$$\mu = \frac{ln\frac{X_t}{X_{t0}}}{t_t - t_o} \quad (1)$$

where X_{t0} and X_t were the dw biomass concentrations (g/L), t_0 (day) and t_t (day) are start-and end- point of exponential phase, respectively.

The generation or doubling time t_d (day), can also be calculated once the specific growth rate is known:

$$t_d = \frac{ln2}{\mu} \quad (2)$$

Cell disruption

The cell disruption was carried out by autoclaving under temperature 125°C for 5 minutes with 5 mL of distillate water. Afterwards, the biomass was dried in an oven at 105 °C for 24h.

Lipid extraction

After 9 days of cultivation lipid extraction was carried out with Bligh and Dyer procedure (Bligh and Dyer, 1959). Dried biomass and methanol/chloroform mixture were vigorously agitated for 25 min and then centrifuged at 4500 pm for 10 min. Obtained mixture had three phases, where lower phase contained the extracted lipids. Extracted lipids were measured gravimetrically drying in an oven at 105°C.

However, in process design at industrial scale lipid productivity is more important parameter than lipid content. The lipid productivity in a microalgae population, P_L, g/(L day), in batch cultures is determined from the harvested biomass using following equation:

$$P_L = \frac{\Delta X}{\Delta t} \quad (3)$$

where ΔX represents the accumulated lipids from inoculation, which is synthetized in the time Δt.

3. Results

3.1 Microalgae growth

The microalgae growth and glucose consumption for *Chorella vulgaris*, *Desmodesmus brasiliensis and Desmodesmus* sp. were shown in the Figures 1-3, respectively.

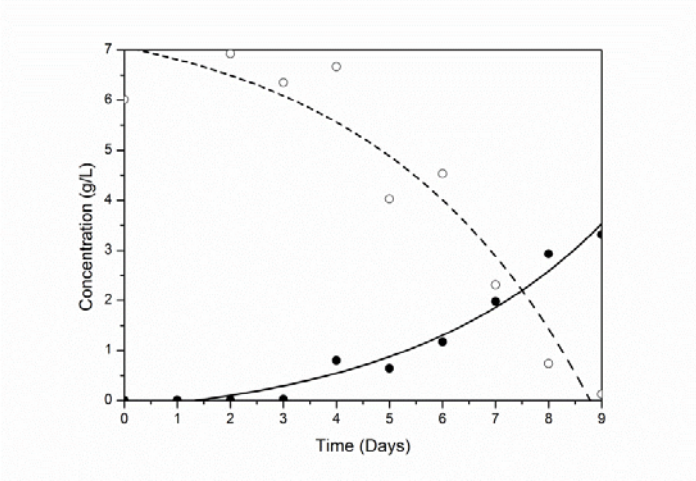


Figure 1: Microalgae mixotrophic growth and glucose uptake of Chlorella vulgaris; (Closed circle — biomass, Open circle — glucose).

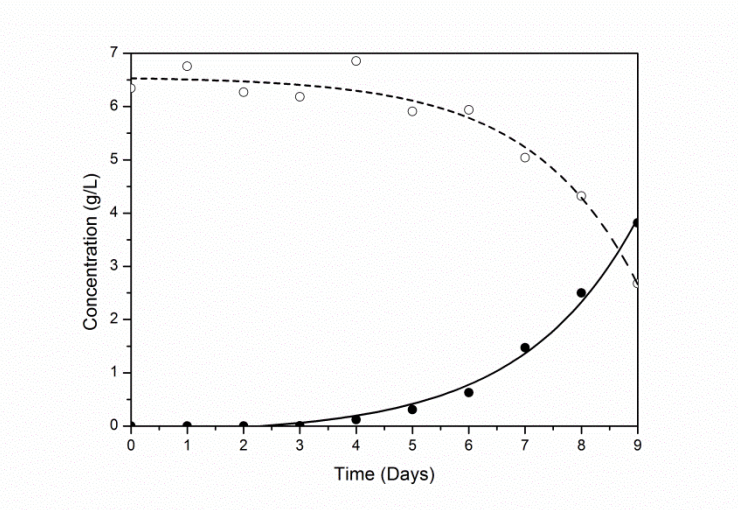


Figure 2: Microalgae mixotrophic growth and glucose uptake of Desmodesmus brasiliensis (Closed circle — biomass, Open circle — glucose).

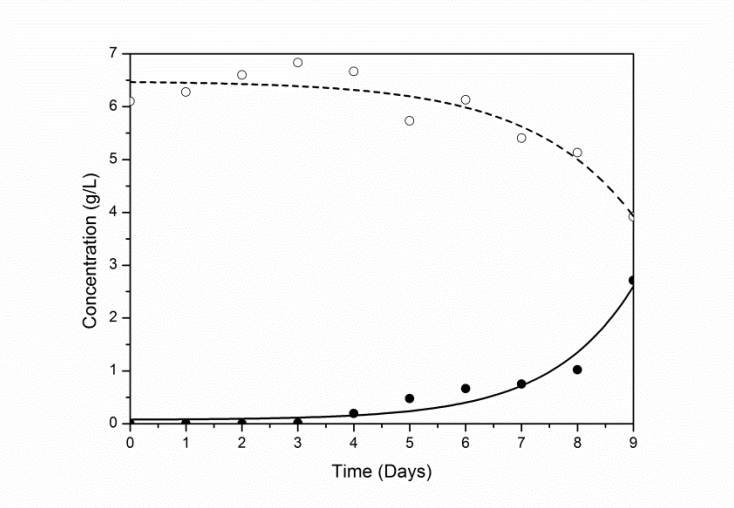


Figure 3: Microalgae mixotrophic growth and glucose uptake of Desmodesmus sp. (Closed circle — biomass, Open circle — glucose).

Table 1: The growth rates of microalgae strains

	Chorella vulgaris	*Desmodesmus brasiliensis*	*Desmodesmus* sp.
μ(day^{-1})	0.661	0.699	0.44607
	0.089[a]	0.101[a]	0,070[a]

[a]photoautotrophic conditions (Almeida et al., 2015)

Desmodesmus brasiliensis was the one with highest growth rate and smallest doubling time, as was the trend under photoautotrophic conditions.

3.2 Lipids concentration

Table 2 showed the results for the lipid extraction. The *Desmodesmus* sp. has accumulated the major while *Desmodesmus brasiliensis* was with the lowest content of lipids. Obtained values of lipid content were in agreement with previously published (Table 2). Comparing to the values obtained by autotrophic cultivation (Cheirsilp and Torpee. 2012. Rios et al., 2015) *Desmodesmus* sp. lipid content under mixotrophic cultivation was higher.

Table 2: Lipids concentration in the three strain microalgae

Strain	*Chorella vulgaris*	*Desmodesmus brasiliensis*	*Desmodesmus* sp.
Lipidis (%)	13.20	5.69	19.45
	13.82 [a]	-	14.50 [b]
	27.38 [a]	-	15.00 [c]

[a]Heredia-Arroyoa et al. 2011.[b]Cheirsilp and Torpee. 2012. [c]Rios et al., 2015.

Lipid productivity for *Clorella vulgaris*, *Desmodesmus brasiliensis* and *Desmodesmus* sp. obtained during these cultivations were 48.69 mg/(L day), 24.15 mg/(L day) and 78.66 mg/(L day), respectively. Higher lipid productivity (54 mg/l/d) was obtained under mixotrophic cultivation with *Chlorella vulgaris* in the Basal culture media enriched with the same glucose concentration of 1% (Liang et al., 2009). The lipid rich microalga *Desmodesmus* sp cultivated in CO_2 (2.5%) mixotrophic conditions (Ho et al., 2014) obtained even more higher lipid productivity values of 113 mg/(L day) – 263 mg/(L day). The values for *Desmodesmus brasiliensis* was not available, since there was no publishes data about using this strain under mixotrophic conditions.

4. Conclusions

According to the most recent scientific opinion, the energetically and economically favourable large scale algal biodiesel production is based on wastewater treatment as primary goal. The wastewaters are in abundance in organic carbon. Thus, the principal aim of the present work was to investigate which algal species were able to grow on a carbon (glucose) rich medium. In this way we can have a good starting point in determination of selected algal strains potential to grow in wastewater effluent.

This paper results indicate that under mixotrophic conditions the *Desmodesmus* strains grow faster than the *Chorella* strain, and that the *Desmodesmus* sp. produces more lipids than the *Desmodesmus brasiliensis* strain. Therefore, the *Desmodesmus* sp. should be considered as a potential strain for the exploitation in biodiesel production. It should be pointed out that although have the highest content of lipids further investigations should confirm which carbon source is better for higher lipid productivity values and if *Desmodesmus* sp.'s lipid composition is suitable for producing standardized quality of biodiesel.

Acknowledgments

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