EFFECT OF CRUDE GLYCEROL CONCENTRATION AND LUMINOSITY ON THE OBTAINING OF BIOPRODUCTS BY *Chlorella Vulgaris*

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ABSTRACT

The cultivation of microalgae, with deprivation of light and the use of organic compounds, can lead to the synthesis of extracellular polymeric substances (EPS) of commercial interest, as well as lipids with potential for biodiesel. For the growth of microalgae and the generation of low-cost EPS and lipids, crude glycerol (CG) can be used as a nutrient source. The objective of this study was to evaluate the influence of CG concentration on EPS and lipids production by *Chlorella vulgaris* IBLC105 in heterotrophic (dark) and mixotrophic (12-hour light/dark photoperiod) cultivation, as well as to characterize the bioproducts obtained under the best conditions. Cultivations were carried in a synthetic medium (BG11) containing different concentrations of CG (0.5, 2.5, and 4.5 g L^{-1}). In the experimental domain, the maximum simultaneous productions occurred in heterotrophic cultivation in the medium containing 4.5 g L^{-1} of CG. Under these conditions, the fatty acid composition of accumulated lipids demonstrated potential for producing qualified biodiesel for use in Brazil, the United States and Europe. Additionally, the EPS exhibited pseudoplastic behavior and rheological characteristics similar to those of a commercial viscosifier, xanthan gum. These results generate the expectation of more competitive EPS and lipids on the market.

Keywords: Microalgae. Residue. Viscosity. Bioproducts. Biodiesel.

1 INTRODUCTION

Environmental problems, the energy crisis and the high costs of natural products encourage global interest in sustainable and economically viable products (1). In this context, as an alternative to the generation of bioproducts based on plant extractivism, products of microbial origin emerge. Microbiological processes are advantageous because they require little labor, eliminate the need for arable land and are constant and renewable sources of resources, as they are independent of climatic conditions (2).

Among microorganisms of technological interest, microalgae stand out for their ability to adapt to adverse conditions and metabolic versatility (3), which makes it possible to obtain bioproducts, such as biomass, which can be used in the production of biodiesel, and extracellular polymers, whose rheological properties justify their wide application as thickeners, emulsifiers and stabilizers (4). When cultivating microalgae, process costs can be minimized by using light-deprived organic waste. In this sense, residual glycerol from biodiesel production emerges as an alternative to supplement heterotrophic microalgae cultivations, enabling the generation of high-value bioproducts (5,6). However, the production and properties of these bioproducts are influenced by cultivation conditions (7).

The objective of this work was to evaluate the influence of CG concentration on the production of cell biomass and EPS by *Chlorella vulgaris* IBLC105 in heterotrophic and mixotrophic cultivation, as well as to characterize the oil and biopolymer obtained in the best condition.

2 MATERIAL & METHODS

Microorganisms and crude glycerol

The microalgae *Chlorella vulgaris* IBL - C105 was donated by LABBIOTEC – UFBA Bioprospection and Biotechnology Laboratory. The microalgae inoculum was propagated in an Erlenmeyer flask with BG-11 medium and incubated in BOD (Biochemical Oxygen Demand), at 28°C, 12h light/dark photoperiod, and aeration with injection of compressed air filtered through glass wool. The crude glycerol for biodiesel was granted by the company Petrobras, Candeias-BA.

Growing conditions

The microalgae, after prior adaptation (1.5 g L^{-1} of sodium nitrate, 2 g.L^{-1} of glycerol, agitation at 140 rpm and absence of light), were used in heterotrophic and mixotrophic cultures (12h light/dark photoperiod) in amount corresponding to 30% (m/v) of the medium. The cultures were carried out in 250mL Erlenmeyer flasks (100 useful mL) incubated in a TE-424 shaker (Tecnal, Brazil) for 24h, at 25ºC, 140 rpm, $NaNO_3$ concentration of 1.5 g L^{-1}, and different concentrations of CG (0.5; 2.5 and 4.5 g L^{-1}). The concentration measurements were carried out by reading the optical density (OD) of the medium, using a spectrophotometer (PerkinElmer Lambda 35 UV/VIS), 670 nm (8).

Biomass, EPS and Lipids

The biomass was recovered by centrifugation (Eppendorf 5702 R) at 4400 rpm for 10 min. The biomass obtained was frozen at -80°C (ColdLab CL580-86V) and lyophilized (Liotop L101). The EPS present in the supernatant was extracted with the addition of absolute ethanol in a ratio of 3:1 (ethanol: medium). The precipitated material was filtered, dried in an oven at 35°C and quantified in g L^{-1}(1). The parameters that attest the biodiesel quality were estimated in relation to the molecular structures of the fatty acid methyl esters (9). The rheological parameters were obtained in a rheometer (Haake Rheotest 2.1). Aqueous solutions of EPS (0.4%, w/v) were prepared and kept under refrigeration for 12 hours. The measurements were obtained as a function of the variation in the shear rate (25 to 1000 s^{-1}), at a temperature of 25°C (1). The data were fitted to the Ostwald-de-Waele model.

3 RESULTS & DISCUSSION

The results presented in Table 1 reveal that in cultivation without light deprivation, the CG concentration did not influence biomass growth and EPS production. The photoperiod was probably insufficient for the energy demand required under the cultivation conditions (10), causing a delay in the growth phase. In light-deprived crops, the amount of glycerol proportionally influenced the production of Biomass (1.12 g L^{-1}) and EPS (0.98 g L^{-1}). Under these conditions, both energy and carbon were obtained from CG consumption. Thus, light deprivation favored biomass and EPS productivity.

Table 1 Biomass and EPS production by *Chlorella vulgaris* IBL-C105 grown under different conditions.

	CG (g L^{-1})	Biomass (g L^{-1})	EPS (g L^{-1})
Heterotrophic	0,5	0,66 $\pm$0,08	0,02$\pm$0,08
	2,5	0,91$\pm$0,12	0,44$\pm$0,02
	4,5	1,21$\pm$0,09	0,98$\pm$0,06
Mixotrophic	0,5	0,19$\pm$0,08	0,21$\pm$0,05
	2,5	0,17$\pm$0,10	0,18$\pm$0,03
	4,5	0,19$\pm$0,12	0,24$\pm$0,05

The fatty acids composition of microalgal biomass is a fundamental characteristic for the quality of biodiesel (11). The cultivation that presented the highest production of biomass and EPS (4.5 g L^{-1} of glycerol) accumulated 51% (m/m) of lipids composed of 39.54% of saturated fatty acids, 35.86% monounsaturated and 24.06% polyunsaturated. Saturated fatty acids are of interest for the production of biodiesel, as they make it possible to obtain an oil with greater resistance to fuel oxidation, and with greater stability. Furthermore, the higher the chain saturation, the higher the cetane number, which is one of the quality parameters of biodiesel (9, 12).

The fatty acid esters obtained showed a cetane number corresponding to 53.44, which is in accordance with reference values established by standards in Brazil (> = 45), European Union (> = 51) and United States of America (> = 47). This result demonstrates the potential for good combustion in an engine, as this parameter is related to the ignition speed. Furthermore, the composition presented a cold filter clogging point of 10.69°C, with values lower than 19°C being acceptable. This parameter indicates the limit temperature for the fuel to flow. As for the iodine value (84.73 g/100g), the value obtained also meets the reference parameter (<= 120 g/100g), which indicates that the fuel is stable and less susceptible to oxidation. (9, 13).

The rheological analysis of EPS produced with 4.5 g L^{-1} of CG was compared with those of Sigma xanthan gum, as shown in Figure 1. The curves revealed that the apparent viscosity decreases as the strain rate increases, which suggests pseudoplastic behavior, which in turn was confirmed with the fluid behavior index values (n<1) (14). Although the solutions demonstrate similar behavior, EPS in mixotrophic cultivation presented consistency index (k) and apparent viscosity values, respectively, 1.2 and 1.5 times lower than those obtained for xanthan gum, a high purity biopolymer, obtained by selected bacteria and costly carbon sources such as glucose.

The EPS from the heterotrophic culture showed a higher apparent viscosity than the EPS from the mixotrophic culture. The composition and chemical structure of natural polymers can vary depending on cultivation conditions, including stress conditions such as lack of light, which may explain the more favorable results found in heterotrophic cultivation (15).

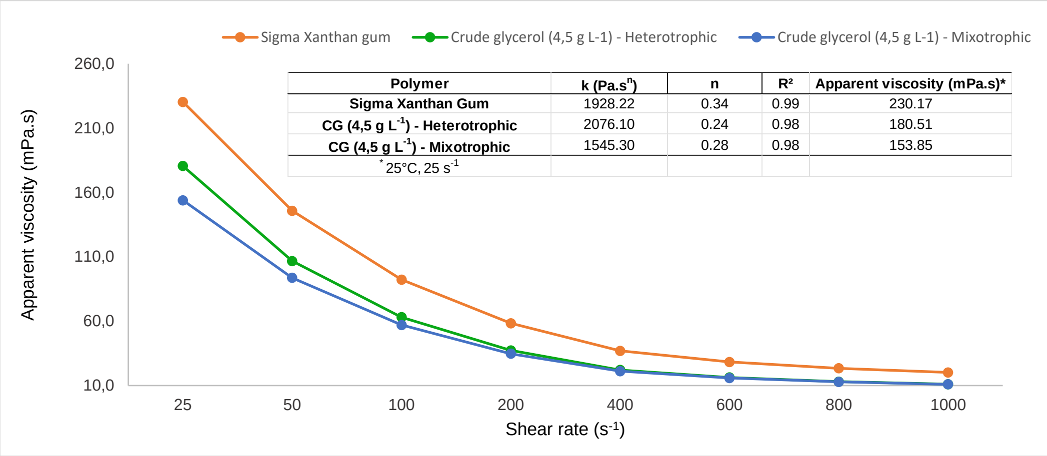

Figure 1 Rheological parameters and effect of strain rate on the viscosity of aqueous solutions (0.4%, w/v) of EPS by *Chlorella. vulgaris* in heterotrophic and mixotrophic cultivation, as well as Sigma xanthan gum.

4 CONCLUSION

The results demonstrate that it is possible to add value to crude glycerol by applying it in the heterotrophic cultivation of *Chlorella vulgaris* IBLC105 to simultaneously synthesize lipids suitable for the production of biodiesel and EPS with viscosifying properties. Furthermore, the relatively low cost generates expectations of more competitive bioproducts on the market.

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