PHAEODACTYLUM TRICORNUTUM CULTIVATED IN ASSOCIATION WITH INDUSTRIAL WASTE FROM FUEL PRODUCTION: BIOMASS PRODUCTION AND BIOPRODUCT EXTRACTION

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ABSTRACT

Microalgae are considered an attractive raw material for producing important bioproducts, such as pigments, polymers, and biofuels, due to their rich composition of organic compounds, including carbohydrates, lipids, and proteins. One of the main challenges in obtaining microalgal biomass is the high cost of the cultivation medium. In this context, using industrial waste as a nutrient source emerges as a promising alternative. This study aims to evaluate the growth of the microalga *Phaeodactylum tricornutum* in a medium containing crude glycerol (CG) and produced water (PW). A central composite rotational design (CCRD) was employed, with the independent variables being the concentration of CG (0-15 g L^{-1}) and PW (0-100% v v^{-1}). The optimal cultivation condition was found to be at 15 g L^{-1} of CG and 50% (v/v) of PW, resulting in a maximum biomass concentration of 0.88 g L^{-1}. Additionally, under these conditions, a carbohydrate content of 28.55 ± 0.10% w/w was observed, similar to the control experiment, along with the production of biopolymers, which was not observed in the control. Finally, it is suggested that further studies be conducted to better characterize the biomass and assess the influence of the alternative medium on the production of significant bioproducts.

Keywords: Microalgae. Glycerol. Produced Water. Bioprocess. Biopolymers.

1 INTRODUCTION

Microalgae are microorganisms with extensive biodiversity, with an estimated 30,000 to 1,000,000 species. They are commonly found in both fresh and saltwater environments and can grow rapidly and adapt to varying environmental and climatic conditions due to their simple cellular structure [1]. These microorganisms are commercially cultivated in synthetic media, making the process quite expensive, with the cost of industrial-scale heterotrophic production being around 22.55 R$ kg^{-1} (4.00 €·kg^{-1}) [2]. Thus, developing economically viable methodologies for obtaining this biomass is a major challenge in producing high-value biomolecules and bioproducts, such as biopolymers [3]. In this context, crude glycerol (CG), a byproduct of biodiesel production, and produced water (PW), a waste effluent from oil extraction, are considered potential carbon sources for microalgal biomass growth [4,5]. However, the presence of toxic contaminants like benzene (0.0395 mg L^{-1}), toluene (0.0720 mg L^{-1}), phenol (165.5 mg L^{-1}), and heavy metals such as arsenic (0.0137 mg L^{-1}), lead (0.0525 mg L^{-1}), copper (0.0182 mg L^{-1}), and cadmium (0.0007 mg L^{-1}) in PW can inhibit the growth of these microorganisms. Therefore, studying microalgae species that are tolerant to these contaminants is a promising alternative from economic, environmental, and technological perspectives [6,7,8]. According to the literature, microalgae can develop mechanisms to protect themselves from the toxicity of undesirable components present in industrial waste. Species like the marine microalga *Phaeodactylum tricornutum* can adapt and grow in various wastewater and waste sources, with concurrent nutrient removal and high biomolecule production. Therefore, this study aimed to develop a bioprocess for obtaining important bioproducts using a blend of PW and CG, without pretreatment, for cultivating *P. tricornutum*.

2 MATERIAL & METHODS

Biomass Production Systems: The *Phaeodactylum tricornutum* strain was provided by the microalgae bank of the Laboratory of Bioprospecting and Biotechnology (LaBBiotec) at the Institute of Biology, Federal University of Bahia (UFBA). It was cultured in 2 L Erlenmeyer flasks containing 1800 mL of culture medium, in a BOD-type climatic chamber with a 12-hour light/12-hour dark photoperiod, illumination of 124.87 µmol photons·m^{-2}·s^{-1}, and a temperature of 25°C ± 2°C for 20 days. Aeration was provided by an air pump with a flow rate of 50 L h^{-1}. To determine the optimal composition of the alternative culture medium, a 2^2 Central Composite Rotational Design (CCRD) with axial points and three repetitions at the central point was used. The parameters analyzed were the concentrations of produced water (PW, %) and crude glycerol (CG, g L^{-1}) as shown in Table 1. The Conway medium served as the control [9] .

Table 1. Composition of alternative culture media evaluated for the growth of *P. tricornutum*.

Studied Parameters	-1,41	-1	0	+1	+1,41
Produced water (v/v,%)	0	14.7	50	85.4	100
Crude glycerol (g L^{-1})	0	2.2	4.5	12.8	15

Monitoring Biomass Concentration: Biomass concentration (X, g L^{-1}) was measured daily by determining optical density (OD) values using a previously established standard curve as per Costa et al. [10], A spectrophotometer (PerkinElmer Lambda 35 UV/VIS) at a wavelength (λ) of 680 nm was employed for daily measurements over 20 days. The initial inoculum concentration

was 0.2 g L^{-1}. Productivity (P, g $L^{-1} \cdot d^{-1}$) was calculated based on X_t and X_0, representing biomass concentrations (g L^{-1}) at time t (days) and initial time t_0 (days), respectively. The growth rate (μ, d^{-1}) was determined by linear regression of the biomass production curve during the logarithmic phase[11]. Maximum biomass production (X_{max}) was identified as the highest biomass concentration achieved during cultivation. The biomass was separated by centrifugation (Centrifuge MPW-351) at 10,000 rpm for 10 minutes. The resulting pellet was removed from the culture medium, frozen at -12°C (Electrolux FE 22), lyophilized (Terroni Enterprise II), and stored at -12°C (Electrolux FE 22) for subsequent characterization

Characterization of Biomass and Bioproduct: Lipid extraction was performed following Folch et al. [12]. Carbohydrate content was determined using the Dubois et al. [13] method. Crude protein content was measured by the micro-Kjeldahl method, with a conversion factor of 4.87, specific to *P.* tricornutum [14]. Biopolymer extraction followed the procedure described by Yellore and Desai [15]. The biopolymer was qualitatively analyzed using Fourier-transform infrared spectroscopy (FTIR) (Perkin Elmer Model Spectrum 100, Perkin Elmer, Waltham, MA, USA) in the range of 4000 cm^{-1} to 400 cm^{-1} with an ATR accessory. Data were evaluated using analysis of variance (ANOVA), t-test, and Tukey test (95% confidence) with Statistica 13.5 software (TIBCO Software Inc.).

3 RESULTS & DISCUSSION

To examine the impact of combining PW and CG in the experimental setup, we considered the response variable as the maximum production (X_{max}). In cases where cultures showed no growth, we considered the initial production (X_0). Our analysis revealed a significant quadratic effect of PW concentration and a significant linear effect of CG concentration (Table 2). The response surface plot vividly illustrates the statistical findings: higher CG concentrations correlate with increased biomass production. However, for PW, we observed a rise in biomass production at intermediate concentrations (50%), while higher concentrations (80 to 100%) led to a decline (Figure 1). Consequently, statistical analysis pinpointed Exp. 8 (50% PW and 15 g L^{-1} of CG) as the optimal cultivation condition among those tested. Notably, neither PW nor CG underwent pretreatment.

Table 2. Effects and regression coefficients for production.

Factor	Maximum production (g L^{-1})		
	Effect	Coefficiente*	p-Value
PW(L)	0.1475	0.0738	0.1952
PW (Q)	-0.3447	-0.1723	**0.0325**
CG (L)	0.2696	0.1348	**0.0412**
CG (Q)	-0.0446	-0.0223	0.7201
PW (L) * CG (L)	0.1150	0.0575	0.4475

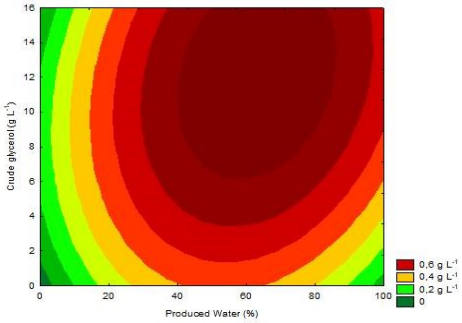

Figure 1. Influence of the concentrations of the mixture of produced water and glycerol on the biomass production of *P. tricornutum.*

Although both cultures commenced with the same cell density (0.2 g L^{-1}), it's noteworthy that Exp 8 displayed a more prolonged adaptation phase, spanning approximately six days, whereas the control exhibited five days (Figure 2). However, it's notable that after the 7th day, Exp 8 demonstrated growth patterns akin to the control, achieving an equivalent value of maximum productivity on the 14th day of cultivation, X_{max} (0.91 g L^{-1}), matching the control's attainment on the 13th day (Table 4). The average productivity parameter of Exp 8 marginally surpassed that of the control, while the specific growth rate (μ_{max}) surged by 44.53% compared to the control. A similar trend was identified by Baldisserotto et al. [16], who investigated the cultivation of the diatom microalga *Thalassiosira pseudonana* supplemented with varying concentrations of crude glycerol (0.5, 1.0, 2.5, and 5.0 g L^{-1}) and also reported elevated growth rates in samples treated with glycerol (0.8 d^{-1}) compared to the control (0.6 d^{-1}), except for the experiment featuring 0.5 g L^{-1} of crude glycerol.

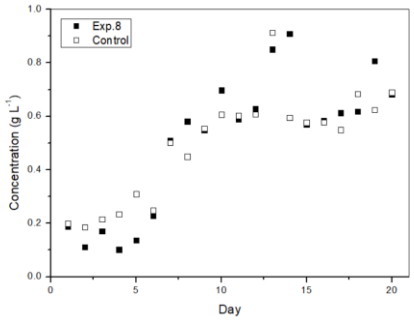

Figure 2. Growth kinetics of *P. tricornutum* cultivated in industrial residues (Exp.8) and synthetic medium (Control).

Table 3. Specific growth parameters of *P. tricornutum* cultivated in industrial residue (Exp.8) and synthetic medium (Control).

Experiment	$\mu_{máx}$ (h^{-1})	Average productivity (g L^{-1} $days^{-1}$)	Final production (g L^{-1})	Maximum production (g L^{-1})
Control	0.14	0.025	0.69	0.91
Exp 8	0.20	0.026	0.68	0.91

Tabela 5 Composition of Biomolecules and Biopolymer Production (%, w/w) of *P. tricornutum* Cultivated in Residues (Exp.8) and Synthetic Medium (Control).

In terms of biomolecular composition within the biomass, lipids emerged as the predominant components in the control experiment, whereas carbohydrates constituted the primary fraction in Exp. 8 (refer to Table 5). When comparing the experiments, a significant reduction of 52% in lipid content and 14.55% in protein content was observed in the medium containing residues compared to the control. This decline in macromolecular constituents is attributed to stress conditions induced by the residues present in the culture medium. It is well-documented that under nitrogen-deficient conditions, microalgae tend to accumulate nitrogen-containing macromolecules and carbon reserve compounds like carbohydrates and lipids. In such scenarios, many microalgal strains have been reported to convert proteins or peptides into lipids or carbohydrates, thereby serving as energy reserve components [17].

Biomolecules and Biopolymer (%, w/w)	Control (Conway medium)	Exp.8 (50% PW;15 g L^{-1} CG)
Proteins	17.63 ± 0.10[a]	14.52 ± 0.48[b]
Carbohydrates	27.52 ± 1.65[a]	28.55 ± 0.10[a]
Lipids	33.73 ± 0.35[a]	17.60 ± 0.10[b]
Biopolymer	-	19.68 ± 0.16

Mean ±standard deviation; means with different letters in the same row are significant different ($p < 0.05$).

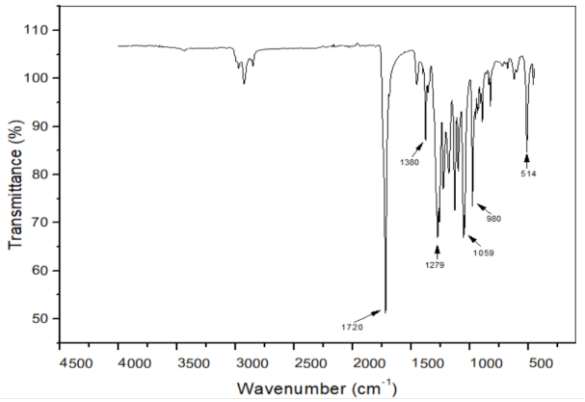

Figure 3. FTIR Spectrum of the Biopolymer.

It's important to highlight that biopolymer production was only observed in the culture containing residues, likely due to the presence of other microorganisms in the medium resulting from the utilization of a non-sterile culture medium. This finding resonates with the outcomes presented by Scheliga et al. [4], who explored the growth of the cyanobacterium *Arthrospira platensis* for industrially relevant compound production in the presence of GB. Their study revealed that Polyhydroxyalkanoates (PHA) production (132 ± 19 mg/g) occurred solely in cultures containing GB, attributed to the natural consortium formed by the cyanobacterium and heterotrophic bacteria. In our investigation, the biopolymer obtained underwent analysis using an FTIR spectrometer, as illustrated in Figure 3. The sample exhibited characteristic bands akin to those of PHA, including bands associated with the stretching of the carbonyl (C=O) ester bond (1721 cm^{-1}), alongside a series of bands in the range between 1000 and 1300 cm^{-1}, indicative of the stretching vibrations of the C–O bond in the ester group [4].

4 CONCLUSION

The findings of this study underscore the potential of utilizing a culture medium comprised of a blend of crude glycerol and produced water for cultivating *P. tricornutum*, all without the need for any prior treatment. This innovative approach capitalizes on impure residues, offering not only a cost-effective cultivation medium but also the prospect of adding value to various stages of the microalgal biomass production, biodiesel manufacturing, and petroleum extraction processes.

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