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Media optimization for *Chlorella* sp. cultivation and carotenoid valorization

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ABSTRACT

Microalgae are photosynthetic microorganisms comprising classes of both eukaryotic and prokaryotic domains. These unicellular organisms are known to grow quickly and to be potential sources of several natural products. The search for natural pigments, for example, has highlighted selected microalgae as carotenoid sources. Besides their use as food colors, carotenoids have important applications in human health, acting as antioxidants and as provitamins. However, cell concentrations are typically low in microalgae culture, and therefore the culture media have an enormous impact on biomass and bioproduct price. Especially for new isolates, production still needs to be optimized to increase productivity and lower the cost. This study aimed at medium optimization based on maximum productivity for a *Chlorella* sp. isolate initially cultivated in the BG-11 medium. The optimized medium was prepared using lower concentrations of the compounds used on BG-11 and did not compromise the *Chlorella* sp. growth. During the experiment, the carotenoid content on biomass increased from 1.28 mg g^{-1} to 2.42 mg g^{-1}, for those cultivated on BG-11 and optimized medium, respectively. The *Chlorella* sp. growth was similar on both BG-11 and optimized BG-11, reaching 2.64 g L^{-1} of biomass after 9 days of cultivation. In terms of economy, the optimized medium used lower amounts of reagents with a reduction of 49.4% in the medium cost.

Keywords: Natural pigment. Antioxidant. Medium optimization. Synthetic media.

1 INTRODUCTION

Microalgae are sources of various natural products like pigments, proteins, starch, lipids, and various antioxidants. Given the great diversity, and the extreme conditions where they can be found, they represent great potential for future bioproducts, meeting human needs [1]. Carotenoids are gaining prominence in the pharmacological market, due to their antioxidant and preventive properties against some diseases. Among this group of pigments, β-carotene, astaxanthin, lutein (including zeaxanthin), lycopene and canthaxanthin are the ones that stand out on the market and have natural origin.

Astaxanthin and β-carotene are mainly of microalgal origin, from *Haematococcus pluvialis* and *Dunaliella salina* [2]. These microalgae are important in the algae market due to their ability to reach high intracellular concentrations of carotenoids through a phase of carotenogenesis, that is, specific to produce these pigments. Carotenogenesis can be induced through stress, caused by external factors such as light, temperature, nutritional deficiency in the environment, or high salinity [3,4].

Cultivation optimization focuses on improving microorganism growth and reducing costs. The planning process to improve cell growth is based on manipulating the cultivation medium, and in the case of microalgae, light and aeration must be included. Furthermore, the specific production of a bioproduct may be related to the induction by factors external to traditional cultivation, leading to a stress capable of inducing specific synthesis, as is the case of *Haematococcus pluvialis* in the production of the pigment astaxanthin and *Neochloris oleoabundans* in the production of lipids [5,6].

The present work sought to optimize the cultivation *of Chlorella* sp. LEB114 focusing on exploring its pigment production capacity. The study on this microalga previously showed its robustness in cultivation with easy maintenance and stability for long periods and an orange appearance possibly related to carotenogenesis, characteristic of other microalgae reported in the literature. In this study *Chlorella* sp. LEB114 was evaluated on its growth kinetics and total carotenoid accumulation in an optimized and more economical synthetic medium.

2 MATERIAL & METHODS

Chlorella sp. LEB114, was gently donated by the Biochemical Engineering Laboratory (LEB) of the Federal University of Rio Grande do Sul (UFRGS). This strain was previously isolated from a treatment pond of a thermoelectric power plant (Candiota, Brazil). The strain was maintained at continuous and low light (20 µmol m^{-2} s^{-1}), 25 °C, in BG-11 medium, in the Microalgae Engineering Laboratory (LEM) at the Bioprocess Engineering and Biotechnology Department of the Federal University of Paraná (DEBB- UFPR). The experimental cultivation was conducted in Erlenmeyers of 250 mL with a working volume of 100 mL (optimization tests) agitated at 110 – 120 RPM in a shaker. Cultivation for kinetics studies was done in 2 L Erlenmeyers with a working volume of 1.5 L aerated at 0,3 vvm. The light intensity was set on 12:12 hours of 10kLux light intensity, approximately 185 µmol m^{-2} s^{-1} (General Electric – Tubular lamp T8 LED 10W – 1050 lm – 4000 K, Brazil). Cultivation systems can be seen on Figure 1. The experimental design for BG-11 optimization followed the Plackett-Burman (PB) design to select relevant nutritional compounds of BG-11 (Table 1 presents the 13 compounds). The PB design recommended a full 16-test model with different

combinations of each of the 13 compounds original to BG11. The selected compounds were optimized using CCD design. Samples were collected and processed for analysis within 24h. The biomass was gravimetrically analyzed. For pigment extraction, microalgae suspensions were centrifugated and washed with deionized water before solvent (acetone 80%) extraction. The extraction was conducted under low light, 25ºC and kept overnighting for complete extraction of carotenoids. The extracts were centrifugated, and the absorbance of the solvent phase was read using a spectrophotometer (Shimadzu UV1200) according to the Lichtenthaler method for acetone 80% extract [7].

3 RESULTS & DISCUSSION

Each 16-test biomass production and pigments were analyzed. An image of solvent extract and results can be seen in Figure 2 and Table 2. From those, Plackett-Burman resulted in four different combinations (highlighted lines on Table 2) with biomass and carotenoid production nearly 1,99 g L^{-1} and 4.36 g L^{-1}, respectively. The *Chlorella* sp. LEB114 could grow with more carotenoids per dry biomass (nearly 2.18 mg g^{-1}) on those media (test numbers 3, 4, 7, and 8) than the others (less than 1.21 mg g^{-1}). In particular, the medium number 4 showed the best results for carotenoid production, 5.17 g L^{-1} for 9 days of cultivation.

Table 1 Media composition for different experiments in this work.

Item	BG-11	Plackett-Burman (level -1 and +1)		CCD optimized BG-11
*$NaNO_3$	*1500	300	*1500	300
K_2HPO_4. $3H_2O$	40	8	72	72
$MgSO_4$. $7H_2O$	75	15	135	157,5
$CaCl_2$. $2H_2O$	36	7,2	64,8	64,8
Citric acid	6	1,2	10,8	3,0
Fe-Citrate + EDTA-2Na	6	1,2	10,8	1,2
Na_2CO_3	20	4	36	0,4
H_3BO_3	2,86	0,57	5,15	0,57
$MnCl_2$. $4H_2O$	1,81	0,36	3,26	0,36
$ZnSO_4$. $7H_2O$	0,22	0,04	0,40	0,40
Na_2MoO_4. $2H_2O$	0,39	0,08	0,70	0,08
$CuSO_4$. $5H_2O$	0,08	0,02	0,14	0,14
$Co(NO_3)_2$. $6H_2O$	0,05	0,01	0,09	0,01

Numeric values are presented in **mg L^{-1}**. *$NaNO_3$ the level +1 were mateined at 1.5 g L^{-1} due to high concentration of nitrate.

Table 2 *Chlorella* sp. LEB114 and pigment production from each 16 diffetent test proposed by Plackett-Burman desing after 9 days cultivation.

Test number	Biomass (g L^{-1})	Carotenoids (mg L^{-1})	Pigments content (mg g^{-1})		
			Chlorophyll a	Chlorophyll b	Total carotenoid
1	1.40	0.46	0.19	0.40	0.33
2	1.31	1.58	0.52	0.36	1.21
3	**1.83**	**3.65**	**1.36**	**1.25**	**1.99**
4	**2.02**	**5.17**	**4.56**	**1.65**	**2.56**
5	1.42	0.50	0.07	0.11	0.35
6	1.35	1.14	0.18	0.20	0.84
7	**1.98**	**4.03**	**1.25**	**1.50**	**2.04**
8	**2.15**	**4.62**	**2.38**	**2.90**	**2.15**
9	1.28	0.20	0.07	0.12	0.15
10	1.29	0.20	0.10	0.25	0.16
11	1.70	1.30	0.68	0.44	0.76
12	1.65	0.81	0.23	0.36	0.49
13	1.25	0.16	0.04	0.06	0.13
14	1.16	0.18	0.13	0.11	0.16
15	1.69	1.52	0.41	0.95	0.90
16	1.84	0.98	0.46	1.87	0.53

After statistical analysis, Plackett-Burman experiments resulted in six significant (p<0.05) factors (media components) for *Chlorella* sp. LEB114 biomass production and carotenoid content: $NaNO_3$, Mg_2SO_4, K_2PO_4, Citric acid, H_3BO_3, and $ZnSO_4 \cdot 7.H_2O$. However, only three were considered relevant for optimization when considering both biomass production *and* carotenoid content. $NaNO_3$ reduction had no impact on biomass production, but it negatively impacted carotenoid production. $MgSO_4$ impacted positively both biomass and carotenoid production. Citric acid had a distinct impact on biomass and cellular counts (data not shown) and a slightly positive impact on carotenoid content. H_3BO_3 affected the biomass negatively and had a slightly positive effect on the carotenoid content, and ZnSO4 affected positively the biomass production but showed a negative effect on the carotenoid content.

Therefore, only Mg_2SO_4, K_2HPO_4, and $ZnSO_4$ were considered relevant for optimization. Citric acid and H_3BO_3 were not optimized since those compounds only slightly affected the biomass and carotenoid content. In addition, citric acid showed no effect on the previous study, with less variation in the concentrations (data not shown). So, for CCD optimization experiments, the following strategies were done: $MgSO_4$, K_2HPO_4, and $ZnSO_4$ were chosen as important for optimization; the citric acid was not optimized as the previous study showed no effect, and its concentration was set on 3 g L^{-1} (low level of previous study); all other compounds with no significant effect of variations were set on the lowest level of concentration.

At the end of CCD optimization, the maximum biomass concentration was 2.64 g L^{-1}, and the carotenoid content was 2.42 mg g^{-1} after 9 days of cultivation. This result was achieved with optimized K_2HPO_4, $MgSO_4$, and $ZnSO_4$ at concentrations of 56 mg L^{-1}, 135 mg L^{-1}, and 0.310 mg L^{-1}, respectively.

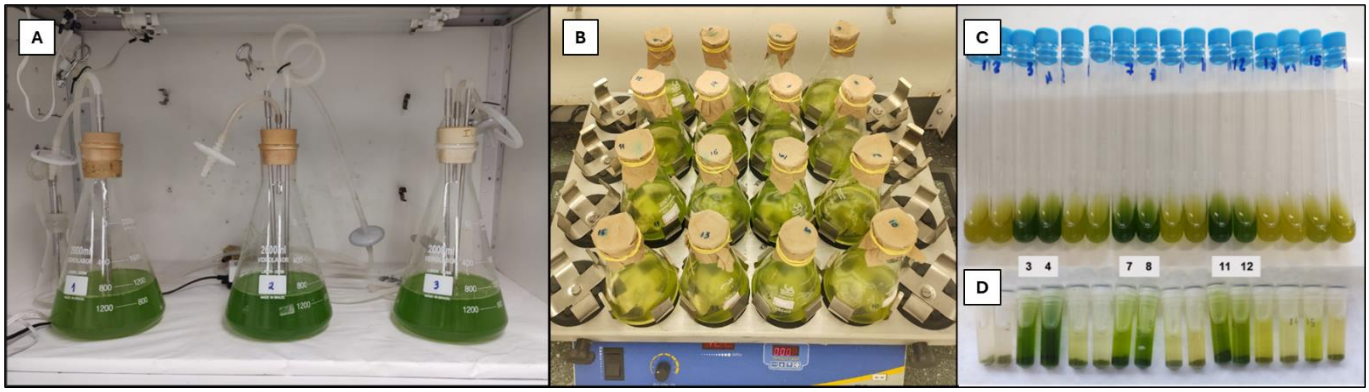

Figure 1 Cultivation systems: *A* – Cultivation of *Chlorella* sp. in triplicate. *B* – Cultivation of *Chlorella* sp. during Plackett-Burman and CCD experiment in a shaker. Carotenoid extraction. *C* – Biomass suspention colected from each 16 experiments. *D* – Solvent extract appearance after centrifugation. Numbers highlights the experiments commented on text.

According to the composition listed in Table 1, the expenses for preparing the two media using reagent compounds were analyzed. The results indicated that creating a 1 m³ culture of BG11 would cost R$976.50, while the same volume of optimized media would cost R$482.70 when using analytical grade salts. The optimized medium is 49.4% more cost-effective than the traditional medium, yet it still supports equivalent biomass and carotenoid production. Moreover, the cost could be reduced further to R$1.30 per m³ if commercial food-grade compounds are used, based on data from ALIBABA (2022). The entry of microalgae into the market is desired both because it is a natural source of various bioproducts, and because it is an alternative for removing pollutants such as CO_2. Its production must be as efficient as possible, avoiding the generation of more post-production waste, which can be improved with optimized means, with reduced cost and essential composition for the growth of microalgae [8].

4 CONCLUSION

The studied microalgae, *Chlorella* sp. LEB114, showed relatively high growth and carotenoid production. This study showed that, although not a hot topic of research, statistical optimization of culture media remains an important step in bioprocess development. Using a Plackett-Burmann design followed by a response-surface optimization, it was possible to reach the same biomass production obtained with traditional BG-11, at *half* the cost.

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