

# Comparative Analysis of three Growth Medium for *Arthrospira platensis* Cultivation based on Lab-Scale Results

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**Abstract** —*Arthrospira platensis* is a rich source of essential amino acids, vitamins and it is used as a feedstock for energy sources. The high cost of the growth medium used in its cultivation is a problem for increasing the production viability. The present study aimed to compare the technical viability and the cost elements in different growth medium for *A. platensis* cultivation. For that purpose, it was proposed the use of three different growth medium, named as M1, M2 and M3 in a lab scale. The growth of the treatments presented a microbial process with characteristic phases. M1, M2 and M3 maximum concentration ( $X_{max}$ ), productivity ( $P_x$ ) and the maximum specific growth rate ( $\mu_{max}$ ) showed no significant difference among treatments. However, M3 presented the lowest cost element, about 45.75% less than the M1 and 38.92% lower than M2. Therefore, the comparison enabled the result that M3 presented the best performance to be used, thus increasing profitability of this production in a lab-scale analysis.

**Keywords**—*Arthrospira platensis*, medium, growth rate, technical viability, costs analysis.

## I. INTRODUCTION

The provision of food security for the growing population, higher oil prices, environmental concerns and increased interest in energy security have encouraged a private and public investment in microalgae biofuels and microalgae food research. It was a challenge to be aware of this potential thus far has been finding a process that can be scaled in a cost competitive manner [1].

Microalgae are simple organisms that are mostly in farm water [2, 3]. Throughout its evolution, microalgae have become a very diverse group of photosynthetic organisms, and a particular example is the *Arthrospira Platensis* (*A. platensis*) [4].

For food, microalgae *A. platensis* represents one of the most promising sources of compounds with biological activities possibly used as functional ingredients [5]. Its use is stimulated in order to increase the nutritional value of the food because it is a source of pigments, unsaturated fat, vitamins, sterols, among others [6].

This microorganism has received considerable interest as a potential feedstock for biofuel production, thereby leading to a marketing appeal by decreasing greenhouse gas emissions, as well as using renewable energy sources. Its big advantage is that it easily adapts to the environmental conditions of cultivation, high photosynthetic efficiency and is not a competitor in food production areas [7]. In addition, it can produce large amounts of polysaccharides (sugar) and triglyceride (oil), these are materials for the production of bioethanol and biodiesel [8].

Regarding the national production of microalgae, companies which started producing bio compounds claim about the lack of investment in production technology and competitive pricing, which ended up preventing the advance of large-scale production projects. Consequently, proposals must concern about lower production costs and high efficiency. Furthermore, the new growth medium sources should be encouraged to achieve this purpose.

Raoof et al. [9] emphasized that the growth medium is a significant part of production costs. Several lab-scale recent studies demonstrated the potential of alternative growth methods in increasing the production of *A. platensis* biomass [1, 3, 5, 6, 7]. However, regarding its importance, a few number of scientific articles concern about comparing a new growth medium in order to verify their gains.

In this context, the objective of this paper is to perform a comparison analysis related to the technical feasibility and the cost element of three different growth medium for

*A. platensis* cultivation using lab-scale experimental results.

## II. REVISION AND STATE OF THE ART

### Microalgae

The cultivation of microalgae is a branch of modern biotechnology, although there are records that it has already grown in 1890. Commercial production began in the 1960s in Japan. Since then, the microalgae biotechnology industry has expanded and diversified significantly [10].

Microalgae are autotrophs that develop from the process of photosynthesis, as well as plants. Photosynthesis is recognized as a natural media of CO<sub>2</sub> sequestration and aquatic microalgae are the fastest photosynthetic organisms that can fix carbon at a moderate rate in comparison to terrestrial plants. Microalgae also have the ability to use free CO<sub>2</sub>, carbonate and bicarbonate ions as growth substrates [11].

This designation includes two types of cell structure: prokaryotic, with representatives in the division *Cyanophyta* (cyanobacteria) and *Eukaryotic*, with representatives of *Chlorophyta* divisions, *Euglenophyta*, *Rhodophyta*, *Haptophyta* (*Prymnesiophyta*), *heterokonts* (*Bacillariophyceae*, and *Chrysophyceae* *Xanthophyceae*) and *Cryptophyta* *Dinophyta* [12, 13].

The microalgae biomass is used for the production of: (1) biosynthesis and ethanol by fermentation using algae hydrogen, (2) carbohydrate biosynthesis (producing ethanol and biobutanol acetone-butanol- via fermentation and ethanol), hydrocarbons (that can be taken to the production of diesel, kerosene, among others) and the production of triglycerides itself for biodiesel production [14].

### *A. platensis*

The specie *A. platensis*, from *Oscillatoriaceae* family, includes multicellular filamentous cyanobacterium group (blue-green microalgae), formed by cylindrical cells arranged in helical trichomes [15].

Humans learned early how to use the *A. platensis* as a power source, by noting the migration of birds being safely fed and also encouraging them to use it [16]. Kebede [17] reported that, in Ethiopia, farmers and shepherds living close to the alkaline lakes made the cattle feed mixed with *A. platensis* water monthly, and they believed it had therapeutic effect and was a supplement in the daily diet.

They are able to adapt in certain environments where other microorganisms would not survive, such as those in media with high salt concentration. Through photosynthesis, it converts nutrients into cellular material and produces oxygen. Necessary nutrients are water and a

source of carbon, nitrogen, phosphorus, potassium, iron and other minerals [15].

Microalgae are also vectors for the production of bioactive agents that can be applied to medical field, compounds with specific biochemical characteristics, and compounds which are energy sources such as bio-diesel, bio-methane, and hydrogen biobutanol, biomethanol [18, 19].

*A. platensis* is legally permitted as a food supplement in Europe, Japan and in the United States by the Food and Drug Administration (FDA), with no toxic effects on the human body [20]. In Brazil, ANVISA (National Health Surveillance Agency) allowed its sale provided that the final product, which microalgae have been added, is properly registered [21].

### Growth Medium

The current interest in expansion of industrial use of microorganisms is leading to a greater attention to the growth medium used. As a matter of profitability and environmental protection, it is essential to ensure the productivity optimization with the lowest cost [22].

The growth of *A. platensis* and the composition of the biomass produced depend on many factors. The most important are temperature, light intensity and specially the amount of available nutrients. Therefore, the costs elements are considered the second major influence that impacts on its production [23].

*A. platensis* growth requires carbon because their cells contain approximately 50% (v/v) of this element. Carbon costs represent a higher impact, for the reason that this element being the most important chemical constitution. For an autotrophic growth (recommended to open large-scale cultivation), carbon can be replaced by CO<sub>2</sub>, carbonate or bicarbonate. If bicarbonate is used, it will represent about 60% of the costs of nutrients [24].

Scientific literature lacks information while proposing alternative growth medium components and their impacts in the synthesis of compounds [25].

## III. MATERIALS AND METHODS

**Microorganism:** It was used the cyanobacterium *A. platensis*, classified as *Arthrospira platensis* (Nordstead) by Gomont [26], obtained from Federal University of Santa Catarina, Brazil.

**Culture medium and cultivations:** The inoculum was prepared according to the criteria established in previous studies: 8-day process, temperature  $\pm 30^\circ\text{C}$  using shaker *Marq Labor CFW 08*, fitted with artificial light. Initial concentration of inoculum was 50 mg.L<sup>-1</sup> [27].

The experimental design was a completely randomized design (C.R.D.), using a factorial 3x4, with three proposed medium and 4 replicates.

Three culture media were proposed and analyzed: a standard medium [29] – Medium 1 (M1); a standard modified medium [30] with no trace elements – Medium

2 (M2); and finally, a medium proposed with alternative commercial substances replacing natural elements – Medium 3 (M3). All three growth mediums elements are presented in Table 1.

Table.1: Composition of Growth Medium for three cultivation treatments.

Elements	Medium 1 (M1)		Medium 2 (M2)		Medium 3 (M3)	
	g.L <sup>-1</sup>	Modified Elements	g.L <sup>-1</sup>	Modified Elements	g.L <sup>-1</sup>	Modified Elements
NaCl	0.92	-	0.92	-	0.92	Commercial Salt
Na <sub>2</sub> SO <sub>4</sub>	1.88	-	1.88	-	1.88	
K <sub>2</sub> HPO <sub>4</sub>	0.50	-	0.50	-	0.50	
Na <sub>2</sub> CO <sub>3</sub>	8.89	-	8.89	-	8.89	
NaHCO <sub>3</sub>	15.15	-	15.15	-	15.15	Commercial Sodium Bicarbonate
CaCl <sub>2</sub> .2H <sub>2</sub> O	0.05	-	0.05	-	0.05	
KNO <sub>3</sub>	2.57	-	2.57	-	2.57	Commercial fertilizer double sodium and potassium nitrate.
MgSO <sub>4</sub> .4H <sub>2</sub> O	0.25	-	0.25	-	0.25	
Fe-EDTA Solution	1.0	-	1.0	-	1.0	
Microelements Solution*	1.0	-	1.0	-	1.0	

\*Microelements Solution – Composed by H<sub>3</sub>BO<sub>3</sub>.4H<sub>2</sub>O (2.86 g.L<sup>-1</sup>), ZnSO<sub>4</sub>.7 H<sub>2</sub>O (0.222 g.L<sup>-1</sup>), NaMoO<sub>4</sub>.2 H<sub>2</sub>O (0.390 g.L<sup>-1</sup>), MnSO<sub>4</sub> (1.543 g.L<sup>-1</sup>), CuSO<sub>4</sub>.5 H<sub>2</sub>O (0.079 g.L<sup>-1</sup>) and CoCl<sub>2</sub>.6 H<sub>2</sub>O (0.038 g.L<sup>-1</sup>).

**Determinations and analyzes:** The parameters monitored were growth with biomass maximum concentration ( $X_{max}$ ), productivity (Px), maximum specific growth rate ( $\mu_{max}$ ) and the cost element. The comparison between these three mediums, with the same day sampling, was conducted by using Tukey test, also considering a significance level of 5% ( $p < 0.05$ ) [28].

**Growth and biomass maximum concentration ( $X_{max}$ ):** Biomass concentration was determined daily by turbidimetry at 560 nm [30] in 8-day process.

**Productivity (Px):**

$$Px = \frac{(X_m - X_i)}{T_c} \quad [\text{equation 1}]$$

In equation 1,  $X_m$  is the final biomass concentration,  $X_i$  is the initial biomass concentration,  $T_c$  is time of analysis (days). Px was expressed in biomass concentration (mg.L<sup>-1</sup>.d<sup>-1</sup>) growth per day.

**Maximum specific rate ( $\mu_{max}$ ):** Determined using Monod model [31]:

$$\ln\left(\frac{C_x}{C_{x_0}}\right) = \mu_{max} \cdot t \quad [\text{equation 2}]$$

In equation 2,  $C_x$  is the final biomass concentration,  $C_{x_0}$  is the initial biomass concentration,  $\mu_{max}$  is expressed in d<sup>-1</sup>.

**Cost Element:** The cost element analysis was about all the nutrients included in the used mediums [32]. It was determined by surveying suppliers directly.

The total capital element investment (TCEI) was calculated according to the cost of each element. This analysis was subjected to variations, as it considered values in American Dollar (US\$).

Afterwards, a direct comparison was made considering the cost of the component medium. For a better analysis, it was calculated the cost for 1 Kg of *A. platensis* biomass production.

## IV. RESULTS AND DISCUSSION

Experimental results obtained from *A. platensis* biomass concentration growth are shown in Figure 1.

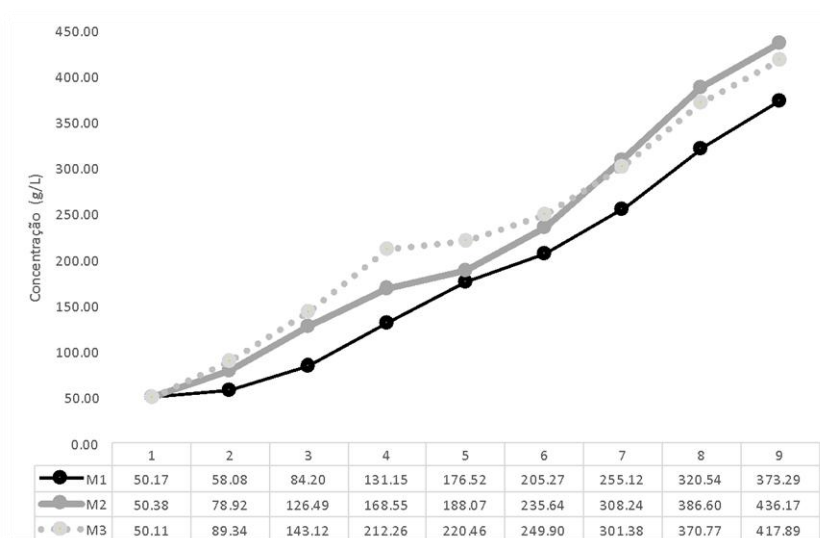


Fig.1: Comparison of biomass growth in the experimental medium

As presented in figure 1, the three analyzed experimental medium initiated biomass concentration around 50 mg.L<sup>-1</sup>. M1 started with a stationary phase and then culminating in a maximum concentration ( $X_{max}$ ) of 373.29 mg. L<sup>-1</sup>. M2 also presented the same phase, culminating in maximum concentration ( $X_{max}$ ), of 436.17 mg L<sup>-1</sup>. M3 had the stationary phase and a maximum concentration ( $X_{max}$ ) of 417.89 mg. L<sup>-1</sup>.

Thirumala [33] also studied *A. platensis* biomass growth by using different growth medium from distinct

combinations. As a result obtained from growth medium, slightly different from this paper findings, it established that it had a maximum concentration on the 9<sup>th</sup> and 12<sup>th</sup> day of incubation.

The experimental results and statistical analysis maximum concentration ( $X_{max}$ ), productivity ( $P_x$ ) and specific maximum growth rates ( $\mu_{max}$ ) are shown in table 2.

Table 2. Comparison of growth parameters.

Growth Medium	$X_{max}$ (mg . L <sup>-1</sup> )	$P_x$ (mg. L <sup>-1</sup> . d <sup>-1</sup> )	$\mu_{max}$ (d <sup>-1</sup> )
M1	373.29 ± 100.62 <sup>a*</sup>	40.39 ± 12.57 <sup>a</sup>	0.27 R <sup>2</sup> = 0.977 <sup>a</sup>
M2	436.17 ± 107.48 <sup>a</sup>	48.22 ± 13.43 <sup>a</sup>	0.30 R <sup>2</sup> = 0.928 <sup>a</sup>
M3	417.88 ± 113.72 <sup>a</sup>	45.97 ± 14.21 <sup>a</sup>	0.31 R <sup>2</sup> = 0.805 <sup>a</sup>

\* Same letters indicate no significant differences between treatments analyzed by Tukey analysis (p <0.05).

#### Maximum concentration ( $X_{max}$ )

As demonstrated in Table 2, maximum concentration in M1 was 373.29 ± 100.62 mg. L<sup>-1</sup>, for M2 the result was 436.17 ± 107.48 mg. L<sup>-1</sup> and for M3 was 417.88 ± 113.72 mg. L<sup>-1</sup>. Although the numerical result of M2 was higher, Tukey test (p <0.05) confirmed that all three treatments resulted in a statistically equal amount.

Several authors found out similar results. Kaushik et al. [34] cultivated *A. platensis* in different dilutions of effluents from industrial activities. Similarly to this paper analysis, after 14 days of growth it achieved maximum concentration values ranging from 460 mg.L<sup>-1</sup>.

Also cultivating *A. platensis* in a low-cost medium, Madkour et al. [23] affirmed maximum concentration values ( $X_{max}$ ) starting from 591 ± 0.018 mg. L<sup>-1</sup> for different concentrations of nitrate ammonia. Finally, Pandey et al. [35] in his research cultivated *A. platensis* in Zarrouk medium confirmed maximum concentration values ( $X_{max}$ ) from 390 ± 0.020 mg. L<sup>-1</sup>.

#### Productivity ( $P_x$ )

In Table 2, the productivity at M1 was 40.39 ± 12.57 mg.L<sup>-1</sup>.d<sup>-1</sup>, M2 was 48.22 ± 13.43 mg.L<sup>-1</sup>.d<sup>-1</sup> and M3 was 45.97 ± 14.21 mg.L<sup>-1</sup>.d<sup>-1</sup>. Results were also expressed in

%  $\text{mg.L}^{-1}\text{d}^{-1}$ , therefore, M1 productivity was  $80\% \text{ mg.L}^{-1}\text{d}^{-1}$ , M2 was  $96.4\% \text{ mg.L}^{-1}\text{d}^{-1}$  and M3 was  $91.9\% \text{ mg.L}^{-1}\text{d}^{-1}$ .

The results indicated that there is no effect of treatments on productivity because, although numerically the result of M2 was higher, statistical Tukey ( $p < 0.05$ ) test analysis showed that the three treatments were equal. Jitendra et al. [37] presented similar results in *A. platensis* cultivation. For productivity results, it was obtained values between  $60\%$  and  $90\% \text{ mg.L}^{-1}\text{d}^{-1}$ . Madkour et al. [24] also found results between  $16 \pm 0.0005 \text{ mg.L}^{-1}\text{d}^{-1}$  and  $52 \pm 0.0005 \text{ mg.L}^{-1}\text{d}^{-1}$ .

#### Specific growth rate ( $\mu_{\text{max}}$ )

According to Table 2, the specific rate for M1 was  $0.27 \text{ R2} = 0.977 \text{ d}^{-1}$ , for M2 was  $0.30 \text{ R2} = 0.928 \text{ d}^{-1}$  and for

M3 it was  $0.31 \text{ R2} = 0.805 \text{ d}^{-1}$ . The results were statistically (Tukey test) the same, in 5% level.

Similar results were found by Borges et al. [38], Soletto et al. [39] and Madkour et al. [24], showing that this specific maximum growth rate found in this study indicates good results, which are similar to those found in this work.

#### Cost Element Analysis

According to the maximum biomass production (table 2), it was established that to produce 1 Kg of *A. platensis* will be needed approximately 2679 liters for M1, 2293 liters for M2 and 2393 liters for M3.

The analysis of cost element for 1 Kg of *A. platensis* biomass in US\$ (quoted at January 27th, 2018) for the treatments is shown in Table 3.

Table.3: Element cost analysis.

Elements	Medium 1 (M1)		Medium 2 (M2)		Medium 3 (M3)	
	US\$	% Total	US\$	% Total	US\$	% Total
NaCl	1.73	0.14	1.48	0.14	1.54	0.24
Na <sub>2</sub> SO <sub>4</sub>	132.34	10.91	113.26	10.92	118.22	18.66
K <sub>2</sub> HPO <sub>4</sub>	51.64	4.26	44.20	4.26	46.13	7.28
Na <sub>2</sub> CO <sub>3</sub>	333.47	27.50	285.40	27.51	297.88	47.02
NaHCO <sub>3</sub>	546.58	45.07	467.78	45.09	39.06	6.17
CaCl <sub>2</sub> .2H <sub>2</sub> O	1.97	0.16	1.68	0.16	1.76	0.28
KNO <sub>3</sub>	42.85	3.53	36.67	3.53	38.27	6.04
MgSO <sub>4</sub> .4H <sub>2</sub> O	15.63	1.29	13.38	1.29	13.96	2.20
Fe-EDTA	85.9	7.08	73.52	7.09	76.13	12.11
Solution						
Microelements	0.60	0.05	-	-	-	-
Solution*						
Total	1212.69		1037.36		633.55	

\*Microelements Solution Costs– Composed by H<sub>3</sub>BO<sub>3</sub>.4H<sub>2</sub>O ( $2.86 \text{ g.L}^{-1}$ ), ZnSO<sub>4</sub>.7 H<sub>2</sub>O ( $0.222 \text{ g.L}^{-1}$ ), NaMoO<sub>4</sub>.2 H<sub>2</sub>O ( $0.390 \text{ g.L}^{-1}$ ), MnSO<sub>4</sub> ( $1.543 \text{ g.L}^{-1}$ ), CuSO<sub>4</sub>.5 H<sub>2</sub>O ( $0.079 \text{ g.L}^{-1}$ ) and CoCl<sub>2</sub>.6 H<sub>2</sub>O ( $0.038 \text{ g.L}^{-1}$ ) was US\$ 0.60.

The cost element for 1 kg of *A. platensis* biomass, at lab-scale, in M1 was US\$ 1212.69 and the biggest costs identified, based on the percentage over the total, were NaHCO<sub>3</sub> (45.07%) followed by Na<sub>2</sub>CO<sub>3</sub> (27.5%) and Na<sub>2</sub>SO<sub>4</sub> (10.91%). For M2 the cost element was US\$ 1037.36 and the biggest costs were NaHCO<sub>3</sub> (45.09%) followed by Na<sub>2</sub>CO<sub>3</sub> (27.51%) and Na<sub>2</sub>SO<sub>4</sub> (10.92%). For M3 the cost was US\$ 633.55 and the biggest costs were Na<sub>2</sub>CO<sub>3</sub> (47.02%), followed by Na<sub>2</sub>SO<sub>4</sub> (18.66%) and Fe-EDTA solution (12.11%).

M1 showed the highest cost (US\$ 1212.69), it is probably because it is needed a higher growth medium amount to reach 1Kg of *A. platensis* biomass. The biggest costs were almost the same as M2.

M2 cost (US\$ 1037.36) represents a 14.5% lower cost than M1 and it is still approximately 38.92% higher than

in M3. The element that most charges the growth medium cost, NaHCO<sub>3</sub> (45.09%), was replaced by commercial sodium bicarbonate in M3.

M3 cost (US\$ 633.55) was the lowest among the three growth medium analyzed. Some of the higher costs in the other growth medium were replaced in M3. It represents about 47.75% lower cost than M1 and 38.92% lower than M2. Several authors have also proposed the use of alternative growth medium for this cyanobacterium growth process.

For the purposes of biofuels production, commercially viable biomass production today is only 5 Kt/ year with a production cost of \$ 25.00 / ton [14]. However, to occupy only 5% of biofuel demand for a country as the United States, it would be required a production of more than



66.000 Kt/year of biomass rich in oil costing less than US\$ 400/t [40].

Analyzing the European market to get more acquainted with the whole transport network of microalgae biodiesel, a 9.25 million ha crop would be required resulting in an output of 40.000 liters/ ha.day [41]. Thus, this number shows that according to the results in this paper, productivity should be improved in order to become viable regarding profitability.

Norsker et al. [42] estimated the cost of producing algal biomass on a commercial scale. As a conclusion, the important factors resulted in the irradiation conditions, mixing, efficient photosynthetic system, the medium and the cost of carbon dioxide, in agreement with this study also demonstrating that carbon source has one of the higher costs.

Acien et al. [43] conducted a cost analysis for the actual production of the microalgae *Scenedesmus almeriensis* and Madkour et al. [24] studied a low cost medium of cultivation for the large-scale production of *A. platensis*, both resulted in the economic analysis showing the production itself and depreciation as the main offenders of the cost. Also concluded that the simplification of the technology used and the scaling tests could increase productivity and reduce production costs.

In lab-scale process, the costs will probably be higher than the commercial value of the product, mainly due to the productivity scale and the small amount of purchased resources [24, 40].

## V. CONCLUSIONS

As stated in experimental results, this research conducted efficient conversion. It had specifically grown from 0,50 mg.L<sup>-1</sup> until for M1 373.29 ± 100.62 mg. L<sup>-1</sup>, for M2 436.17 ± 107.48 mg. L<sup>-1</sup> and for M3 417.88 ± 113.72.

M1 biomass productivity had results statistically identical to the other culture medium (M1 and M2) which did not contain macroelements confirming such a no need of these elements in order to increase *A. platensis* biomass. Productivity (Px) and specific rate (μ<sub>max</sub>) also showed good results regarding the ones presenting their similarity among other carried out researches.

Even though M3 cost element for 1 kg of *A. platensis* production was the lowest between them, its maximum concentration, productivity and specific growth rate had no statistical difference from the others. Therefore, the use of M3 is strongly recommended in this comparative study.

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