https://dx.doi.org/10.22161/ijaers.5.6.32 *ISSN: 2349-6495(P) | 2456-1908(O)*

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

Fernando Caixeta[1,2], Monica Hitomi Okura [2], Marlei Barbosa [2], Lucia Helena Pelizer [2]

[1]Federal Institute of Education, Science and Technology from Triângulo Mineiro – Campus Uberlândia, BRAZIL
Email: fernandocaixeta@iftm.edu.br
[2]Technological Innovation Science Master Program (PMPIT), Federal University from Triângulo Mineiro, BRAZIL
Email: moni@mednet.com.br;Email: marlei.uftm@gmail.com; Email: lucia.pelizer@gmail.com

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 elementsin 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 (Px) and the maximum specific growth rate (μ_{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

https://dx.doi.org/10.22161/ijaers.5.6.32 *ISSN: 2349-6495(P) | 2456-1908(O)*

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_2 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 abilityto use free CO_2, 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 Xantophyceae*) 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 importants 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_2, 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 ± 30 ° C using shaker *Marq Labor CFW 08*, fitted with artificial light. Initial concentration of inoculum was 50 $mg.L^{-1}$ [27].

https://dx.doi.org/10.22161/ijaers.5.6.32 *ISSN: 2349-6495(P) | 2456-1908(O)*

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^{-1}	Modified Elements	g.L^{-1}	Modified Elements	g.L^{-1}	Modified Elements
NaCl	0.92	-	0.92	-	0.92	Commercial Salt
Na_2SO_4	1.88	-	1.88	-	1.88	
K_2HPO_4	0.50	-	0.50	-	0.50	
Na_2CO_3	8.89	-	8.89	-	8.89	
$NaHCO_3$	15.15	-	15.15	-	15.15	Commercial Sodium Bicarbonate
$CaCl_2.2H_2O$	0.05	-	0.05	-	0.05	
KNO_3	2.57	-	2.57	-	2.57	Commercial fertilizer double sodium and potassium nitrate.
$MgSO_4.4H_2O$	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 $H_3BO_3.4H_2O$ (2.86 g.L^{-1}), $ZnSO_4.7\ H_2O$ (0.222 g.L^{-1}), $NaMoO_4.2\ H_2O$ (0.390 g.L^{-1}), $MnSO_4$ (1.543 g.L^{-1}), $CuSO_4.5\ H_2O$ (0.079 g.L^{-1}) and $CoCl_2.6\ H_2O$ (0.038 g.L^{-1}).

Determinations and analyzes: The parameters monitored were growth with biomass maximum concentration (X_{max}), productivity (Px), maximum specific growth rate (μ_{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):

$$Px = \frac{(Xm - Xi)}{Tc} \quad \text{[equation 1]}$$

In equation 1, *Xm* is the final biomass concentration, *Xi* is the initial biomass concentration, *Tc* is time of analysis (days). Px was expressed in biomass concentration (mg.L^{-1}.d^{-1}) growth per day.

Maximum specific rate (μ_{max}): Determined using Monod model [31]:

$$Ln\left(\frac{Cx}{Cx_0}\right) = \mu_{max} . t \quad \text{[equation 2]}$$

In equation 2, *Cx* is the final biomass concentration, Cx_0 is the initial biomass concentration, μ_{max} is expressed in d^{-1}.

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.

https://dx.doi.org/10.22161/ijaers.5.6.32 *ISSN: 2349-6495(P) | 2456-1908(O)*

IV. RESULTS AND DISCUSSION

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

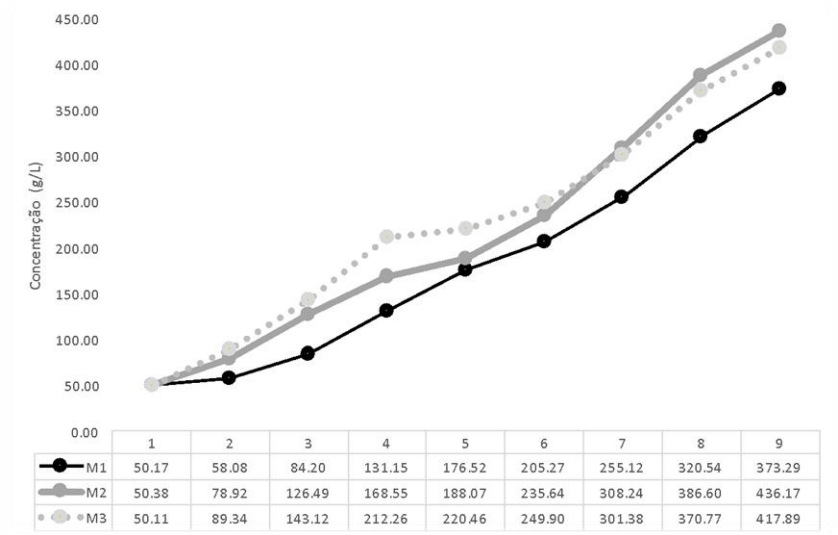

Fig.1: Comparison of biomass growth in the experimental medium

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

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 9th and 12th day of incubation.

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

Table 2. Comparison of growth parameters.

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

*** 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^{-1}, for M2 the result was 436.17 ± 107.48 mg. L^{-1} and for M3 was 417.88 ± 113.72 mg. L^{-1}. 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^{-1}.

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^{-1} 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^{-1}.

Productivity (Px)

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

https://dx.doi.org/10.22161/ijaers.5.6.32 *ISSN: 2349-6495(P) | 2456-1908(O)*

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

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% $mg.L^{-1}.d^{-1}$. Madkour et al. [24] also found results between 16 ± 0.0005 $mg.L^{-1}.d^{-1}$ and 52 ± 0.0005 $mg.L^{-1}.d^{-1}$

Specific growth rate (μ_{max})

According to Table 2, the specific rate for M1 was 0.27 R2 = 0.977 d^{-1}, for M2 was 0.30 R2 = 0.928 d^{-1} and for M3 it was 0.31 R2 = 0.805 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_2SO_4	132.34	10.91	113.26	10.92	118.22	18.66
K_2HPO_4	51.64	4.26	44.20	4.26	46.13	7.28
Na_2CO_3	333.47	27.50	285.40	27.51	297.88	47.02
$NaHCO_3$	546.58	45.07	467.78	45.09	39.06	6.17
$CaCl_2.2H_2O$	1.97	0.16	1.68	0.16	1.76	0.28
KNO_3	42.85	3.53	36.67	3.53	38.27	6.04
$MgSO_4.4H_2O$	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 $H_3BO_3.4H_2O$ (2.86 $g.L^{-1}$), $ZnSO_4.7\ H_2O$ (0.222 $g.L^{-1}$), $NaMoO_4.2\ H_2O$ (0.390 $g.L^{-1}$), $MnSO_4$ (1.543 $g.L^{-1}$), $CuSO_4.5\ H_2O$ (0.079 $g.L^{-1}$) and $CoCl_2.6\ H_2O$ (0.038 $g.L^{-1}$) 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 $NaHCO_3$ (45.07%) followed by Na_2CO_3 (27.5%) and Na_2SO_4 (10.91%). For M2 the cost element was US$ 1037.36 and the biggest costs were $NaHCO_3$ (45.09%) followed by Na_2CO_3 (27.51%) and Na_2SO_4 (10.92%). For M3 the cost was US$ 633.55 and the biggest costs were Na_2CO_3 (47.02%), followed by Na_2SO_4 (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, $NaHCO_3$ (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

https://dx.doi.org/10.22161/ijaers.5.6.32 *ISSN: 2349-6495(P) | 2456-1908(O)*

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^{-1} until for M1 373.29 ± 100.62 mg. L^{-1}, for M2 436.17 ± 107.48 mg. L^{-1} 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_{max}) 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, it 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.

VI. ACKNOWLEDGMENT

The authors are grateful for the support provided by Universidade Federal do Triângulo Mineiro (UFTM); Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro – Campus Uberlândia (IFTM); and Universidade Federal de Santa Catarina (UFSC).

REFERENCES

[1] Mcbride, R. C., Merrick, D. S. (2014). Innovations in open pond algae agriculture for biofuel production. Industrial Biotechnology, 10 (3), 162-163. DOI: 10.1089/ind.2013.1614.

[2] Ozkurt, L. (2009). Qualifying of safflower and algae for energy. Energy Education Science and Technology, 23 (2), 145-151.

[3] Demirbas, A. (2010). Use of algae as biofuel sources. Energy Conversion and Management, 51 (12), 2738-2749. DOI: 10.1016/j.enconman.2010.06.010.

[4] Franceschini, I. M., Burliga, A. L., Reviers, B., Prado, J. F., Rezig, S. H. (2010). Algas: uma abordagem filogenética, taxonômica e ecológica. Porto Alegre: Artmed.

[5] Marco, E. R., Steffolani, M. E., Martinez. C. S., Leon. A. E. (2014). Effects of Spirulina Biomass on the technological and nutritional quality of bread wheat pasta. LWT – Food Science and Technology, 58 (1), 102-108. DOI: 10.1016/j.lwt.2014.02.054.

[6] Batista, A. P., Nunes, M. C., Fradinho, P., Gouveia, L., Souza, I., Raymundo, A., Franco, J. M. (2012). Novel foods with microbial ingredientes – effect of gel setting conditions on the linear viscoelasticity of Spirulina and Haematococcus gels. Journal of Food Engineering, 110 (2), 182-189. DOI: 10.1016/j.jfoodeng.2011.05.044.

[7] Zhu, L. D., Hiltunen, E., Antila, E., Zhong, J. J., Yuan, Z. H., Wang, Z. M. (2014) Microalgal biofuels: Flexible bioenergies for sustainable development. Renewable and Sustainable Energy Reviews, 30, 1035-1046. DOI: 10.1016/j.rser.2013.11.003.

[8] Slade, R., Bauen, A. (2013) Microalgae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. Biomass and Bioenergy, 53, 29-38. DOI: 10.1016/j.biombioe.2012.12.019.

[9] Raoof, B., Kaushik, B. D., Reinher, R. P. (2006). Formulation of a lowcost medium for mass production of Spirulina. Biomass and Bioenergy, 30 (6), 537-542. DOI: 10.1016/j.biombioe.2005.09.006.

[10] Borowitza, M. A. (1999). Commercial production of microalgae: ponds, tanks, tubes and fermenters. Journal of Biotechnology. 70 (1-3), 313-321. DOI: 10.1016/S0168-1656(99)00083-8.

[11] Martis, R. T., Singh. R., Ankita. K., Pathak. A. N., Guria. C. (2013). Solubility of carbon dioxide using aqueous NPK 10:26:26 complex fertilizer culture medium and Spirulina platensis suspension. Journal

https://dx.doi.org/10.22161/ijaers.5.6.32 *ISSN: 2349-6495(P) | 2456-1908(O)*

of Environmental Chemical Engineering, 1 (4), 1245-1251. DOI: 10.1016/j.jece.2013.09.013.

[12] Olaizola, M. (2003). Commercial development of microalgal biotechnology: from the test tube to the marketplace. Biomolecular Engineering, 20 (4-6), 459-466. DOI: 10.1016/S1389-0344(03)00076-5.

[13] Pulz, O., Gross, W. (2004). Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology, 65 (6), 635-648. DOI: 10.1007/s00253-004-1647-x.

[14] Raslavicius, L., Semenov, V. G., Chernova, N., Kersys, A., Kopeyka, A. (2014). Producing transportation fuels from algae: In search of synergy. Renewable and Sustainable Energy Reviews, 40, 133-142. DOI: 10.1016/j.rser.2014.07.176.

[15] HENRIKSON, R. (1989). Earth food Spirulina. California: Ronore Enterprises.

[16] AMARA, A. A., Steinbuchel, A. (2013). New Medium for Pharmaceutical grade Arthrospira. International Journal of Bacteriology, 1-9. DOI: 10.1155/2013/203432.

[17] KEBEDE, E. (1997). Response of Spirulina platensis (Arthrospira fusiformis) from Lake Chitu. Ethiopia. to salinity stress from sodium salts. Journal of Applied Phycology, 9 (6), 551– 558. DOI: 10.1023/A:1007949021786.

[18] XU, Y., Isom, L., Hanna, M. A. (2010). Adding value to carbon dioxide from ethanol fermentations. Bioresource Technology, 101 (10), 3311–3319. DOI: 10.1016/j.biortech.2010.01.006.

[19] Harun, R., Singh, M., Forde, G. M., Danquah, M. K. (2010). Bioprocess engineering of microalgae to produce a variety of consumer products. Renewable and Sustainable Energy Reviews, 14 (3), 1037–1047. DOI: 10.1016/j.rser.2009.11.004.

[20] VON DER WEID, D., Dillon, J. C., Falquet, J. (2000). Malnutrition: a silent massacre. Antenna Technology.

[21] BRAZIL. (2009). National Health Surveillance Agency. VII List of new approved ingredients - Techno-scientific Advisory Committee on Functional Foods and Novel Foods. http://www.anvisa.gov.br/alimentos/comissoes/novos_ingredientes.htm. [Accessed January 17. 2015].

[22] Cogne, G., Lehmann, B., Dussap, C. G., Gros, J. B. (2003). Uptake of macrominerals and trace elements by the cyanobacterium Spirulina platensis (Arthrospira platensis PCC 8005) under photoautotrophic conditions: culture medium optimization. Biotechnology and bioengineering, 81 (5), 588-593. DOI: 10.1002/bit.10504.

[23] Madkour, F. F., Kamil, A. E. W, Nasr, H. S. (2012). Production and nutritive value of Spirulina platensis in reduced cost media. Egyptian Journal of Aquatic Research, 38 (1), 51-57. DOI: 10.1016/j.ejar.2012.09.003.

[24] Alava, D., Mello, P. C., Wagener, K. (1997). The Relevance of the CO2 Partial Pressure of Sodium Bicarbonate Solutions for the Mass Cultivation of the Microalga Spirulina. Journal of Brazilian Chemistry Society, 8 (5), 447-450. DOI: 10.1590/S0103-50531997000500004.

[25] TARKO, T., Duda-chodak, A., Kobus, M. (2012) Influence of Growth Medium Composition on Synthesis of Bioactive Compounds and Antioxidant Properties of Selected Strains of Arthrospira Cyanobacteria. Czech Journal of Food Science, 30 (3), 258-267.

[26] SILVA, P. C., Basson, P. W. E., Moe, R. L. (1996). Catalogue of the benthic marine algae of the Indian Ocean. University of California publications in botany. Berkeley: University of California Press.

[27] PELIZER, L. H., Danesi, E. D. G., Rangel, C. O., Sassano, C. E. N., Carvalho, J. C. M., Sato, S., Morais. I. O. (2003). Influence of inoculum age and concentration in Spirulina platensis cultivation. Journal of Food Engineering, 56 (4), 371-375. DOI: 10.1016/S0260-8774(02)00209-1.

[28] NORMAN, G. R., Streiner, D.L. (1994). Biostatistics: the bare essentials. St. Louis: Mosby-Year Book.

[29] PAOLETTI, C., Pushparaj, B., Tomaselli, F. L. (1975). Ricerche sulla nutrizione minerale di Spirulina platensis. Atti XVII Congr. Naz. Soc. It. Microbiol. Padova, 2, 845-853.

[30] LEDUY, A., Therien, N. (1977). An improved method for optical density measurement of the semicroscopic blue alga Spirulina máxima. Biotechnology and Bioengineering Journal, 19, 1219-1224.

[31] CHOJNACKA, K., Noworyta, A. (2004). Evaluation of Spirulina sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme and Microbial Technology, 34 (5), 461-465. DOI: 10.1016/j.enzmictec.2003.12.002.

[32] KALK, J., Langlykke, A. (1986). Cost estimation for biotechnology projects. Manual of Industrial Microbiology and Biotechnology. Washington DC.

[33] THIRUMALA, M. (2012). Optimization of Growth of Spirulina Platensis LN1 for production of Carotenoids. International Journal of Life Sciences Biotechnology and Pharma Research, 1(2), 152-157.

[34] KAUSHIK, R., Prasanna, R., Joshi, H. C. (2006) Utilization of anaerobically digested distillery effluent for the production of Spirulina platensis

https://dx.doi.org/10.22161/ijaers.5.6.32 *ISSN: 2349-6495(P) | 2456-1908(O)*

(ARM 730). Journal of Scientific & Industrial Research, 65 (6), 521-525.

[35] PANDEY, J. P., TIWARI, A., MISHRA, R. M. (2010). Evaluation of Biomass Production of Spirulina Maxima on Different Reported Media. Journal of Algal Biomass Utilization, 1 (3), 70-81.

[36] JITENDRA, M., Madhulika, J., Mohsina, S., Komal, M., Neha, K. (2012). Impact of different Physical and Chemical Environment for mass Production of Spirulina platensis- An Immunity Promoter. International Research Journal of Biological Sciences, 1 (6), 49-56.

[37] BORGES, J. A., Rosa, G. M., Meza, L. H. R., Henrard, A. A., Souza, M. R. A. Z., Costa, J. A. V. (2013). Spirulina sp. LEB-18 culture using effluent from the anaerobic digestion. Brazilian Journal of Chemical Engineering, 30 (2), 277-288. DOI: 10.1590/S0104-66322013000200006.

[38] SOLETTO, D., Binaghi, L., Ferrari, L., Lodi, A., Carvalho, J. C. M., Zilli, M., Converti, A. (2008). Effects of carbon dioxide feeding rate and light intensity on the fed-batch pulse-feeding cultivation of Spirulina platensis in helical photobioreactor. Biochemical Engineering Journal, 39 (2), 369-375. DOI: 10.1016/j.bej.2007.10.007.

[39] CHISTI, Y. (2007). Biodiesel from microalgae. Biotechnology Advances, 25 (3), 294 – 306. DOI: 10.1016/j.biotechadv.2007.02.001.

[40] WIJFFLES, R. H., Barbosa, M. J. (2010). An outlook on microalgal biofuels. Science, 329 (5993), 796-799. DOI: 10.1126/science.1189003.

[41] NORSKER, N. H., Barbosa, M. J., Vermue, M. H., Wijffels, R. H. (2011). Microalgal production – a close look at the economics. Biotechnology Advances, 29 (1), 24 –27. DOI: 10.1016/j.biotechadv.2010.08.005.

[42] ACIEN, F. G., Fernández, J. M., Mágan. J. J., Molina, E. (2012). Production cost of a real microalgae production plat and strategies to reduce it. Biotechnology Advances, 30 (6), 1344 – 1353. DOI: 10.1016/j.biotechadv.2012.02.005.