Environmental Engineering and Management Journal

February 2020, Vol.19, No. 2, 353-358
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

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STUDY OF DIFFERENT LIQUID MEDIA INFLUENCE ON *Arthrospira platensis* MICROALGAE CULTIVATION FOR ENVIRONMENTAL APPLICATIONS

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Abstract

Growth rate and biomass yield in the case of *Arthrospira platensis* depend on nutrient availability, temperature and light, which can determine changes in the metabolism and therefore in the biomass composition. The aim of this study is to evaluate the behavior of *A. platensis* during the growing time in several liquid culture media and their influence on the yield of biomass production. The following culture media have been investigated in this sense: ARS, Zarrouk (Z), modified Zarrouk (MZ), economic (EM) and UTEX media. Experiments have been carried out by using Erlenmeyer flasks containing 100 mL medium inoculated with 5% cellular suspension of *A. platensis*. Monitoring of microalgae development has been performed for 30 days at room temperature, 12/12 light/dark alternation regime and daily intermittent shaking. Optical density (OD) has been measured every three days. Dry substance, protein and antioxidant enzymes (catalase and dehydrogenase) were determined at the end of the cultivation period. Results of OD monitoring relieved a very fast acclimatization and exponential growth of *A. platensis* on UTEX, ARS and Zarrouk media, while a higher lag period and a slower growth have been observed on MZ and EM. The algal dry weight (DW) was greatly enhanced on UTEX, ARS and Z media, where the highest amounts of protein were recorded. The lack of carbon source in EM and MZ media has increased the activity of antioxidant enzymes, which suggests that these conditions could be considered in further investigations related to the antioxidants harvesting from *A. platensis*. Overall, economic media in the microalgae-based systems used in environmental applications can sustain biomass development and the increase of the enzymatic activity, at a reasonable yield and lower costs.

Key words: Arthrospira platensis, catalase, culture media, dehydrogenase, dry mass, protein

Received: January, 2019; Revised final: February, 2020; Accepted: February, 2020; Published in final edited form: February, 2020

1. Introduction

Of all cyanobacteria, *Arthrospira platensis* is most often used for biomass production, because of its high cell growth rate, easy process control and biomass recovery, ability to grow on alkaline and high-salt media, reduced risks of contamination and flexibility and resistance to adverse or suboptimal conditions (Vonshak, 1997). *Arthrospira (spirulina) platensis* is a mesophilic microalga that grows naturally in many places, including freshwater, saltwater lakes and marine environments. It usually develops under photoautotrophic conditions, but can also adapt to mixotrophic and heterotrophic conditions (Chen and Zhang, 1997). Undoubtedly, *A. platensis* is one of the most cultivated microalgae in the world. Overall, cultivation conditions dictate the biomass yield, quality and production costs, with a significant impact on the *A. platensis* industry (Delrue et al., 2017) and related applications. The growth of *such microalgae* and the composition of the produced biomass depend on several key factors, such as the availability of nutrients, temperature, light and pH (Fagiri et al., 2013). Particularly, *A. platensis* growth

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is favored at relatively high pH values e.g. between 9.5 and 11.0 with an optimum at 10.5, which also inhibits the culture contamination (Delrue et al., 2017; Madkour et al., 2012). Therefore, high amounts of sodium bicarbonate must always be present in the culture medium to sustain the high pH and prevent fluctuation. The composition of the culture medium plays an important role when referring to biomass yield and other compounds of interest such as protein content, antioxidant enzymes, pigments etc. (Marrez et al., 2013). In biotechnological processes, the components of culture media are responsible for high costs and exert a strong influence on the behavior of microorganisms. Several studies have been conducted to reduce these costs by using alternative components or by changing their concentration (Pandey and Tiwari, 2010). Zarrouk medium is a standard culture medium used for the development of *A. platensis* (de Castro et al., 2015). Manipulation of cultivation conditions can promote the biosynthesis of various compounds and, in addition, these variables can be controlled to reduce the cost of the production process.

Particularly, cultivation aspects play an important role for further tailoring of appropriate conditions in the perspective of involving *A. platensis* in environmental applications (e.g. wastewater or air treatment). In fact, using microalgae for the decontamination of gaseous or aqueous streams is a very promising trend in the field of environmental biotechnology (Markou et al., 2012; Șoreanu et al., 2018; Zinicovscaia et al. 2013). Despite this potential, such microalgae-based environmental applications are in the early stage of development (Șoreanu et al., 2018). In a sustainable process, biomass production should be correlated with the economical and the environmental benefits. Thus, *A. platensis* cultivation can be improved taking into account three criteria: productivity, quality and cost. The productivity of spirulina biomass can be significantly improved by using the suitable medium.

Therefore, the present study has been conducted to assess the growth of *A. platensis* on different culture media and to compare the growth characteristics, productivity and biochemical composition of the organism developed on these nutrient substrates, while depicting their implication from the environmental application perspective.

2. Materials and methods

2.1. Microorganism and inoculum preparation

The strain *A. platensis* (Algae Research Supply) was supplied as kit culture comprising the 1.7 mL algae and the standard culture medium (ARS) required for obtaining the inoculum used in the experiments.

An axenic stock culture of 50 mL standard medium (ARS) and 1.7 mL algae was maintained in 250 mL sterilized Erlenmeyer flask at room temperature, pH 10.1± 0.2 with 12/12 light/dark alternation regime and daily intermittent shaking. Thus, the inoculum used in the subsequent experiments was obtained.

2.2. Culture media and experiments

Batch experiments were conducted in 300 mL Erlenmeyer flasks containing 100 mL medium inoculated with 5% cellular suspension of *A. platensis*, to evaluate the effects of media composition on the growth profile, productivity and biochemical composition of the *A. platensis*. The following culture media have been investigated in this sense: ARS (available from Algae Research Supply), Zarrouk (Z) (adapted from Nyabuto et al., 2015), modified Zarrouk (MZ), economic (EM) and UTEX media (available from UTEX Culture Collection of Algae). The chemical composition of these media is presented in Table 1.

Table 1. Ingredients of synthetic media used in experiments

No.	***Ingredients***	***Culture media***				
		ARS	***Zarrouk***	***MZ***	***EM***	***UTEX***
		Amount (g/L)				
1	$NaHCO_3$	13.61	18.00	-	4.50	13.61
2	Na_2CO_3	4.03	-	-	-	4.03
3	Sodium bicarbonate food	-	-	18.0	5.00	-
4	$NaNO_3$	2.50	2.50	2.50	1.50	2.50
5	K_2HPO_4	0.50	0.50	0.50	0.05	0.50
6	K_2SO_4	1.00	1.00	1.00	1.00	1.00
7	NaCl	1.00	1.00	1.00	1.00	1.00
8	$CaCl_2$• $2H_2O$	0.04	0.04	0.04	0.04	0.04
9	$MgSO_4$• $7H_2O$	0.20	0.20	0.20	0.20	0.20
10	$FeSO_4$•$7H_2O$	0.0007	0.01	0.01	0.015	-
11	$FeCl_3$• $6H_2O$	-	-	-	-	0.00058
12	Na_2- EDTA•$2H_2O$	0.0008	0.08	0.08	-	0.00450
13	**Micronutrients***	***a***	***b***	***b***	***b***	***c***
14	Vitamin B12	0.0005 mg/L	-	-	-	0.135 mg/L
15	**Other adjuvants****	-	-	-	-	d
16	Distilled water	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL

***Micronutrients**

No.	Ingredients	a	b	c
		(mg/L)		
1	H_3BO_3	0.010	2.860	0.620
2	$MnCl_2 \cdot 4H_2O$	-	1.810	0.012
3	$MnSO_4 \cdot 7H_2O$	0.002	-	-
4	$ZnSO_4 \cdot 7H_2O$	0.001	0.222	0.044
5	$CuSO_4 \cdot 5H_2O$	0.000005	0.079	0.020
6	$Na_2MoO_4 \cdot 2H_2O$	0.001	0.390	0.012
7	Na_2- EDTA $\cdot 2H_2O$	-	-	0.050
8	$CoCl_2 \cdot 6H_20$	-	-	0.020
9	$Co(NO_3)_2 \cdot 6(H_2O)$	0.001	-	-

***Other adjuvants (d)**: $MnCl_2 \cdot 4H_2O$ - 0.246 mg/L; $ZnCl_2$ - 0.030 mg/L; $CoCl_2 \cdot 6H_2O$ - 0.012 mg/L; $Na_2MoO_4 \cdot 2H_2O$ - 0.024 mg/L; HEPES buffer pH 7.8 – 0.012 g/L.*

2.3. Growth, biomass and biochemical analysis

Monitoring of microalgae development has been performed for 30 days at room temperature, 12/12 light/dark alternation regime and daily intermittent shaking. The development of submerged microalgae cultures was determined every three days by measuring the optical density at λ 600 nm, using a UV- VIS spectrophotometer "Helyos ε" (Thermo Electron Corporation).

Cell productivity (CP_x) of *A. platensis* was evaluated using the equation:

$$CP_x(mg/L/day) = (X_m - X_i/t_m), \quad (1)$$

where: X_i = initial cell concentration (mg/L); X_m = maximum cell concentration (mg/L); t_m = cultivation time related to maximum cell concentration (days).

After the incubation period, *A. platensis* cultures were harvested by centrifugation at 7000 rpm for 15 min at 4°C, and the cell mass obtained were washed with 10 mM Na_2-EDTA and then twice with distilled water. The algal pellets were homogenized with an equal volume of glass beads at 4°C in 2 mL of extraction buffer containing 50 mM of phosphate buffer (pH 7.0). A further centrifugation is performed under the same conditions. The supernatant was used to measure the protein content according to Lowry et al. (1951) and modified by Clayton et al. (1988). A calibration curve was prepared using bovine standard albumin at a concentration range of 0 to 1500 μg mL^{-1}. In order to measure the protein content, 0.2 mL of each standard or samples containing the crude protein extract was withdrawn and then 1 mL of modified Lowry reagent was added to each sample. Each sample was then vortexed and incubated for 10 min. After incubation, 100 μL of Folin-Ciocalteu Reagent (1 N) was added and again vortexed and incubated for 30 min. The blue color solution was then measured at 750 nm with a UV-VIS Helyos ε spectrophotometer.

Also, the activity of the antioxidant enzymes (catalase and dehydrogenase) was determined. The activity of the catalase was assayed according to the method of Kar and Mishra (1976). One unit of catalase activity is defined as the amount of enzyme that decomposes 1 mmol of H_2O_2 in 1 min under the assay conditions described. The activity was expressed in U/mg protein.

Dehydrogenase activity was determined based on the reduction of 2,3,5-triphenyltetrazolium (TTC) chloride to triphenylformazan (TPF) colored in red. The amount of formazan formed was determined spectrophotometrically at 480 nm. Dehydrogenase activity was expressed in micrograms of TPF formed on one milligram of dry biomass (Kar and Mishra, 1976). To estimate the dry biomass a known volume of culture was sampled and weighed. Then it was centrifuged at 7000 rpm for 10 minutes. The supernatant was discarded and the pellets were washed three times with sterile distilled water, dried in oven at 50-60^{0}C and weighed. The difference in weight before and after drying represented the biomass and was expressed as mg/L.

3. Results and discussions

In experiments conducted to evaluate *A. platensis* growth and biomass production, five culture media (ARS, Z, MZ, EM, UTEX) with different chemical composition were used. All trials have been performed in triplicates. The presented results represent the average values. The other parameters (pH, temperature, light) were kept constant during the cultivation period so that growth differences and active metabolic products (protein, enzymes) were due exclusively to the composition of the culture medium. Growth performance of *A. platensis* under different culture media was measured in terms of optical density. The results are shown in Fig. 1.

The growth curves lacked a lag phase for ARS, UTEX and Z media. In contrast the lag phase on the EM and MZ media keep up to three days. On these media, culture grows exponentially until 24 days, after which the stationary phase is established, due to the lack of nutrients. A rapid increase in biomass values was observed in UTEX, ARS and Z media, giving the maximum biomass values at the end of the cultivation period, which indicates that culture is still in the exponential growth phase due to the availability of nutrients. The maximum growth belongs to the UTEX media. The OD of these cultures was 1.821 after 30 days, but close values were also recorded on ARS (1.728) and Z (1.711), respectively. It might be

possible that the higher content of vitamin B12 in UTEX medium contributed to its superior performances among these performant mediums, otherwise their composition is quite equilibrated (Table 1) and could explain the close values.

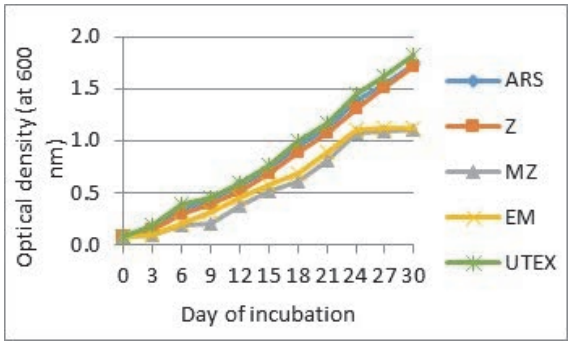

Fig. 1. The growth pattern of *A. platensis* in different liquid media by measured optical density

In a study conducted by Murugan and Radhamadhavan (2010) using Zarrouk medium with four different supplements and four different concentrations to increase *A. platensis* growth, an exponential increase in OD (0.6 -0.7 maximum) was recorded throughout the cultivation period (20 days) at optimal nutrient concentrations. These values were lower than the maximum OD observed in the present study, which is comparable to those obtained by Soni et al., (2012) that cultivated *A. platensis* under three different conditions i.e. F.G.C. (Fibre Glass Chamber), outdoor conditions and lab conditions.

Table 2 shows the cell productivity, protein and dry mass found for the five experimental variants. The biochemical composition of the microalgae greatly depends on the type and amount of nutrients present in the culture medium, especially the carbon and nitrogen sources. Cellular productivity reflects the daily growth rate of *A. platensis* culture, which is much more intense in UTEX, ARS and Z media compared to EM and MZ, which also led to different biomass (Fig. 2). The values of proteins varied from 46.07% dw^{-1} (MZ) to 52.31 % dw^{-1} (UTEX), depending on the composition of the culture media. Higher levels of protein content (60-67%) were reported by other authors (Danesi et al., 2002), but in other experimental conditions in which the nitrogen source increased. El-Baky et al., (2008) reported that the use of 1.875 g/L sodium nitrite, maintaining the culture at 35° C, decreases biomass production but results in higher protein, lipid and phenolic compounds. However, these results were obtained varying the concentration of nitrates without simultaneous variation of bicarbonate, a factor of particular importance in the process of cyanobacterial development. Also, the amount of protein varies from one experimental variation to another and is in direct correlation with the determined biomass.

The obtained biomass concentration was 2.8932 g/L (UTEX), 2.6827 g/L (ARS) and 2.4911g/L (Z). For EM and MZ, the recorded biomass values were 2.0372 g/L and 1.9553 g/L. The reduction of algae production in EM and MZ can be explained by the existence of an insufficient amount of CO_2 due to a small contribution of bicarbonate as a carbon source.

Fig. 2. General aspect of the *A. platensis* culture growing on liquid media

Because of the stress from carbon dioxide deficiency, the free radicals or ROS (reactive oxygen species) levels in algal cells may increase, which causes the algal cells to undergo oxidative stress (Choo et al., 2004; Ismaiel et al., 2016). The source of carbon is the main nutrient needed for *A. platensis* growth. Vonshak et al. (1982) have shown that, apart from the biotechnological process, the second major cost of biomass production of *A. platensis* is the cost of nutrients, mainly the carbon source. Experimental results showed that when higher concentrations of sodium bicarbonate were used, even at a lower luminous intensity, there was an increase in the amount of biomass, confirming that $NaHCO_3$ is the most influential factor in obtaining a large amount of biomass.

Cyanobacterial cells prefer to capture HCO_3^- rather than CO_2, although this is a poorer carbon source when it is quantitatively limited in the culture medium. Biomass productivity increases with the concentration of CO_2 in the gas mixture to a certain percentage, after which productivity decreases (Kumar et al., 2011). This preference for bicarbonate favors the continuous growth of cyanobacteria, fact demonstrated by the existence of culture in the exponential phase, even after 30 days of cultivation on the UTEX, Z and ARS media (Fig. 1).

Table 2. Cell productivity (CP_x), protein and dry mass of *A. platensis* grown on different media

No.	*Culture media*	*CP_x (mg/L/day)*	*Protein (%dw^{-1})*	*Dry mass (g/L)*
1	ARS	0.0655 ± 0.0025	51.72 ± 1.33	2.6827 ± 0.0257
2	Z	0.0648 ± 0.0013	51.7 ± 1.50	2.4911 ± 0.0189
3	MZ	0.0418 ± 0.0009	46.07 ± 1.02	1.9553 ± 0.0292
4	EM	0.0427 ± 0.0009	47.62 ± 1.02	2.0372 ± 0.0264
5	UTEX	0.0690 ± 0.0008	52.31 ± 1.00	2.8932 ± 0.0192

Madkour et al. (2012) conducted a study to assess the production of *A. platensis* biomass under different nutritive conditions, with comparable behaviour to that in the present study. Data obtained in this study shows that the growth and biomass yield of *A. platensis* were clearly affected by the composition of the culture medium, especially the carbon source, which is a limiting factor in the EM and MZ media, becoming a stress factor for the development of cyanobacteria. As a result, it can increase the enzymatic activity, especially antioxidant enzymes such as catalase and dehydrogenase.

The values obtained by the determination of catalase and dehydrogenase (Figs. 3-4) reveal the existence of oxidative stress in the cultures of EM and MZ media, where the largest activities were recorded. In this case, the relative reduction of *A. platensis* growth on EM and MZ culture media could be compensated by the higher antioxidant content, which is beneficial for the growth of microalgae in the presence of toxic compounds. Thus, the antioxidant enzyme activity will accelerate the detoxification and removal of contaminants (El-Baky et al., 2007).

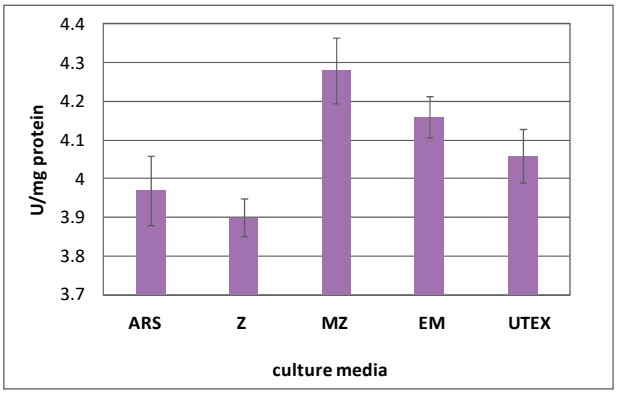

Fig. 3. Catalase activity of *A. platensis* grown on different liquid media

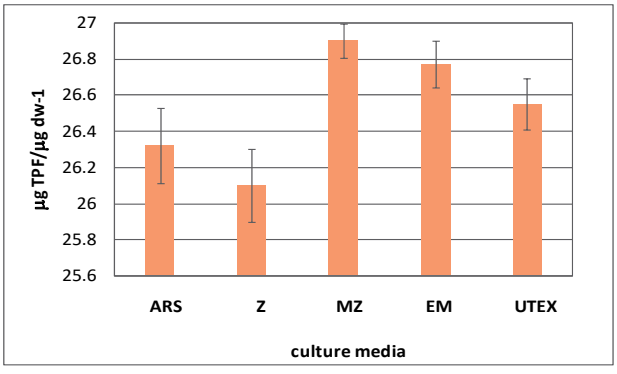

Fig. 4. Dehydrogenase activity of *A. platensis* grown on different liquid media

Environmental application perspectives

The obtained results in this study show that a low cost media can sustain a reasonable biomass development and favors the increase of the enzymatic activity. These aspects can be thus considered when developing sustainable microalgae-based biosystems for environmental applications. Soreanu et al. (2018) investigated CO_2 uptake from indoor air by microalgae in a sparged photobioreactor and shown that using nutrient/substrate - limiting culture can favor the contaminant uptake from the gas phase, thus creating perspectives for developing a cost-effective and environmental-friendly process. Under non-limiting conditions, microalgae tend to use the substrate readily available in the culture, rather than from the gas phase, which is in a good agreement with the results of the present study.

4. Conclusions

In order to optimize *A. platensis* cultivation conditions, it must be taken into account three criteria: quality, productivity and cost. The productivity of biomass can be significantly improved by using the suitable medium.

Although the highest biomass yield and dry substance, respectively, are obtained on the UTEX medium, the ARS and Zarrouk medium lead to good development performances of *A. platensis*. It should be also noted that the other alternative screened options (EM and MZ) allow a reasonable yield of biomass production at lower costs. Protein content in biomass developed on UTEX, ARS and Z medium was higher than in the case of EM and MZ medium, while an increased enzymatic activity was noticed for these last two medium.

Such information contributes to the development of cost-effective methods for biomass production based on *A. platensis*, with benefits for many practical applications, including those related to environmental biotechnology.

Acknowledgments

This work is supported by ROSA (Romanian Space Agency) - RDI Program for Space Technology and Advanced Research - STAR, project no. 157/2017.

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