

CULTIVATION OF *ARTHROSPIRA (SPIRULINA) PLATENSIS* IN DESALINATOR WASTEWATER AND SALINATED SYNTHETIC MEDIUM: PROTEIN CONTENT AND AMINO-ACID PROFILE

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ABSTRACT

Arthrospira (Spirulina) platensis was cultivated in laboratory under controlled conditions (30°C, photoperiod of 12 hours light/dark provided by fluorescent lamps at a light intensity of 140 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and constant bubbling air) in three different culture media: (1) Paoletti medium (control), (2) Paoletti supplemented with 1 $\text{g}\cdot\text{L}^{-1}$ NaCl (salinated water) and (3) Paoletti medium prepared with desalinator wastewater. The effects of these treatments on growth, protein content and amino acid profile were measured. Maximum cell concentrations observed in Paoletti medium, Paoletti supplemented with salinated water or with desalinator wastewater were 2.587, 3.545 and 4.954 $\text{g}\cdot\text{L}^{-1}$, respectively. Biomass in medium 3 presented the highest protein content (56.17%), while biomass in medium 2 presented 48.59% protein. All essential amino acids, except lysine and tryptophan, were found in concentrations higher than those required by FAO.

Key words: *Arthrospira platensis*, desalinator wastewater, aminogram.

INTRODUCTION

Cyanobacterium *Arthrospira (Spirulina) platensis* is commercial product with high nutritional value, serving as a source of nutrients for food, chemical and pharmaceutical industry (9,10,19,20). It is the major known source of vitamin B₁₂, having high protein content (6). It presents immune-promoting effects, such as enhancement of macrophage function and *in vitro* and *in vivo* antioxidant activity (19). Due to lack of cellulose in its cell wall, 85-95% is assimilated by the organism (2). It is also used in aquaculture as feed in fish, crustacean, shellfish and bivalve cultures (9). Also, it is applied in wastewater treatment and agriculture (9,10). *Arthrospira platensis* is naturally found in tropical regions inhabiting alkaline lakes (pH 11) with high concentration of NaCl and bicarbonates (4,13,14,17). These limiting conditions for other microorganisms allow cultivation of this microalga in opened reactors (10).

In the Northeast of Brazil, a semi-arid area of 900,000 Km² is also known as Polygon of Drought. This area corresponds approximately to the isoyhets (lines of equal precipitation) of

800 mm of annual rainfall. Attempting to reduce the drought problem in the area and set up alternatives, Brazilian government is encouraging the captation of ground water. However, reservoirs from this region present high levels of salinity, making the use of desalinators mandatory. Nevertheless, these processes generate residues with high saline concentration with great environmental impact. The aim of this work was to cultivate *Arthrospira platensis* in this wastewater and in salinated synthetic medium, evaluating the amino acid profile and the protein content of the cells.

MATERIALS AND METHODS

Microorganism

Arthrospira platensis strain used in this work was given by the Laboratório de Bioquímica from the Departamento de Química of the Fundação Universidade Federal do Rio Grande – FURG/RS, Brazil. The strain was kept in Paoletti medium according to Ferraz *et al.* (7), with modifications, presenting the following composition ($\text{g}\cdot\text{L}^{-1}$): 0.92 NaCl; 1.88 Na₂SO₄; 0.50

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K_2HPO_4 ; 8.89 Na_2CO_3 ; 15.15 $NaHCO_3$; 0.05 $CaCl_2 \cdot 2H_2O$; 2.57 KNO_3 ; 0.25 $MgSO_4 \cdot 7H_2O$; 1.0 $mL \cdot L^{-1}$ of micronutrient solution (2.86 H_3BO_3 ; 1.54 $MnSO_4 \cdot H_2O$; 0.22 $ZnSO_4 \cdot 7H_2O$; 0.39 $NaMoO_4 \cdot 2H_2O$; 0.079 $CuSO_4 \cdot 5H_2O$ and 0.038 $CoCl_2 \cdot 6H_2O$) and 1,0 $mL \cdot L^{-1}$ Fe-EDTA solution (29.8 EDTA- Na_2 ; 24.9 $FeSO_4 \cdot 7H_2O$).

Culture media

Three different media were prepared for cultivation of *A. platensis*. Paoletti medium was used as control medium. Salinated water, the second medium, was produced by adding 1 $g \cdot L^{-1}$ NaCl to Paoletti medium. The third medium (desalinator wastewater) was a modified Paoletti medium in which distilled water was replaced by desalinator wastewater and the concentration of the components of Paoletti medium was reduced by 50%. This medium was produced as follows: the components were solubilized in wastewater, centrifuged at 4,000 rpm for 15 min and the precipitate discarded. Desalinator wastewater was given by the Laboratório de Referência em Dessalinização, Campina Grande/PB, Brazil, and presented the following composition, determined according to United States Environmental Protection Agency ($mg \cdot L^{-1}$): Bicarbonate 0.00; Calcium 585.00; Carbonates 38.10; Chloride 4,340.04; Phosphate <0.05; Magnesium 433.00; Nitrate 779.27; Potassium 101.50; Sodium 1245.00 and Sulfate 919.65. The pH of the three media was adjusted to 9.4 ± 0.2 . The experiments were done in triplicate.

Inoculum preparation

Previous inocula of *A. platensis* were prepared for three media. Microalgae were cultivated in 4 L Erlenmeyer flasks, with photoperiod of 12 hours light/dark provided by fluorescent lamps at a light intensity of 140 $\mu mol \text{ photons} \cdot m^{-2} \cdot s^{-1}$ and temperature of $30 \pm 1^\circ C$. Experiments were initiated with 10% (v/v) of inoculum. Media agitation was carried out by bubbling air.

Growth evaluation

Cell concentration was estimated at 72 hours intervals using a calibration curve (560 nm) for each medium. Experiments finished when decreasing of cell growth was observed. At the end of cultivation, biomass was filtered (0.45 μm cellulose acetate filter), lyophilized and stored at $-20^\circ C$. Cell productivity was also determined.

pH determination

pH of the media was measured at each 72 hours using a potentiometer.

Protein content and aminogram

Total protein content in the lyophilized biomass was determined by the Kjeldahl method (1). Amino acid profile was carried out according to White *et al.* (18) and determination of tryptophan was performed according to Spies (16) using HPLC technique.

Statistical analysis

Results of the analyses were submitted to analysis of variance (ANOVA) with confidence level of 95% ($p < 0.05$) in order to verify significant differences among media. Statistical analyses were carried out using Statistica® 6.0 software.

RESULTS AND DISCUSSION

Effect of culture medium in growth

Fig. 1 shows the growth of *Arthrospira platensis* in the three tested culture media. Despite having started with a similar initial inoculum, after 9 days of cultivation, the growth on desalinator wastewater was more intense than in the other two media. The difference became more evident on day 23, where cell concentration reached $4.954 g \cdot L^{-1}$ which is 91.5% higher than that observed in Paoletti medium. An intermediate production of biomass was observed for microalgae cultivated in salinated water, which presented increased growth from day 15 on, reaching a final cell concentration of $3.545 g \cdot L^{-1}$ which is 37.03% higher than that in Paoletti medium. Regarding to Paoletti medium, the values for maximum cell concentration obtained are close to those reported by Oliveira *et al.* (9), $2.4 g \cdot L^{-1}$, but lower than values reported by Rafiqul *et al.* (13), who used Zarrouk medium and reached $2.7 g \cdot L^{-1}$ at day 20. However, values attained were 49.0% higher than those reported by Pelizer *et al.* (11) ($1.3 g \cdot L^{-1}$).

ANOVA tests indicated that the differences observed between treatments were significant ($p = 0.008722$). Yet, the differences observed in final cell concentration in salinated water and Paoletti medium were not significant, nor were those observed in salinated water and desalinator wastewater. However, the difference in cell concentration in Paoletti medium

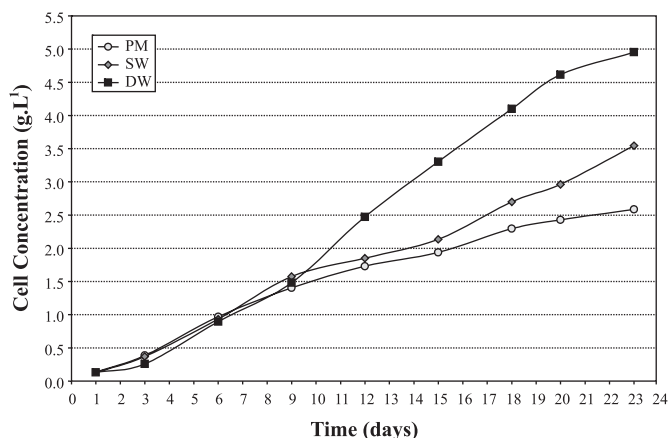


Figure 1. Cell concentration of *Arthrospira platensis* in different culture media (PM – Paoletti medium; SW – salinated water and DW – desalinator wastewater).

and desalinator wastewater was significant. Microalgae cultivated in Paoletti medium, salinated water and desalinator wastewater presented cell productivities of 0.109 g.L⁻¹.day⁻¹, 0.150 g.L⁻¹.day⁻¹ and 0.210 g.L⁻¹.day⁻¹, respectively. It is possible to note that the cell productivities of desalinator wastewater was higher (92.9%) than Paoletti medium. pH values ranged from 9.6 to 10.5 for Paoletti medium, from 9.6 to 10.6 for salinated water and from 9.4 to 10.2 for desalinator wastewater.

Effect of culture medium on protein and amino acid content

A. platensis cultivated in salinated water presented a total protein content of 48.59% in dry weight, while in desalinator wastewater the content was 56.17%. The differences between these results were significant (p=0.001661). Similar values for total protein, ranging from 46% to 50% in dry weight, were reported by Richmond (13) in *A. platensis*, but the values in desalinator wastewater were lower than that presented by Oliveira *et al.* (9). Pelizer *et al.* (11), studying different initial inocula, reported 55.0 - 61.0% protein content. Similar values were found by Rafiqul *et al.* (12), who found 58.6% of total protein content when using Zarrouk medium. Zeng and Vonshak (20) reported that cells under stress conditions, including salinity-stress, have a lower protein synthesis capacity. This fact can probably explain lower protein contents found in biomasses grown in salinated water and desalinator wastewater. Besides, an alternative for salinity-stress adaptation is the increase in carbohydrates metabolism (20).

Among analyzed amino acids, four were responsible for 39.33% (desalinator wastewater) and 40.34% (salinated water) of the total amino acid content (Table 1). Regarding to desalinator wastewater, the main amino acids were: leucine, alanine, glutamine and histidine. Concerning salinated water, the main amino acids were: leucine, asparagine, glutamine e histidine. Tryptophan was the amino acid found in lowest content: 0.082 and 0.060g/100g of protein in desalinator wastewater and salinated water, respectively.

The contents of all amino acids were higher than those reported by Grinstead *et al.* (8), except tryptophan (0.6%) and cysteine (0.7%). As can be observed in Table 1, 57.60% of the amino acids in biomass grown in desalinator wastewater were essential amino acids. In biomass grown in salinated water this content was of 52.10%. Values obtained for biomass grown in desalinator wastewater are close to those reported by Babadzhanov *et al.* (2), who observed that the content of essential amino acids in *A. platensis* biomass was around 60%.

As shown in Table 1, the essential amino acid content were above the minimum values required by FAO (16) with histidine overcoming in 483% (biomass grown in salinated water) and 548% (biomass grown in desalinator wastewater) the FAO's requirements. However, lysine and tryptophan did not reach the minimum requirements: lysine supplied 76% (salinated

Table 1. Total amino acids of *Arthrospira platensis* (g/100 g of protein)

Amino Acids	Richmond ^a	Salinated Water	Desalinator Wastewater	FAO/WHO ^b
Isoleucine*	6.70	5.71	6.49	2.80
Leucine*	9.80	9.26	10.17	6.60
Valine	7.10	6.45	6.54	3.50
Lysine*	4.80	4.42	4.99	5.80
Phenylalanine*	5.30	4.45	5.16	6.30
Tyrosine	5.30	5.26	5.69	
Methionine*	2.50	2.05	2.16	2.50
Cysteine	0.90	0.59	0.57	
Tryptophan	0.30	0.06	0.08	1.10
Threonine*	6.20	4.65	5.31	3.40
Histidine*	2.20	9.17	10.41	1.90
Alanine*	9.50	8.51	9.27	
Arginine*	7.30	7.09	8.00	
Asparagine*	11.80	9.86	3.90	
Glutamine*	10.30	13.40	9.47	
Glycine*	5.70	1.10	2.98	
Proline*	4.20	3.33	3.75	
Serine*	5.10	4.59	5.00	

^a – Richmond (15);

^b – Recommendation for children of 2-5 years old, according to FAO/WHO (apud 16);

*significant differences were observed between the treatments salinated water and desalinator wastewater (p<0.05).

water) and 86% (desalinator wastewater) of FAO's requirements, while tryptophan supplied only 5.45% (salinated water) and 7.45% (desalinator water). These results are in disagreement with data presented by Danesi *et al.* (5) who reported that, among all amino acids of *A. platensis*, only methionine content was below the FAO's minimum requirements.

It is important to notice that according to Campanella *et al.* (3) tryptophan levels in *A. platensis* are usually not high, with contents ranging from 0.139 - 0.144g/100g of protein.

Regarding to amino acids profile, biomass from salinated water presented higher content of non essential amino acids, such as asparagine and glutamine. However, the difference in contents of valine, tyrosine, cysteine and tryptophan in the two types of biomass was not significant.

According to these data, cultivation of *A. platensis* in desalinator wastewater produces high levels of biomass (4.954 g.L⁻¹). However, the produced biomass contains only two essential amino acids, lysine and tryptophan, both below FAO's minimum requirements. Thus, cultivation of *A. platensis* in this medium is possible but more studies are necessary in order to better evaluate the application in large scale production.

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RESUMO

Cultivo de *Arthrospira (Spirulina) platensis* em rejeito de dessalinizador e meio sintético salinizado: teor protéico e perfil de aminoácidos

Arthrospira (Spirulina) platensis foi cultivada em laboratório sob condições controladas (30°C, intensidade luminosa de 140 $\mu\text{mol f\acute{o}tons.m}^{-2}.s^{-1}$, 12 horas claro/escuro e insuflação constante de ar atmosférico), em três meios de cultivo: (1) meio de Paoletti (controle), (2) meio de Paoletti suplementado com 1,0 g.L⁻¹ de NaCl (água salinizada) e (3) meio de Paoletti preparado com rejeito de dessalinizador. Foi verificado o efeito destes tratamentos no crescimento, teor de proteínas e aminoácidos. As concentrações celulares máximas obtidas foram de 2,587; 3,545 e 4,954 g.L⁻¹ no meio controle, meio de Paoletti suplementado com água salinizada ou com rejeito de dessalinizador, respectivamente. Com relação às concentrações protéicas, estas foram maiores na biomassa cultivada no meio 3, com 56,17%, enquanto que a biomassa cultivada no meio 2 apresentou 48,59%. A maioria dos aminoácidos essenciais encontrou-se acima dos limites requeridos pela FAO, com exceção apenas de lisina e triptofano.

Palavras-chave: *Arthrospira platensis*, rejeito de dessalinizador, aminograma

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