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Influence of desalinator wastewater for the cultivation of *Arthrospira platensis*. Fatty acids profile

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RESUMEN

Influencia de agua de desecho de desalación para el cultivo de *Arthrospira platensis.* Perfil de ácidos grasos.

En la región nordeste de Brasil, debido a la elevada salinidad de los acuíferos, existe la necesidad de utilizar procesos de desalación. Sin embargo, estos procesos generan residuos con alta concentración salina, con significativo impacto ambiental. El objetivo de este trabajo fue el cultivo de *Arthrospira platensis* en tres medios de cultivo diferentes: medio de Paoletti, medio de agua salinizada y medio de agua de desecho obtenida en el proceso de desalación de acuíferos. Las microalgas fueron cultivadas en laboratorio, con condiciones controladas, en fotobiorreactores de 4 L, a 30±1°C y periodos de 12 horas de claridad/oscuridad con iluminación de 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ e inyección constante de aire (0,5 $L \cdot L^{-1} \cdot min^{-1}$). Fue verificado el efecto de los diferentes medios en la concentración celular, productividad, contenido total de lípidos y perfil de ácidos grasos. La mayor concentración celular, así como la productividad máxima fueron encontradas en el medio de agua de desecho de desalación, 4,954 (±0,554) $g \cdot L^{-1}$ y 0,225 (±0,042) $g \cdot L^{-1} \cdot día^{-1}$ respectivamente. En relación a los lípidos totales, se encontraron valores de 4,54% en el medio de agua de desecho de desalación y 4,69% en el medio de agua salinizada. En cuanto a los ácidos grasos, se obtuvieron altos índices de ácidos grasos saturados con ambos tratamientos. Con relación al ácido γ-linolénico, se encontró en un 13,09% en el medio de agua de desecho de desalación y en un 11,95% en el medio de agua salinizada.

PALABRAS-CLAVE: Acidos grasos – Agua de desecho de desalación – Arthrospira platenses.

SUMMARY

Influence of desalinator wastewater for the cultivation of *Arthrospira platensis*. Fatty acids profile.

The need for a desalination processes in northeastern Brazil is due to the salinity of its water sources. However, these processes produce residues with high saline concentrations and a significant environmental impact. These wastewaters can be used for *Arthrospira platensis* cultivation. This work aimed to cultivate *Arthrospira platensis* in three different media: Paoletti Synthetic Medium (PSM), Salinated Water Medium (SWM) and Desalinator Wastewater Medium (DWWM). Microalgae were cultivated under controlled conditions, in 4 L photobioreactors, 30±1°C, 12 hours of light/dark photoperiod provided by fluorescent lamps at a light intensity of 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and constant bubbling of air (0.5 $L \cdot L^{-1} \cdot min^{-1}$). The effect of different media on cell concentration, productivity, total content of lipids and fatty acids profile was verified. Higher cell concentration, as well as higher productivity was observed in DWWM: 4.954 (±0.554) $g \cdot L^{-1}$ and 0.225 (±0.042) $g \cdot L^{-1} \cdot day^{-1}$, respectively. Concerning total lipids, the contents of 4.54% and 4.69% were observed in DWWM and SWM, respectively. High levels of saturated fatty acids were observed in both treatments. Concerning γ-linolenic acid, the contents of 13.09% (DWWM) and 11.95% (SWM) were found.

KEY-WORDS: Arthrospira platensis – Desalinator wastewater – Fatty acids.

1. INTRODUCTION

Large semi-arid areas of northeastern Brazil have serious problems with low pluviometric precipitations and seasonal droughts, besides a frequent occurrence of high levels of saline in its water sources. This problem can be minimized through the captation of groundwater and subsequent desalinization by reverse osmosis. However, the back-washing of membranes produces a highly salinated wastewater with a problematic destination. The present work aims to use this wastewater for composing a medium for *Arthrospira platensis* cultivation. The production of food and other compounds of interest from microalgae in non conventional systems present several advantages once it allows high levels of production, operating in controlled conditions and low levels of contamination. *Arthrospira platensis* is produced worldwide for obtaining proteins, carotenoids, vitamins, minerals and PUFAs (Polyunsaturated Fatty Acids), mainly γ-linolenic acid (Quoc and Dubacq, 1997; Xue *et al.*, 2002; Hongsthong *et al.*, 2003), being the major known source of vitamin B12 (Estrada *et al.*, 2001; Duarte Filho *et al.*, 2002). It presents compounds of

pharmaceutical interest, having immuno-promoting effects, such as enhancing macrophage functions besides in vitro and in vivo antioxidant activity (Xue *et al*., 2002). Moreover, it is 85-95% assimilated by the organism, due to the lack of cellulose in its cell wall (Babadzhanov *et al*., 2004). Therapeutical significance of ω-3 e ω-6 PUFAs has been recently suggested through clinical and epidemiological investigations (Medina *et al*., 1998; Renaud *et al*., 2002; Kroes *et al*., 2003; Wen and Chen, 2003). Studies, mainly with γ-linolenic acid, suggest effects such as reduction in blood cholesterol, protection against some cancers, enhancement of the immune system, reduction of hyper lipidemia and obesity and partial inhibition of HIV-1 replication (Jiménez *et al*., 2003). These findings led to a great interest in the commercial development of a process for the production and extraction of these lipids. PUFAs can be obtained from animal and plant sources, being fish oil its main source. However, fish oils presents a production lower than the required demand, besides its unpleasant odor, contamination with heavy metals, presence of cholesterol, variable production and a complex fatty acid profile (Medina *et al*., 1998; Renaud *et al*., 2002; Kroes *et al*., 2003; Wen and Chen, 2003). On the other hand, PUFAs extracted from microalgae and other microorganisms lack these disadvantages and a simpler fatty acid composition facilitates purification (Medina *et al*., 1998; Zittelli *et al*., 1999; Wen and Chen, 2003).

Nevertheless, the PUFA content of algae depends not only on the species, but also on factors related to culture conditions (Volkman *et al*., 1989; Medina *et al*., 1998). Knowing that, the objective of this work was to evaluate the lipid and fatty acid contents of *Arthrospira platensis* grown in different media.

2. MATERIAL AND METHODS

2.1. Microorganism and cultivation conditions

The Arthrospira platensis strain used in this work was given by the Laboratory of Biochemistry of Chemistry Department from the Federal University of Rio Grande Foundation – FURG/RS. It was kept in a Paoletti Synthetic Medium according to Ferraz *et al.* (1985), with modifications (Table 1).

2.2. Culture Media

Three different media were prepared for cultivation. Paoletti Synthetic Medium (PSM) was used as control medium. Salinated Water Medium (SWM), the second medium, was produced by adding 1.0 $g \cdot L^{-1}$ of NaCl to PSM and the third medium Desalinator Wasterwater Medium (DWWM) was produced by dissolving 50% of all components of PSM in the desalinization wastewater. This medium was produced as follows: after solubilization of the components in the wastewater, it was centrifuged at 4,000 rpm for 15 min and the precipitate was discarded. Desalinator wastewater was given by Reference Laboratory in Desalination, Campina Grande/PB, Brazil, and its composition is presented in Table 2, which was determined according to United States Environmental Protection Agency methods.

The pH of the media was adjusted to 9.4±0.2, with 3.0 M KOH solution using a potentiometer (Quimis, Q400A).

Table 1
Composition of Paoletti Synthetic Medium.

Component		Concentration ($g \cdot L^{-1}$)
NaCl		0.92
Na_2SO_4		1.88
K_2HPO_4		0.50
Na_2CO_3		8.89
$NaHCO_3$		15.15
$CaCl_2 \cdot 2H_2O$		0.05
KNO_3		2.57
$MgSO_4 \cdot 7H_2O$		0.25
Fe-EDTA sol. ($g \cdot L^{-1}$):		
EDTA-Na_2	29.8	1.0 mL
$FeSO_4 \cdot 7H_2O$	24.9	
Micronutrients sol. ($g \cdot L^{-1}$):		
H_3BO_3	2.86	
$MnSO_4 \cdot H_2O$	1.54	
$ZnSO_4 \cdot 7H_2O$	0.22	1.0 mL
$NaMoO_4 \cdot 2H_2O$	0.39	
$CuSO_4 \cdot 5H_2O$	0.079	
$CoCl_2 \cdot 6H_2O$	0.038	

2.3. Inoculum preparation

Inocula of *A. platensis* were prepared for three media in 500 mL Erlenmeyer flasks. Microalgae were cultivated in 4 L photobiorreactors (working volume), with a photoperiod of 12 hours light/dark provided by fluorescent lamps (Philips, 20W) at a light intensity of 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and constant temperature of 30±1°C (climate controlled room). Experiments were initiated with 10% (v/v) of

Table 2
Composition of desalinator wastewater.

Component	Desalinator wastewater ($mg \cdot L^{-1}$)
Bicarbonate	0.00
Calcium	585.00
Carbonates	38.1
Chloride	4340.04
Phosphate	<0.05
Magnesium	433.00
Nitrate	779.27
Potassium	101.50
Sodium	1245.00
Sulfate	919.65

inoculum. Media agitation was carried out by a constant bubbling of air (0.5 $L \cdot L^{-1} \cdot min^{-1}$).

2.4. Evaluation of growth

The cell growth was measured each 72 hours by absorbance readings at 560 nm in spectrophotometer (Hitachii, U-1800), according to these equations: $y = -0{,}4404x^2 + 2{,}3763x - 0{,}0438$ (PSM), $y = 0{,}2811x^2 + 1{,}925x - 0{,}0128$ (SWM) and $y = 0{,}6135x^2 + 1{,}1365x - 0{,}0013$ (DWWM). The equations were elaborated from the correlation of absorbance readings and dry weight taken on the 15th day of a previous experiment under the same conditions. These data were subjected to Polynomial Regression Analysis using Statistica® 6.0 software.

The experiment ended with the decreasing of cell growth. At the end of cultivation, biomass was filtered (0.45 µm cellulose acetate filter), lyophylized (Terroni, LT 1000/8) and stored at –20°C. Specific growth rate and productivity were also determined.

2.5. pH determination

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

2.6. Total lipid content and fatty acid profile

Total lipid content and fatty acid profile were determined only for SWM and DWWM. Lipids were extracted in chloroform:metanol (2:1) according to Folch *et al*. (1957) and quantified by gravimetry.

After lipid quantification, samples were esterified with boron trifluoride (1:10) as derivatizing agent according to Metcalfe *et al*. (1966). The methyl esters from fatty acids were identified on the basis of retention times corresponding to standards (Sigma Chemical Company). Identification was confirmed with Ackerman diagram. Quantification was performed by normalization of peak areas followed by estimation of percentages of each fatty acid in samples.

Gas chromatography of methyl esters was performed using a CG Varian Star (model 3400 CX) equipped with a flame ionization detector, using a DB-WAX capillary column (30 m × 0.25 mm; 0.25 mm film thickness). The column was temperature programmed under the following conditions: initial temperature, 100°C; initial isotherm, 3 min; temperature increasing 5°C per min up to 180°C; intermediate isotherm, 1 min, then temperature increasing 1°C per minute up to 200°C; intermediate isotherm, 4 min, then temperature increasing 2°C per minute up to 210°C; intermediate isotherm, 3 min, then temperature increasing 5°C per minute up to 230°C; final isotherm, 12 min; injector and detector temperatures were 250 and 300°C, respectively. Helium was used as gas carrier (0.5 $mL \cdot min^{-1}$).

2.7. 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.

3. RESULTS AND DISCUSSION

3.1. Effect of culture medium in growth

Maximum cell concentrations (Figure 1) were observed in day 23: 2.587 (±0.082) $g \cdot L^{-1}$ in PSM (control), 3.545 (±0.169) $g \cdot L^{-1}$ in SWM and 4.954 (±0.554) $g \cdot L^{-1}$ in DWWM. Values obtained in DWWM and SWM were 91.49% and 37.03% higher respectively than those observed in the control. Values of maximum cell concentration observed in PSM are close to those reported by Oliveira *et al.* (1999) (2.4 $g \cdot L^{-1}$), but lower than the values reported by Rafiqul *et al*. (2005), who used Zarouk medium and reach 2.7 $g \cdot L^{-1}$ at day 20. However, the obtained values were 49% higher than values reported by Pelizer *et al.* (2003) (1.3 $g \cdot L^{-1}$), where significant differences were observed between control (PSM) and DWWM ($p<0.01$). Relating to specific growth rate, obtained data were: 0.393 (±0.101) day^{-1} for PSM, 0.324 (±0.049) day^{-1} for SWM and 0.244 (±0.155) day^{-1} for DWWM. Significant differences were not observed among treatments.

Microalgae cultivated in DWWM presented lower productivity in the first days of cultivation, when compared with other treatments. However, it presented higher concentrations from day 10, reaching a maximum of 0.225 (±0.042) $g \cdot L^{-1} \cdot day^{-1}$ at day 20 (Figure 2). Maximum values found for PSM and SWM were 0.148 (±0.019) $g \cdot L^{-1} \cdot day^{-1}$

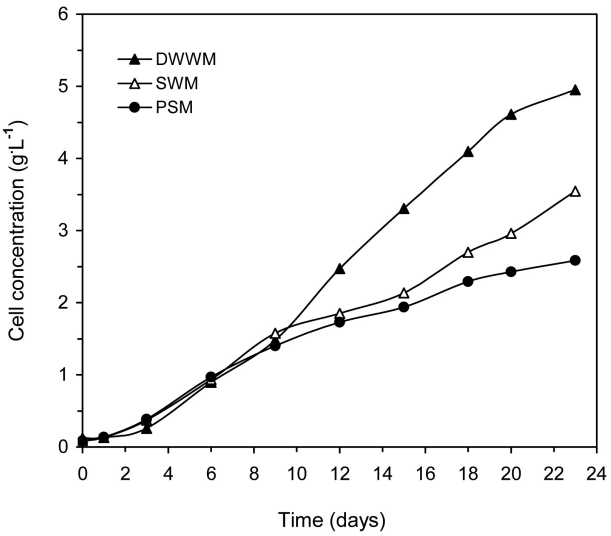

Figure 1
Cell concentration ($g \cdot L^{-1}$) of *Arthrospira platensis* in Paoletti Synthetic Medium (PSM), Salinated Water Medium (SWM) and Desalinator Wastewater Medium (DWWM).

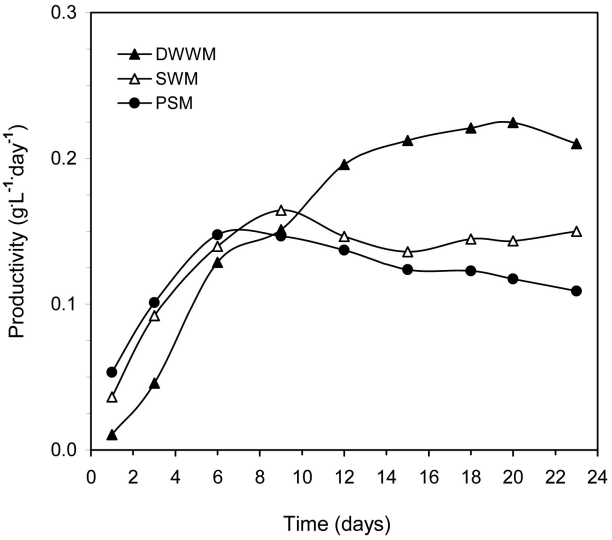

Figure 2
Productivity ($g \cdot L^{-1} \cdot day^{-1}$) of *Arthrospira platensis* in Paoletti Synthetic Medium (PSM), Salinated Water Medium (SWM) and Desalinator Wastewater Medium (DWWM).

(day 6) and 0.165 (±0.020) $g \cdot L^{-1} \cdot day^{-1}$ (day 9), respectively. Significant differences ($p<0.05$) were found between PSM and DWWM. The Salinated Water Medium did not present significant differences when compared to other treatments. Microalgae cultivated in DWWM presented higher productivity than those reported (0.175 $g \cdot L^{-1} \cdot day^{-1}$) by Oliveira *et al.* (1999) but lower when compared to Travieso *et al.* (2001) who found a maximum productivity of 0.40 $g \cdot L^{-1} \cdot day^{-1}$ for *A. platensis* cultivated in BG 11 medium.

pH values ranged from 9.6 to 10.5, 9.6 to 10.6 and 9.4 to 10.2 for PSM, SWM and DWWM, respectively. This increase in pH can be correlated to the carbon source consumption. The bicarbonate ions are assimilated by *A. platensis* and subsequently converted into carbon dioxide and carbonate. During the first one is utilization in photosynthesis and excretion of ion carbonate into the medium; an increase in the pH of the system is generated due to the shift of the bicarbonate-carbonate equilibrium.

3.2. Effect of culture medium in lipidic content and fatty acids profile

Total lipid content was 4.54% and 4.69% in biomass cultivated in DWWM and SWM respectively, and did not present significant differences ($p>0.05$). These values are in agreement with those presented by Richmond (1990) but are lower than 6.96%, 6.38% and 7.09-8.03% reported by Oliveira *et al.* (1999), Xue *et al.* (2002) and Tokuşoglu and Ünal (2003), respectively.

Fatty acid profiles found in cells grown in DWWM and SWM presented 12 and 24 types of fatty acids, respectively (Table 3). Lauric (C12:0) and margaric (C17:0) acids were found in higher proportions in

Table 3
Fatty acid composition (%) of *Arthrospira platensis* biomass cultivated in Paoletti Synthetic Medium (PSM), Desalinator Wastewater Medium (DWWM) and Salinated Water Medium (SWM).

Fatty Acid	Culture Media		
	PSM[a]	DWWM	SWM
C4:0		nd	nd
C6:0		nd	nd
C8:0		nd	nd
C10:0		nd	0.38
C11:0		nd	9.40
C12:0*		36.11	15.35
C14:0		nd	0.50
C14:1		nd	1.99
C16:0	30.38	1.26	1.00
C16:1	3.39	nd	0.98
C17:0*		17.29	12.63
C17:1		0.80	0.54
C18:0*	2.76	0.97	12.19
C18:1c	20.92	5.62	5.06
C18:1t*		8.67	0.54
C18:2c	8.69	12.34	11.49
C18:2t		nd	2.14
C18:3γ	13.65	13.09	11.95
C18:3		nd	0.42
C20:0		nd	2.01
C20:1		nd	0.57
C20:2		nd	0.75
C20:3		nd	nd
C20:5		nd	nd
C22:0		1.11	0.48
C22:1		0.60	0.61
C22:6		nd	6.65
C24:0		0.95	0.85
C24:1		nd	0.33
NI		1.87	1.17

[a] the data were taken from Oliveira et al. (1999); nd: not detected; NI: total of fatty acids not identified; *statistically significant differences between SWM and DWWM ($p<0,05$)

biomass of both cultivations, the sum of both representing 53.40% and 27.98% of total content of fatty acids from DWWM and SWM, respectively. The presence of margaric acid in *A. platensis* was reported by Xue *et al.* (2002) and Babadzhanov *et al.* (2004), but in contents of 0.1% and 1.2%, respectively. Studying forty different strains of *A. platensis*, Mühling *et al.* (2005) verified that 16 did not present the C17:0 fatty acid, indicating variability among them. Statistically significant differences were found between stearic (C18:0) and oleic (C18:1t) fatty acids.

The contents of γ-linolenic acid were of 13.09% (DWWM) and 11.95% (SWM) and are in agreement with Oliveira *et al.* (1999). However, these values are lower than those reported by Deshnium *et al.* (2000), who found contents of 20% when cultivating *A. platensis* in Zarrouk medium at 35°C, and Xue *et al.* (2002) who analyzed dehydrated *Arthrospira*. On the other hand, Tokuşoglu and Ünal (2003) found values ranging from 3.64% to 5.52% when cultivating *Arthrospira* in Conway medium at 20°C.

Docosahexaenoic acid (C22:6) occurred only in the biomass cultivated in SWM. Langdon and Önal (1999) and Tadesse *et al.* (2003) did not find this fatty acid in *Arthrospira platensis* biomass. Tokuşoglu and Ünal (2003) found values ranging from 2.30% to 3.51% of total lipid content.

A high content of saturated fatty acid was found in DWWM (57.69%) and in SWM (54.79%) (Figure 3). The saturated and monounsaturated fatty acids contents were higher in desalinator wastewater biomass while polyunsaturated content was higher in salinated water biomass. This can be due to the presence of C18:2t, C18:3 (α-linolenic), C20:2 and C22:6 (DHA) which were found only in the salinated water biomass. Tokuşoglu and Ünal (2003) showed a better distribution of fatty acids in these three groups presenting ranges from 36.40% to 39.29% of monounsaturated, 34.09% to 35.89% of saturated and 22.30% to 25.12% of polyunsaturated. Variations found in fatty acid profiles can be due to different or stressing conditions of cultivation. Walsh *et al.* (1997) mention an increasing in concentration of saturated fatty acids of microalgae under irradiance stress.

In conclusion, the data obtained in this work indicate that *A. platensis* cultivation in Desalinator Wastewater Medium is possible. It produced almost twice (91.49%) as much biomass as the control and presented expected lipid levels for this species. The presence of higher concentrations of saturated fatty acids and lower concentrations of γ-linolenic can be due to cultivation conditions. However, the use of this microalga in Desalinator Wastewater Medium large scale cultivations must be studied in order to determine its economical viability.

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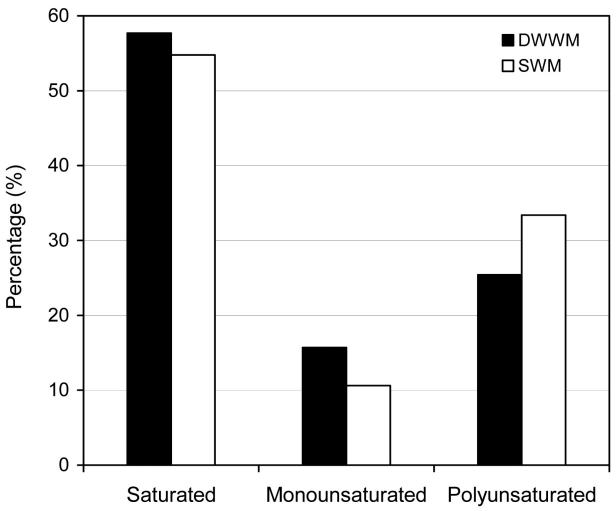

Figure 3
Proportion of saturated, monounsaturated and polyunsaturated fatty acid (% of total lipids) in *Arthrospira platensis* biomass cultivated in Salinated Water Medium (SWM) and Desalinator Wastewater Medium (DWWM).

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