

Osmotic Stress Effect over Carbohydrate Production in a Native Starin of *Scenedesmus* sp.

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ABSTRACT

The production of biofuels is currently presented as a possible answer in the search for sustainable alternatives for the total or partial substitution of fossil fuels. One of the most successful biofuels that have been developed is bioethanol. However, bioethanol production has been limited since it relies on the use of sugar cane or cereals. These materials are important sources of food and their demand as both a biofuel and a foodstuff has led to the price increase and may lead to possible shortages. Our group has focused on searching for native microalgae as sources of carbohydrates and bioethanol, with the goal of finding a sustainable source of bioethanol. Currently, twelve different strains which reach growth rates between 0.7 - 1.8 g/L and present carbohydrate production under osmotic shock conditions have been isolated. In this work, we demonstrate the results obtained with the *Chlorella* sp. [1] strain and the results obtained with the *Scenedesmus* sp. strain. The *Scenedesmus* sp. strain showed an increase in the production from 22 to 650 mg/sugar/g of biomass (dry weight), after 24 hours of osmotic shock with 0.1 M NaCl. The osmolytes which were produced after osmotic shock were identified as sucrose and trehalose, both of which are fermentable. These results demonstrate that this strain, through the photosynthetic pathway and osmotic shock, is a potential source of fermentable sugars.

KEYWORDS

Biofuels; Bioethanol; Microalgae; *Scenedesmus* sp.; Carbohydrates

1. Introduction

Microalgae are oxygenic photosynthetic organisms that are found in diverse environments such as salt water and fresh water. They are even found in environments as extreme as deserts. The majority of these organisms are photosynthetic species, as such they are capable of producing sugars from CO₂ and light [2-4]. Sugar production in these algae has been shown to increase as a response to osmotic shock generated by high salt concentrations, a mechanism known as osmoregulation [5,6].

Much is known about the large quantity of compounds that the algae produces as osmoregulators. These compounds range from simple carbohydrates to amino acids

and even complex compounds such as digenins [7,8].

Some of these osmoregulators synthesized by algae can have commercial uses. This is the case for glucose, sucrose, and trehalose which can even be fermented to produce bioethanol [1].

The production of bioethanol is a technology that has been established in countries like Brazil and the United States [9], and usually is obtained from either the fermentation of starch from different materials such as wheat or direct fermentation of carbohydrates using sugar cane or even from lignocellulose residues [10]. However, the production of bioethanol from primary sources that are food is not sustainable due to inevitable competition with human consumption. Hence, the production of bioethanol from microalgae biomass could be an attract-

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tive alternative. One possible way to take advantage of this alternative is to increase the amount of fermentable carbohydrates in the biomass [11]. The object of this work was to calculate the production of fermentable carbohydrates during osmotic shock with NaCl by an isolated strain of *Scenedesmus*.

2. Materials and Methods

2.1. Isolation and Cultivation

Twelve sites of four aquifers were sampled: Texcoco Lake, Guadalupe Lake, Lake Tabasco, Tames River, Canada. The pH and salinity of each sample was determined.

The samples were enriched with four culture media, according to the salinity of the site of their acquisition, BG11, BG11/saline (25 g/L) and Guillar F/2 media [12], in the culture conditions reported [1]. After replanting two times in liquid media, the algae that demonstrated the largest growth were planted in the solid culture media with 10% agar-agar. Upon isolation the colonies were replanted in liquid media.

2.2. Growth Kinetics

250 mL beakers with an operational volume of 150 mL were inoculated with 10% of a preculture of each isolated microalgae. Each culture was grown for ten days in the same conditions. Growth was monitored through absorbance (at 600 nm) in a Genesis colorimeter 10 UV and by dry weight every 24 h.

2.3. Analysis of Intra- and Extracellular Sugars

The determination of extracellular sugars was done through the separation of the supernatant followed by the Dubois method [13].

To determine intracellular sugars, a modified Muller method was used for the extraction [1], followed by quantification through the Dubois method.

The sugars were identified through HPLC, through a method described by Bremauntz *et al.* [1].

2.4. Effects of Stress on Growth and the Effect of Osmotic Shock on the Production of Sugars

To test the effects of NaCl on growth, the strains were cultivated in BG11 with 0.2, 0.4 and 0.6 M NaCl under the previously described conditions. The intra and extracellular sugar concentrations were determined during the analysis of the growth kinetics.

The osmotic shock showed effects as previously described in Bremauntz *et al.* [1].

3. Results and Discussion

3.1. Isolation and Growth

The isolation of twelve strains, which were characterized by optical microscopy, was accomplished. Of the twelve strains, three were identified as cyanobacteria and nine were identified as eukaryotic microalgae. Seven of the strain showed growth greater than 1 g/L (Table 1).

Growth kinetics showed *Cystococcus* sp, *Synechocystis* sp, *Scenedesmus* sp, and *Chlorella* sp. had the largest amount of growth (Figure 1). All of these strains showed their maximum growth between days nine and eleven. Specifically, *Scenedesmus* sp. presented a maximum growth of 1.58 g/L with a μ_{max} of 0.0059 [14]; however, other authors reached values up to 1.069 (h^{-1}), and growth of 2.63 g/L [15,16]. From this we are able to determine that the BG11 culture media and that cultivation conditions were appropriate for the growth of *Scenedesmus* sp.

3.2. Production of Sugars

For the production of sugars the efficiency of production of each isolated strains was determined (mg of sugar produced per liter of culture). These determined values established that the strains with the greatest efficiency were: *Chlorella*, *Chrysoisphaera*, *Trebouxia*, and *Scenedesmus*, with values of 29.11, 6.22, 5.44, and 5.37 mg/L, respectively (Figure 2).

For the four strains the intra- and extracellular sugars

Table 1. Identification of algae that presented the most growth and their biomass production.

Order y Genus	Origen	Maximun Biomass (g/L)	μ_{max} (h^{-1}) ^a
<i>Oscillatoriales Lyngbya</i>	Texcoco Lake	2.56	0.928
<i>Chroococcales Cystococcus</i>	Texcoco Lake	1.76	0.126
<i>Chroococcales Synechocystis</i>	Texcoco Lake	1.74	0.301
<i>Chlorococcales Scenedesmus</i>	Tamesi River Canada	1.58	0.479
<i>Chlorococcales Chlorella</i>	Guadalupe Lake	1.36	0.628
<i>Oscillatoriales Oscillatoria</i>	Tamesi River Canada	1.24	0.313
<i>Chroococcales Synechocystis (T)</i>	Camarón Lake, Tabasco	1.18	0.413

^a μ_{max} at 25°C, medium BG11, aereation 4 vvm e light intensity 100 μ moles de fotons/m²s.

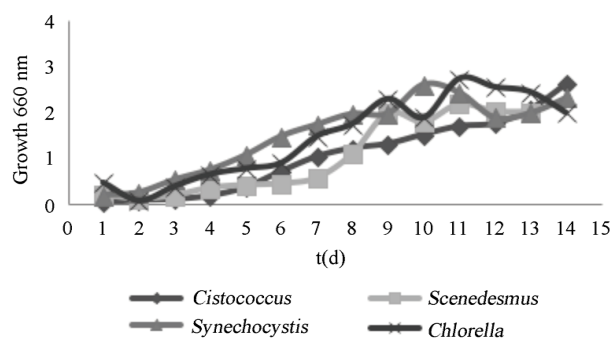


Figure 1. Growth of isolated strains in BG11 medium at 25°C, 4 vvm, y 100 μmol photons/m².

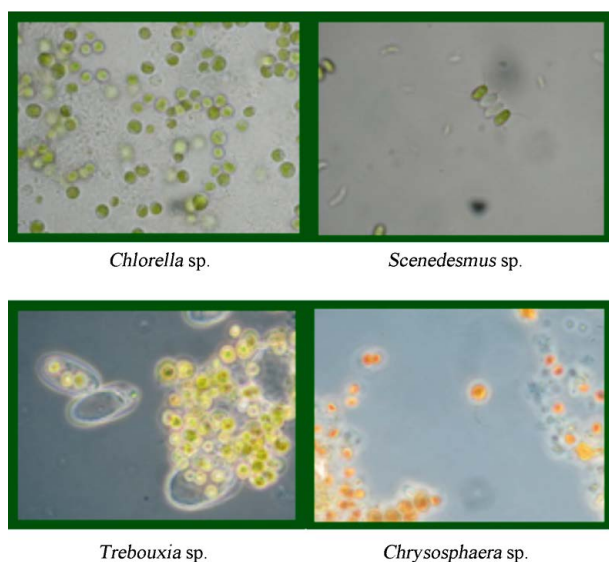


Figure 2. Optical Microscope images (100×) of the four strains with the greatest sugar production efficiency.

concentrations were determined. For *Scenedesmus* sp. only intracellular sugars, were found. The intracellular sugars a concentration of 5.48 mg/L, a sugar content of 38% g/g which is a value greater that that reported by Miranda *et al.* [11]. Miranda *et al.* reported that in studies of geometric and design optimization of bioreactors for *Scenedesmus* sp. reached a production of sugars of 29%; however, results reported by Shih-Hsin *et al.* [15] reached carbohydrate production of 46.65% with nitrogen limitations in the media. It has been observed in the literature that the formulation of the culture media as well as the quantity and intensity of the light used and even the design of the bioreactor can influence the production of sugars, lipid, proteins, and even the morphology of the genus *Scenedesmus* [17].

3.3. Effect of the NaCl Concentration on Growth

In all three NaCl concentrations used the growth of *Scenedesmus* sp. was inhibited (Figure 3). In other studies, *Scenedesmus incrassatulus* Bohl, growth was decreased

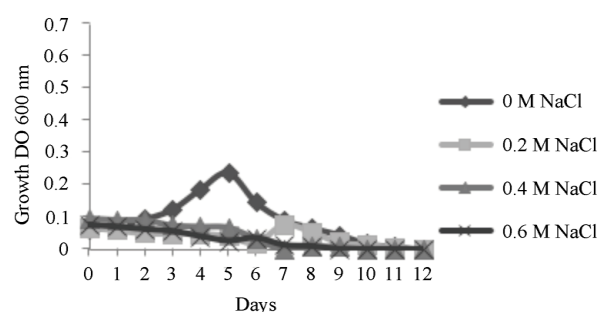


Figure 3. Effects of NaCl concentration on the growth of *Scenedesmus* sp.

with a NaCl concentration of 0.175 M [18], showing a reduction in the fixation of carbon dioxide and an increase in the production of proline. On the other hand, Demetriou *et al.* [19], reported inhibited growth, by measuring the production of pigments in *Scenedesmus* when the concentration of NaCl in the media was greater that 0.2 M; however, the production of sugars and proteins was increased when the concentration of salt was increased to 0.4 M [20]. Sheng Shu and his group completed studies with salt near metabolic levels with NaCl concentrations of 0.075 M, demonstrating that it affects photosynthetic metabolism. They found that osmotic stress inhibits the maximum production of photochemical PSII (F_v/F_m), through the competition of the receptor site of PSII. Through the addition of saline solution the destruction of thylakoids in the chloroplasts was observed [21]. This was observed through the growth experiments where after 24 h after inoculation in salt media, coloration was lost and the media went from green to white. Authors such as El-Sayed have reported a decrease of 50% of the chlorophyll content after immersion of a strain of *Scenedesmus* sp. in 1.5 M NaCl [22].

3.4. Effects of Osmotic Shock on Sugar Production

When the *Scenedesmus* sp. strain underwent osmotic shock an increase in the extracellular sugar production rate from 24 h after the shock 22 to 650 mg/g p.s. as observed 24 h after the shock (Figure 4). The osmotic solution had a concentration of 0.1 M. However, after 48 h or exposure, the increase in sugar production slows notably, where the production reaches a rate of 235 mg/g p.s. This represents a 10 times increase in the rate of production of sugars when compared to the production rate without an osmotic shock treatment and after 72 h of treatment. This matches results from experiments on the effects of salinity on the growth of this strain, where it was shown that NaCl inhibited growth after 72 h (Figure 3).

These results differ from those obtained by other authors for other species of *Scenedesmus* [22,23]. Kirrolia reported an increase in total carbohydrates from 0.04884

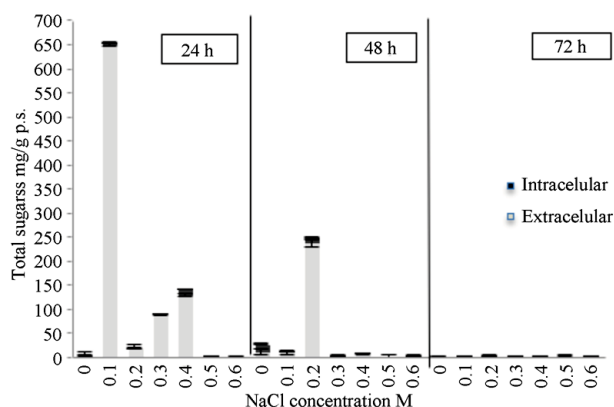


Figure 4. The effect of osmotic shock on the production of total sugars in *Scenedesmus* sp. in media BG11. Intracellular sugars and extracellular sugars.

to 0.06237 mg/ml in an isolated strain of *Scenedesmus quadricauda* in an osmotic shock solution of 0.2 M NaCl during the course of 10 days.

Analysis of both intracellular and extracellular carbohydrates (Figures 5 and 6) showed that the concentration of sucrose and trehalose increased notably in the extracellular fraction. There was 76% increase in sucrose concentration and a 37% increase in trehalose concentration. In the intracellular fraction, sucrose concentration increased 92% and there was no increase in trehalose concentration.

These results indicate that the principal osmoregulator produced by this strain of *Scenedesmus* sp. is sucrose and trehalose, while to a lesser extent than sucrose, also functions as an osmoregulator. The discovery of trehalose as an osmoregulator in *Scenedesmus* sp. has not been reported by any other group. Other groups have reported increases in the production of sucrose and trehalose in the initial phase of osmotic shock followed by stabilization after 48 h of the shock for generic *Scytonema* [24]. They also mentioned that the production of trehalose contributes to the stabilization of proteins in the cell membrane.

4. Conclusions

This work demonstrates that osmotic shock of *Scenedesmus* sp. with a concentration of 0.1 M can increase the production of carbohydrates 24 h after the shock. The carbohydrates produced are sucrose (0.464 mg/g p.s.) and trehalose (0.112 mg/g p.s.). These carbohydrates were obtained in amounts larger than previously reported, or in the case of trehalose, not having been previously reported.

These simple sugars were highly attractive as possible as prime material for the production of bioethanol through simple fermentation. Direct fermentation of these sugars has been shown to be more efficient than

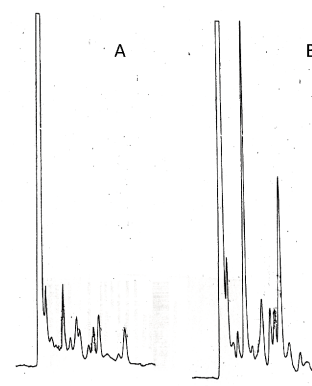


Figure 5. HPLC chromatograms of extracellular extract in *Scenedesmus* sp. without osmotic shock treatment (A) and with osmotic shock treatment of 0.1 M after 24h (B).

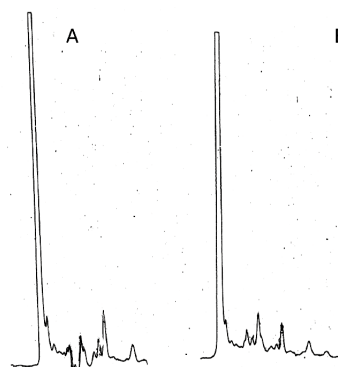


Figure 6. HPLC chromatograms of intracellular extract in *Scenedesmus* sp. without osmotic shock treatment (A) and with osmotic shock treatment of 0.1 M after 24h (B).

strategies proposed by other groups who have tried producing bioethanol through an increase in cellulose/starch in the membrane of various microalgae, including *Scenedesmus* [17,25-27].

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