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Yield and Quality of Cyanobacteria *Spirulina maxima* in Continuous Culture in Response to Sodium Chloride

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ABSTRACT

The objective of this article is to determine the yield and chemical composition of the biomass of *Spirulina maxima* cultures incubated in different concentrations of sodium chloride in steady state.

Index Entries: *Spirulina maxima;* cyanobacteria; algal biomass.

INTRODUCTION

Spirulina, a filamentous alga, has been used as a model organism for outdoor cultivation of algal biomass as a source of protein and chemicals (*1–5*). *Spirulina* has been the subject of intense ecological and physiological studies necessary for development and improvement of large-scale applications (*6,7*). Laboratory studies have been limited to growth kinetics (*8,9*) and environmental factors (*10*). In the United States, *Spirulina* has been recently recommended (*11*) and is under investigation (*12*) for the revitalization of air, waste processing, and production of food for the space program Controlled Ecologial Life Support System (CELSS). Cyanobacteria occupy a unique taxonomic position, since they combine an autotrophic mode of growth that is common to eukaryotic plant cells with a metabolic system that is generally regarded as bacterial, rather than plant-like.

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Increasing the efficiency of the yield of algal culturing in a bioregenerative life-support system is one of the primary concerns of a CELSS. In order to design and operate such a culture system, it is necessary to understand how the macroparameters of a culture system, e.g., productivity and quality, are related to the physiological aspects of the algal culture. Photosynthetic organisms physiologically adapt to variations in growth conditions. In *Anacystis nidulans*, increasing salt concentrations led to decreased photosynthesis (*13–15*). Fresh-water and marine cyanobacteria accumulate sucrose, trehalose, or glucosylglycerol, while hypersaline strains accumulate mainly glycine-betaine (*16–18*). Photoadaptive responses have also been characterized by changes in their metabolic accumulation products and pigments (*19–26*). The objective of this work was to determine the yield and chemical composition of the biomass of *Spirulina maxima* cultures incubated in different concentrations of sodium chloride in steady state.

MATERIALS AND METHODS

Culturing

Cyanobacteria *S. maxima* (UTEX LB 2342) was cultured in Zarrouk medium (*26,27*). BRL's Airlift Fermenter was used for culturing the alga. It consists of a reactor vessel (2 L). The culture was aerated with air at a flow rate of 1000 mL/min. Two peristaltic pumps were used, one for feeding the fermenter with fresh medium, and the second for removal of the overflow. The pH of the medium remained almost unchanged at 9.3–9.4 in all experiments by using 4*N* sodium hydroxide. The temperature of the culture was maintained at 35°C. Batch cultures were used for inoculating the reactor. The alga was precultured in small bottles (250-mL capacity) containing 100 mL medium at 35°C for 4–5 d. The precultures were set up under the same conditions of the reactor experiment. The alga was evaluated for growth rate and yield in response to different concentrations of sodium chloride.

Principles of Continuous Culturing

A continuous culture is a constant-volume cell system in which the rate of cell growth is controlled by the dilution rate of nutrient solution. The constant volume was maintained by ensuring that the rate of culture outflow equaled the inflow rate of the fresh medium. When steady-state conditions were reached, there existed a constant cell number and biomass within the vessel, since the specific growth rate equaled the dilution rate. The fresh medium was kept refrigerated to avoid contamination. Sterile medium was pumped from a reservoir into the vessel by a peristaltic pump. The outflow from the culture was collected in sterile bottles

plugged with sterile cotton-wool filters. The culture was assumed to be in steady state when the cell concentration remained constant for at least 96 h after the initial flow rate was adjusted. In steady-state culture: Dilution reat (D) = F/v, where F is inflow rate (mL/h), v is culture volume (mL), and D is growth rate.

Analytical Methods

Harvesting of Cells

Cells were collected by filtration, washed with buffer solution (pH 8), diluted to known volume, and processed for further analysis.

Dry Weight (Dry Wt) Measurements

A volume from the culture was filtered, dried for 4 h at 80°C in previously dried, preweighted filter paper, and weighed after cooling in a desiccator.

Ash-Free (AF) Dry Wt

After recording the dry wt, the dried cells were ashed at 500°C for 2 h. The difference between dry wt and ash wt gave the organic wt of the sample.

Chemical Analysis

The total carbohydrates and proteins were determined according to the methods of Kochert (*28*) and Lowery et al. (*29*), respectively. Total lipid of the cells was extracted and quantified according to the Bligh and Dyer method (*30*).

Productivity

Productivity is defined as the product of dry wt (g/L) of the culture and the overflow rate (L/h).

Oxygen (μM)

Oxygen evaluation of the algal cells was measured by using a Clark Oxygen Electrode according to Graves and Greenbaum (*31*). All of the above analytical data were related to the organic wt of the algae. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Growth Characteristics of *Spirulina* Maintained in Steady State as a Function of Sodium Chloride Concentrations

Experiments were incubated at various concentrations of sodium chloride. The results are presented in Table 1.

Table 1
Growth Characteristics of *S. maxima* Maintained in Growth Medium of Different Concentrations of Sodium Chloride in Steady-State Cultures (Light Intensity 30 $\mu E/m^2/s$

OD	NaCl concentration, *M*	Dilution rate, h	Total dry wt, g/L	Productivity, g/h/L	Chlorophyll, mg/L	Oxygen, μM O_2, dry wt/h
0.490	0.0	0.016	0.249	0.0039	6.48	900
0.490	0.1	0.016	0.257	0.0041	6.60	1000
0.490	0.2	0.016	0.267	0.0042	8.46	1300
0.485	0.5	0.010	0.245	0.0026	6.74	1800

The dilution rate remained stable with increasing concentration of sodium chloride in growth medium up to 0.2*M*. However, when increasing the concentration of sodium chloride beyond 0.2*M*, the dilution rate started to decrease. These data show that the growth of the culture or cell division was almost inhibited with increasing the concentration of sodium chloride in the growth media.

The total dry wt of the cells increased when the concentration of sodium chloride increased up to 0.2*M*. Then it started to decrease when increasing the concentratioan of sodium chloride beyond 0.2*M*. The productivity of the cultures increased with increasing the dry wt and then decreased.

The total chlorophyll increased with increasing sodium chloride concentration up to 0.2*M*, and then started to decrease with increasing sodium chloride (Table 1). Erdmann et al. (*32*) found decreased photosynthesis and chlorophyll a contents in *Microsystis firma* and *Synechocystis sp.* during the first days after a salt shock. In *Anacystis nidulans*, increasing salt concentrations led to decreased photosynthesis (*13–15*).

The results also show the enhancement of oxygen evolution with increasing concentration of sodium chloride (Table 1). The results coincide with the previous reports (*17*). The role of respiration during adaptation to salt in cyanobacteria has been discussed extensively (*33–35*), and it is generally agreed that respiration is directly involved in maintaining low intracellular sodium concentrations (*36,37*). Salinity increases respiration in plants (*38*).

Chemical Composition of Cultures as a Function of Sodium Chloride Concentration (Fig. 1)

It was noted that the total protein content decreased with increasing concentration of sodium chloride, whereas the total carbohydrate increased. The increase in the total carbohydrate was at the expense of the low protein content. The total lipids of the cultures decreased somewhat. Apte and Bhawat (*39*) observed diminished methionine incorporation by *Anabaena* strains during salt stress, whereas protein synthesis was almost

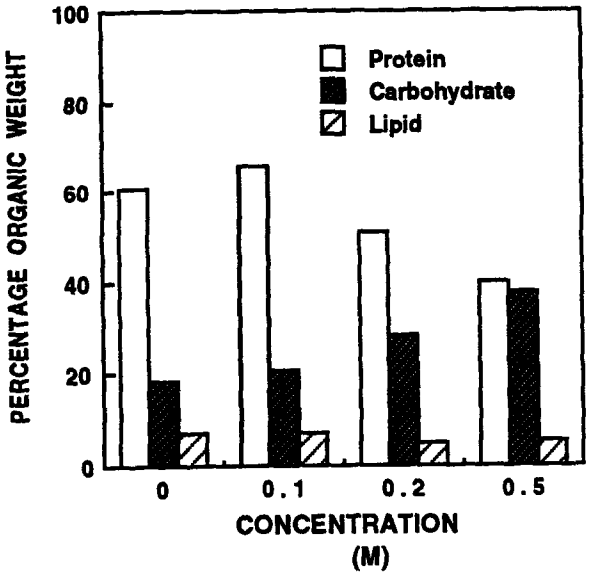

Fig. 1. Growth characteristics of *S. maxima* in culture maintained at steady state as a function of sodium chloride concentration: chemical composition.

blocked in *Synechocystis* sp. (*40*). Halotolerant and halophilic microorganisms accumulate organic osmolites of low molecular weight to counterbalance the decrease in the extracellular water potential. Such osmoregulatory mechanisms have been characterized in a large number of microorganisms, including photosynthetic bacteria and algae, and cyanobacteria (*41–43*). A survey of major organic solutes by 13C NMR spectroscopy revealed that fresh-water and marine cyanobacteria accumulate sucrose, trehalose, or glucosylglycerol, whereas hypersaline strains accumulate mainly glycine-betaine (*16–18*). Rawson et al. (*44*) and Myers et al. (*45*) have concluded it is unlikely that the primary inhibitory effects of salinity on growth are attributable to effects on photosynthetic processes. Although the primary limiting process for salt toxicity remains elusive, the protein turnover during salt stress could be important in the toxicity of plants to saline condition. Hurkman et al. (*46*) have noted a change in the levels of translatable mRNA with salt treatment, indicating altered gene regulation by salt stress. Salinity inhibits general protein synthesis (*47*), induces specific stress proteins (*40*), and increases chlorophyll (*48*) and protein degradation by stimulating protease activity (*49*).

The data we present suggest that carbohydrates that are accumulated by cyanobacteria as a reaction to high concentration of sodium chloride are synthesized as a reaction to water stress as well, and may play a role in the mechanism of drought resistance of many cyanobacteria. In our earlier study (*26*), *S. maxima* responded to changes in growth irradiation levels by altering the quality of yield. The protein of the algal cells increased with decreasing light irradiation of the culture. At low-growth

irradiate levels, the photosynthetic response was associated with increased light-utilization efficiencies. Increasing the light irradiation was associated with increasing the total carbohydrate of the cultures. The results of this work indicate also that nutritional quality of an algal product can be controlled during the production process to the extent that the chemical composition of that species is variable (*50*).

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