

**MANIPULATING CYANOBACTERIA: SPIRULINA FOR
POTENTIAL CELSS DIET**Sp-51
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ABSTRACT

Spirulina sp. as a bioregenerative photosynthetic and an edible alga for spacecraft crew in a CELSS, was characterized for the biomass yield in batch cultures, under various environmental conditions. The partitioning of the assimilatory products (proteins, carbohydrates, lipids) were manipulated by varying the environmental growth conditions. Our experiments with Spirulina have demonstrated that under "stress" conditions (i.e. high light $160 \text{ uE m}^{-2} \text{ s}^{-1}$, temperature 38°C , nitrogen or phosphate limitation; 0.1 M sodium chloride) carbohydrates increased at the expense of proteins. In other experiments, where the growth media were sufficient in nutrients and incubated under optimum growth conditions, the total proteins were increased up to almost 70% of the organic weight. In other words, the nutritional quality of the algal could be manipulated by growth conditions. These results support the feasibility of considering Spirulina as a subsystem in CELSS because of the ease with which its nutrient content can be manipulated.

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Introduction

Pursuit of our national goals in space exploration will eventually require man's long-duration tenancy of celestial vehicles and planetary bases. Requirements for life support could be met through expenditure of stored supplies and by regeneration and reuse of the waste products of human metabolism. The logistics necessary for regeneration for extended space missions are well documented (1). The primary source of all man's food and organic raw materials is solar energy. Conventional food sources consist of higher plants and animals. Unconventional food sources for human consumption are photosynthetic algae and bacteria and non-photosynthetic bacteria, yeast and fungi. Conventional food sources are highly palatable, but require a long time to produce. Algae, on the other hand, grow rapidly; their metabolism can be controlled; they produce a high ratio of edible to nonedible biomass; and their gas-exchange characteristics are compatible with human requirements. The biological components of Controlled Ecological Life Support System (CELSS) will serve as subsystems for the revitalization of air for the long term space flight.

Cyanobacteria single cell protein (SCP) has been used as a food source in various parts of the world (e.g. Mexico, China and Africa) since ancient times; in fact, dried cyanobacteria and cyanobacterial tablets are now sold in health food stores in Japan, North America and Europe because they are recognized for their nutritional value. The nutritional quality of all cyanobacteria which have been tested appears to be very high. The protein of S. maxima is easily digestible and approximately 65% of the protein is assimilable.

In order to evaluate the potential of Spirulina for a CELSS diet, it is essential to have background information on the environmental tolerance of the species. The purpose of this project was to evaluate the chemical composition of Spirulina under different growth conditions in batch cultures. This paper presents the results of one year's work.

Materials and Methods

Spirulina maxima (UTEX LB 2342) was cultured in Zarrouk medium (3). For mass culturing, algal cells were grown in bottles. Cultures were illuminated continuously by placing them in front of a bank of two cool white fluorescent lamps (40W). Light irradiation, measured at the surface of the culture bottles, was $80 \text{ uE m}^{-2} \text{ s}^{-1}$. The cultures were grown in a water bath kept at 29-30°C by the use of a heater-thermostat combination. Cultures were grown under different conditions. Cells were collected after five days growth and analyzed for total proteins, carbohydrates and lipids.

Results and Discussion

Physiological Characterization of Cultures, under Stress Conditions: The results of analysis were expressed on the basis of organic weight (Ash Free Dry Weight: AFDW) and are represented in Table 1.

Light Irradiance and Temperature: Increasing the light irradiance to $120 \mu\text{E m}^{-2} \text{s}^{-1}$, led to an increase in the total carbohydrate content and a decrease in protein content: *S. Maxima* 19.58%, 29.06%. Increasing the temperature of culture incubation to 38°C influenced the composition of the strain in a similar manner to the light irradiation experiment: *S. maxima*, 45.28%, 18.75%, for protein and carbohydrates, respectively. The culture produced a low percentage of lipids when grown in high temperature experiments. **Nutrient Limitation:** Media limited in nitrate-N and phosphate-P favored the accumulation of carbohydrate rather than protein. Nitrate and phosphate limited cultures: *S. maxima* had 37.52%, 35.21% carbohydrate and 21.56%, 41.25% protein. When the cultures were transferred to media limited in nitrogen and phosphate, cultures changed in color from blue to yellow-green. N-limited cultures of *Anacystis nidulans* (7), and P-limited cultures of *Oscillatoria agardhii* (8), showed elevated levels of polysaccharide storage. **Sodium Chloride:** As Zarrouk (3) media were enriched with 0.1M and 0.5M NaCl, the carbohydrate content of the cells increased, when compared to that of the control (Zarrouk: 0.01M NaCl), to 26.24%, 36.73% in *S. maxima*. On the other hand, the total protein decreased respectively to 52.62%, 45.64% in *S. maxima*. The lipid percentages showed little increase when compared to those of the complete media (control). **Bicarbonate:** When bicarbonate concentration of Zarrouk media was reduced to one quarter (4.g/L), the culture showed much difference in the chemical composition as compared with the control media except their yield was somewhat below the control. The carbohydrates increased to 38.53% when 0.03% CO_2 in air was used for aeration and to 40.23% when 1% CO_2 air was used for aeration.

It can be concluded that through manipulating environmental conditions of the algal growth, one can modify the photosynthetic products. Thus, *Spirulina* can be, through manipulating growth factors, used as palatable diet compared to higher plants.

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Table 1. Molecular Composition of *Spirulina maxima*

Growth Conditions	% Organic Wt. (AFDW)		
	Protein	Carbohydrate	Lipids
*Sufficient Nutrients	69.75	11.5	4.68
High Light (160uEm-2s-1)	29.06	19.58	3.56
High Temperature (38°C)	45.28	18.75	3.75
N-limited	21.56	37.52	4.68
P-limited	41.25	35.21	5.20
Sodium Chloride 0.1M	52.62	26.25	4.68
0.1M	45.64	36.73	7.52
Bicarbonate (4.4g/L) (0.03% CO ₂)	45.67	38.53	6.22
(1% CO ₂)	43.52	40.23	6.53

*Experimental conditions were:
 temperature 30°C; light irradiance 80uEm⁻²s⁻¹;
 air flow rate 300 ml/min.;
 The values shown are averages of four independent determinations.