APPLICATION OF PHOTOSYNTHETIC N_2-FIXING CYANOBACTERIA TO THE CELSS PROGRAM

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ABSTRACT

The feasibility of using photosynthetic microalgae (cyanobacteria) as a subsystem component for the CELSS program, with particular emphasis on the manipulation of the biomass (protein/carbohydrate) has been addressed. Using factors which retard growth rates, but not photosynthetic electron flux, the partitioning of photosynthetically derived reductant may be dictated towards CO_2 fixation (carbohydrate formation) and away from N_2 fixation (protein formation). Cold shock treatment of fairly dense cultures markedly increases the glycogen content from 1% to 35% (dry weight), and presents a useful technique to change the protein/carbohydrate ratio of these organisms to a more nutritionally acceptable form.

INTORDUCTION

The use of biological components in the CELSS program as subsystems for air revitalization, waste processing or the production of food has been proposed for long-term space flight /1/. Employment of algae (particularly cyanobacteria), which generate biomass from relatively simple components (minerals and light) and their ability to fix atmospheric N_2, make them an attracive component for incorporation into the CELSS program. However, if use of cyanobacteria is envisaged as a major food source, manipulation of the composition of the biomass is required. Cyanobacteria (aptly described as single cell protein) are approximately 50% protein, with varying levels of carbohydrates, 1% (dry weight) in freshwater non-nitrogen fixers /2/, and up to 30% in some nitrogen fixing strains /3/. The average human nutritional daily requirement is for 20% protein and 50% carbohydrate (table I).

MATERIALS AND METHODS

Nostoc muscorum was grown in BG11 medium minus nitrate and Synechoccus 6311 was grown in KMC medium, in a 2 litre Bethesda Research Laboratories Airlift Fermentor at 30°C unless otherwise stated, 150 u $Es^{-1}m^{-2}$ light (using Bethesda Research Laboratories 2201 LB day light white 300-700nm) with an airflow rate of 2 litres/min, supplemented with 0.5% CO_2 . 200 ml aliquots were withdrawm daily, the fermentor volume made up by addition of 200ml of sterile medium. Cells were centrifuged at 10,000xg/10 min and resuspended to 2 ml in BG11 or KMC medium supplemented with 10 mM Tes buffer pH 7.0.

O_2 evolution was monitored polarographically in BG11 or KMC plus 10mM Tes pH7.0 with a cell density equivalent to 1-2 ug chlorophyll/ml. Nitrogenase activity was determined in whole filaments by monitoring acetylene reduction using a Varian Model 3700 gas chromatograph fitted with a Poropak T column. Cells (3 ug chlorophyll in 3 ml) were assayed in a 5 ml vial under air plus 10% (v/v) C_2H_2 in a shaking waterbath at 28° under 50uEs^{-1}m^{-2} light.

Light intensities were measured using a Li-Cor inc. integrating quantum/radiometer/photometer Li-188B, with a Li-190SB quantum sensor. Glycogen was extracted and determined colorimetrically by the method of Van Handel /4/.

We have previously reported on the affects of salt shock on cellular glycogen content of a freshwater non-nitrogen fixing cyanobacterium,

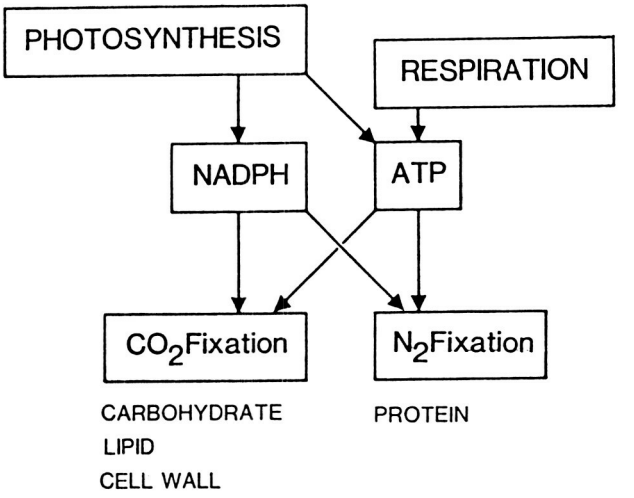

Fig.1 Scheme for the sources of reductant and ATP for CO_2 and N_2 fixation.

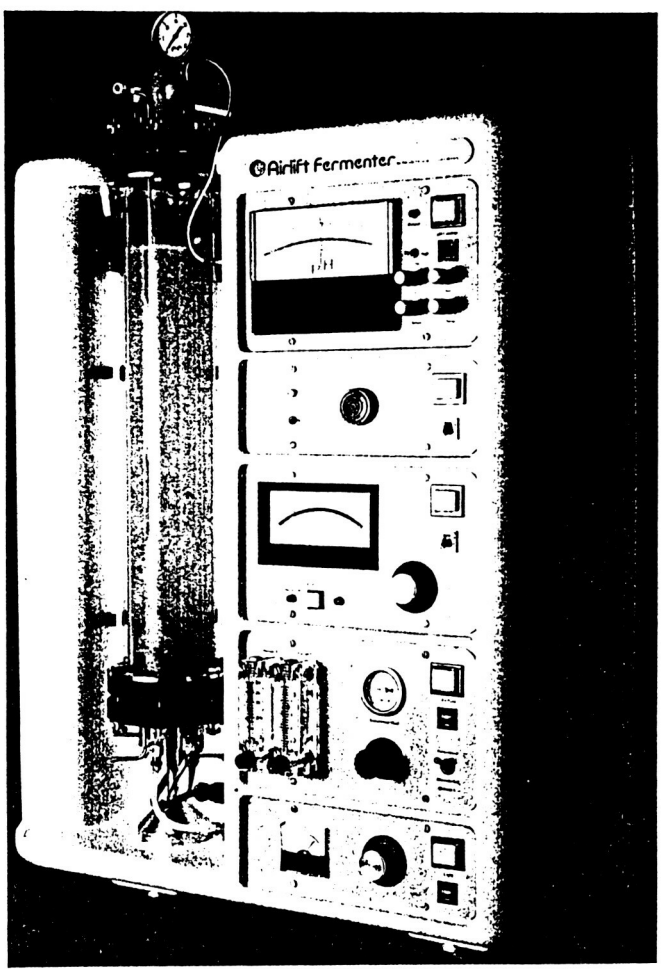

Fig.2 A 21 Bethesda Research Laboratory Air-Lift fermentor.

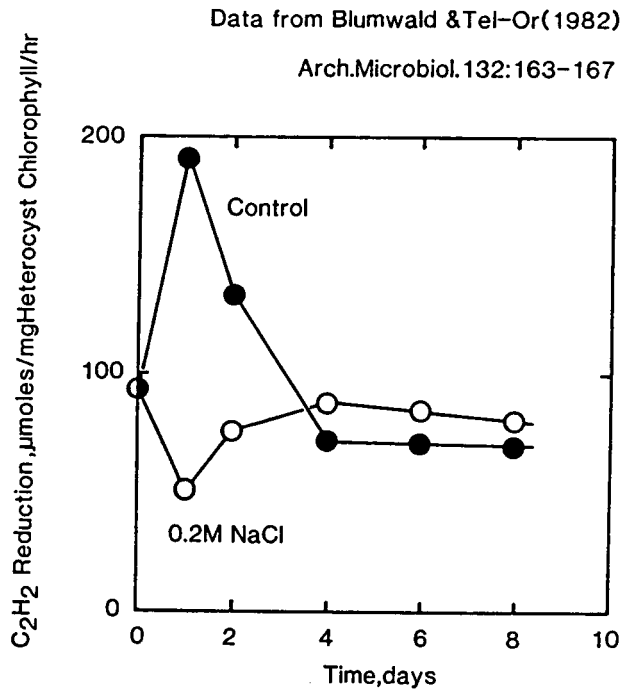

Fig.3 Nitrogenase activity during exposure to salinity of Nosoc muscorum.

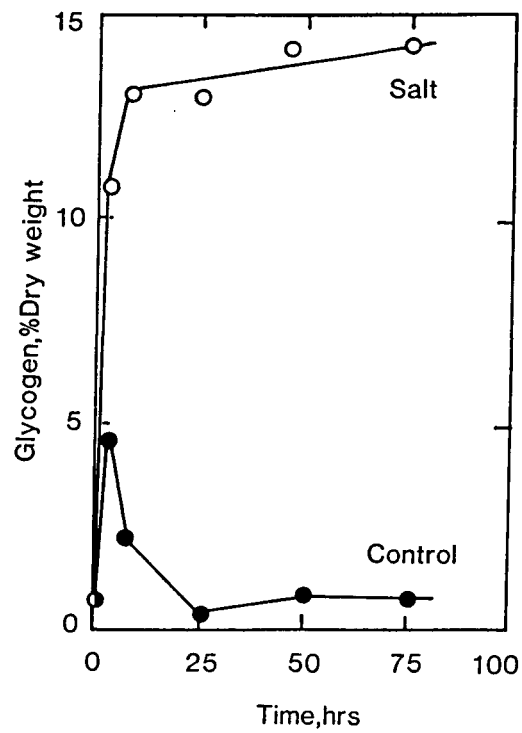

Fig.4 Cellular glycogen content of Synechococcus 6311 during growth under saline conditions.

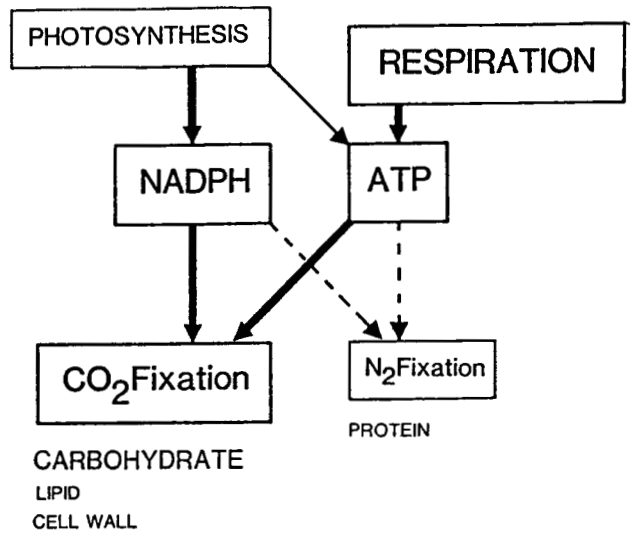

Fig.5 Redirection of photosynthetically derived reductant under stress conditions.

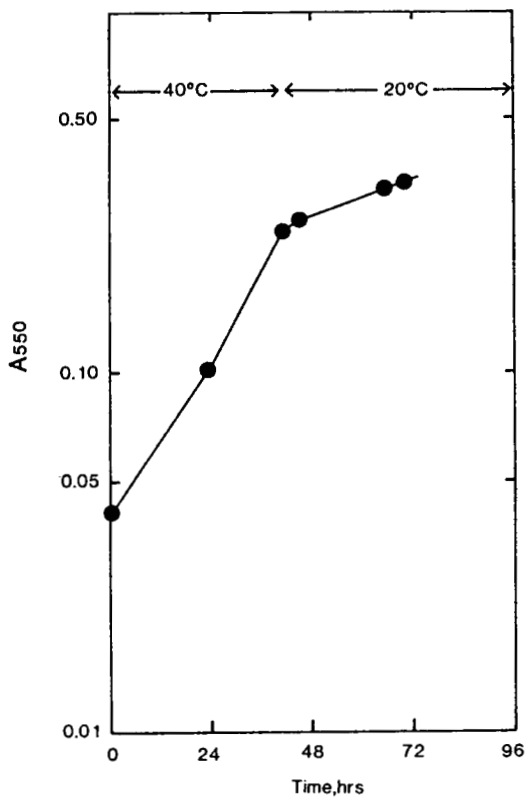

Fig.6 Effect of temperature on the growth of Synechococcus 6311.

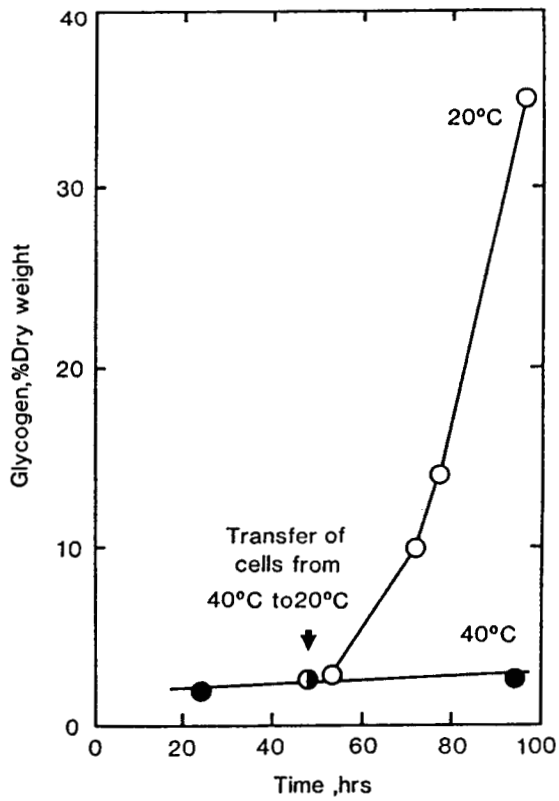

Fig.7 Effect of temperature on the glycogen content of Synechococcus 6311.

TABLE I Average Daily Requirements For Humans

Food Type	Daily Requirement*	
	Grams	%
Protein	330	21
Carbohydrate	825	52
Lipid	429	27
TOTAL	1584	100

Assumptions:

- Body weight of 70kg (150 lbs.)

TABLE II Rates of N_2 Fixation and Photosynthesis During Growth in Airlift Fermenter

	Maximal Rate*	Fermentor Rate
Photosynthetic O_2 evolution (umoles O_2/mg·chlorophyll/hr)	300	72+
N_2 fixation (umoles C_2H_2 reduced/mg·chlorophyll/hr)	25 (air) 46 (N_2)	43#

* direct measurements from diluted samples

\+ calculated from total CO_2 plus N_2 fixed (as carbohydrate and protein)

\# calculated from the total N_2 fixed as protein

TABLE III Factors Affecting The Carbohydrate Content Of Synechococcus 6311

Growth Condition ($30^{o}C$)	Generation Time (hr.)	Respiration (umoles O_2/mg chl/hr.)	Glycogen (% Dry Wt.)
Control	19.7	2.9	0.9
+0.5M NaCl	24.0	20.0	12.0
+60ppm SeO_4^{2-} (SO_4^{2-} = 14ppm)	32.0	8.0	2.2
Grown at $20^{o}C$	33.1	41.3	35.0

Photosynthesis (after 48 hrs) is at control rates in all cases

TABLE IV Effect Of Growth Temperature On Synechococcus 6311

Growth Temp. (^{o}C)	Generation Time (hr.)*	Photosynthesis (umoles O_2/mg chl/hr.) * #	Respiration (umoles O_2/mg chl/hr.) * #	Glycogen (% Dry Wt.)
40	20.1	180.7	3.6	1.7
30	19.7	178.2	2.9	0.9
20	33.1	198.8	41.3	35.0

* Determintions made on 72 hr culture

Assayed at growth temperature (Q_{10} (photosynthesis)=35%, Q_{10} (respiration)=68%, between $30^{o}C$ & $40^{o}C$ for $30^{o}C$ & $40^{o}C$ grown Cultures)

Synechococcus 6311 /2/, and we have continued to use this organism, in addition to the nitrogen fixing cyanobacterium Nostoc muscorum. Both CO_2 fixation and N_2 fixation utalize the pools of photosynthetic reductant and ATP (fig. 1), and we have investigated environmental facators such as salinity, growth inhibition and temperature effects on the distribution of the reductant between N_2 or CO_2 fixation, to determine the feasibility of using such effects to direct the photosynthate to one particular pool of macromolecule.

RESULTS

Measurements of photosynthetic electron transport (O_2 evolution) and nitrogenase activity (C_2H_2 reduction) in Nostoc muscorum show that only 8% of maximum electron transport ($4e^-$ per O_2 evolved, $2e^-$ per C_2H_2 reduced) is utalized for N_2 fixation (Table II) and one would expect high levels of CO_2 fixation to occur. However, estimations of rates of actual photosynthesis during growth in an airlift fermentor (fig. 2), calculated from the rates of carbohydrate and protein formation, show that the total photosynthetic rates are much lower, probably due to a cut off effect, the attenuation of light by the density of the culture during growth. It is interesting to note that, even under this reduced photosynthetic activity, the rate of N_2 fixation is maximal (Table II). Clearly, N_2 fixation, under these conditions, has priority for photosynthetic reductant, and is probably the limiting factor for growth under these conditions.

SALINITY EFFECTS

Blumwald and Tel-Or have reported the effects of salinity or N_2 fixation (5) which is clearly inhibited during the first 2 days (fig. 3). Under similar conditions, using Synechococcus 6311, we have reported the marked accumulation of glycogen (/2/ & fig. 4). In both cases, salt resulted in a retardation of growth /2,5/ and to a much lesser degree, photosynthesis /2/. Under conditions where growth rate is reduced more than photosynthesis, reductant is directed towards CO_2 fixation rather than N_2 fixation (fig. 5).

FACTORS AFFECTING CELLULAR GLYCOGEN CONTENT

Table III presents the effects of several regimes employed to reduce growth rates, while not affecting photosynthesis. Both NaCl and selenate (a competative inhibitor of sulfur metabolism) reduce growth rates and stimulate glycogen content. The greatest effect is observed, however, when cells are grown at below optimal temperature (20^oc).

TEMPERATURE EFFECTS

Growth of Synechococcus 6311 at different temperatures is shown in table IV, and while photosynthesis is relatively unaffected by growth temperature, the generation time is almost doubled and glycogen content increased by a factor of 39 at 20^oc.

Although this presents a useful tool for the modification of biomass towards carbohydrate, growth rate (and therefore total biomass production) is slow at 20^oc. Therefore, cultures of Synechococcus 6311 were grown at 40^oc for two days, the temperature then being reduced to 20^oc. Fig. 6 demonstrates the rapid inhibition of growth during cold treatment of an already dense culture of Synechococcus 6311. Analysis of samples taken before and after transferto 20^oc, show a marked accumulation of glycogen during the period of growth inhibition (fig. 7).

CONCLUSIONS

Results so far indicate that temperature modification is the most effective tool for the manipulation of the biomass in favor of glycogen. This technique is particularly attractive, since the algae subsystem would only require adjustment of the cooling system, without manipulation of the nutrient supply. Future experimentation will continue along this line of research, using established food compatible systems (e.g. Spirulina).

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