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OPTIMISATION OF NUTRIENT REQUIREMENTS OF *SPIRULINA SUBSALSA* OERST (EX GOMNT)

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Unialgal culture of *Spirulina subsalsa* was raised by micropipette method. Its nutrient requirements were evaluated and Zarrouk's medium was proved to be the best. 60% Zarrouk's medium supplemented with 40% slurry yielded highest biomass. A pH of 10.2, low light intensity and prolonged photoperiods at 28±2°C proved optimum for the yield of *S. subsalsa.*

Keywords: BGSE; Chl-a; Growth; Nutrition; Optical density; *Spirulina subsalsa.*

Introduction

Single cell protein has proved to be a cheap source of food and feed for Millenia[1-4]. The cyanobacterium *Spirulina* is known for its high protein contents and quality with maximum number of essential amino acids and has been considered to be a rich source of single cell protein. *Spirulina* has already been commercially exploited in several countries for health foods and therapeutic preparations, because of its valuable constituents[5]. The establishment of suitable nutrient medium happened to be a prime requirement for achieving optimal growth of the alga, as such few suggested inorganic media have been experimented upon.

Materials and Methods

Cyanobacterium *Spirulina subsalsa* Oerst a fresh water puddle isolate of Jaipur was isolated and raised into unialgal cultures, following micropipette method[6]. Five different inorganic media[7-11] were employed. Five sets of 2 test tubes (15 x 120 nm) each containing 8 ml of different inorganic media and 2 ml of freshly growing homogenous cultures were prepared. Of the two tubes one was used for optical density records with the help of photochem colorimeter at 650 nm and other one was used for microscopic investigations. Simultaneously five conical flasks (250 ml), each having 80 ml of the medium added with 20 ml freshly growing cultures were also placed. These cultures were used to analyse Chl-a contents. All tubes and conicals were subjected to continuous illumination at 28±2°C at 522 Lux light intensity.

After initial growth evaluation, the high growth yielding medium was supplemented with biogas slurry extract (BGSE). For preparation of BGSE 100 gm of slurry was added to 500 ml distilled water and left overnight. The supernatent was decanted and filtered through Whatman 1 filter paper several times, till a clear solution was obtained. This was stored as stock solution. 10-90% of the medium was substituted with the slurry extract. The experiment was run over a period of 25 days. Record of optical density, Chl-a estimation and microscopic investigations were carried out on every 5th day.

Observation

In Zarrouk's medium, the growth of the alga was almost doubled within five days. The enhancement was linear and finally on 25th day, it was 30 times the initial density (Fig. 1). Similarly Chl-a contents increase was 6 times the initial value. In Hughe's medium cultures showed 29 times increase in density and 5 times increase in Chl-a contents (Fig. 1). In CFTRI medium; growth rate was slightly

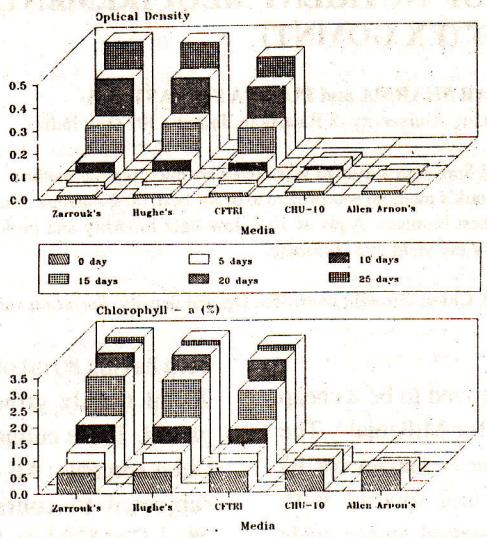

Figure 1. Growth evaluation of *Spirulina subsalsa* in various inorganic media.

slower than Zarrouk's and Hughe's media. In CHU-10 and Allen Arnon's medium growth was initially equal to other media, upto 5th day, thereafter, it declined and by 30th day cultures were dead. Zarrouk's medium proved to be the best for optimum biomass yield of *S. subsalsa*.

In order to reduce the cost input in the cultivation of this commercial alga, the Zarrouk's medium was added with different dilutions of BGSE. In 10% BGSE with 90% Zarrouk's medium, density and Chl-a contents both enhanced 30 times and 5 times respectively (Fig. 2). In 40% BGSE supplemented medium growth was maximum. It was about 33 times more than in minimal medium. 6.5% increase was noted in Chl-a contents. The density increase was equal to control with 50% addition of slurry. In rest of the BGSE supplemented media, growth declined linearly (Fig. 2).

Results and Discussion

The growth of *S. subsalsa* through optical density and Chl-a contents indicated that maximum biomass has been obtained in Zarrouk's medium, followed by Hughe's, CFTRI, CHU-10 and Allen Arnon's media. Almost all the inorganic solutions supported the growth of the alga for initial 5 days, but Zarrouk's medium had an edge over all others with a 30% increase in density and 6 times increase in Chl-a contents. More than any other factor, chemical composition of the medium may be credited for the growth of the alga (Table 1). Zarrouk's solution compared to other solutions had highest amount of $NaNO_3$ (2.5 gm/l). This was followed by Hughe's medium (1.5 mg/l) which seemed to have triggered the division rate[12]. Earlier, the need of nitrates for the enhanced growth of cyanobacteria has been emphasised[13-15]. However without it Chu-10 and Allen Arnon media have not supported the growth of *S. subsalsa*. This fact draws its support from the earlier report where even upto 0.5 gm/

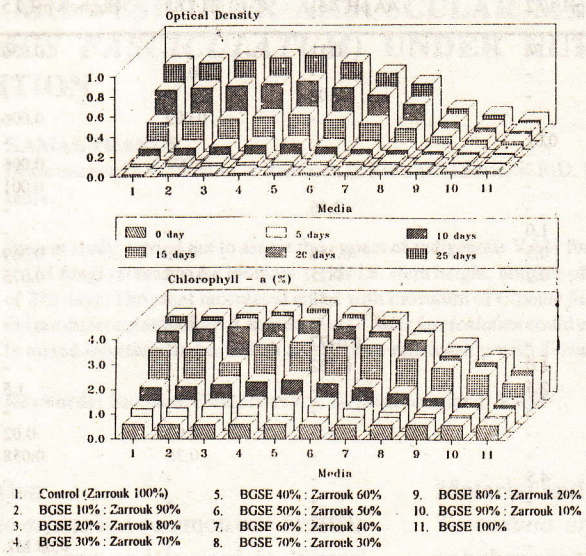

Figure 2. Growth evaluation of *Spirulina subsalsa* in biogas slurry supplemented Zarrouk's medium.

l $NaNO_3$ could not increase the biomass. Nitrogen happened to be an essential component required for protein synthesis. Present alga contained protein as high as 58%. Phosphates in the form of K_2HPO_4 was 0.5 gm/l in Zarrouk's, 0.358/l in Allen Arnon's, 0.01/l inChu-10 and 0.369 in Hughe's medium. Growth was proportionate to phosphate contents. Phosphorus, in the form of phosphates was needed for the growth of this species of *Spirulina*, as phosphorylated compounds were found to be essential for metabolic activities[16]. Magnesium in the form of $MgSO_4$ was present in all the five media. It is an essential component of Chlorophyll. The brilliant blue green colour of the cultures in this medium may be assigned to this salt. Although *Spirulina* is a fresh water alga, it required high amounts of sodium salts. Zarrouk's medium only provided 1% NaCl. The most essential requirement of *S subsalsa* was $NaHCO_3$ as high as 4.5% which none other media had in their composition[17-19].

Although Zarrouk's and Hughe's medium equally favoured the growth of *S. subsalsa*, but Hughe's medium included many and also costly chemicals as against Zarrouk's medium. Therefore, Zarrouk's medium was chosen for further reduction of the cost by adding BGSE. The pure BGSE has supported the life of the alga but not the growth. The slurry extract was dark brown in colour, which seemed to have played a role in abstracting the light penetration upto culture level. This probably led to the reduced photosynthetic activity[20-21]

BGSE added to Zarrouk's medium linearly enhanced the density and Chl-a of the

Table : 1 Chemical composition of various media g/l.

Chemicals	Zr pH 10.2	AA pH 7.31	C-10 pH 7.65	Hughe's pH 9.5	CFTRI pH 10.0
$CaCl_2$	0.04	0.11	-	0.036	-
$Ca(NO_3)_2.H_2O$	-	-	0.04	-	-
$CuSO_4.5H_2O$	-	0.02	-	-	-
Ferric Citrate	-	-	0.003	0.006	-
$FeSO_4.7H_2O$	0.01	-	-	-	-
Citric acid	-	-	0.003	0.006	-
EDTA	-	-	-	0.001	-
H_3BO_3	-	2.86	-	-	-
K_2SO_4	1.0	-	-	-	-
K_2HPO_4	0.5	0.358	0.01	0.369	-
$MgSO_4.7H_2O$	0.2	0.25	0.025	0.075	0.2
$MnCl_2.4H_2O$	-	1.81	-	-	-
$MnSO_4.4H_2O$	-	-	2.23	-	-
MoO_3	-	0.0177	-	-	-
NaCl	1.0	0.232	-	-	-
$NaNO_3$	2.5	-	-	1.5	-
N:P:K 15:15:15	-	-	-	-	1
Na_2CO_3	-	-	0.02	0.02	-
$Na_2SiO_3.9H_2O$	-	-	0.25	0.058	-
Na_2HCO_3	4.5	-	-	-	4.0
Super phosphate	-	-	-	-	0.1
$ZnSO_4.4H_2O$	-	0.222	-	-	-
Minor element Soln.	-	-	-	0.08 ML	-

Hughes = Hughe's medium; Zr=Zarrouk's medium; A.A = Allen Arnon's medium; C-10 = CHU-10 medium; CFTRI = Central Food Technological Research Institute.

cultures upto 40% level, when compared to the controlled sample. These results are in conformity with the observations on *S. platensis*[20]. The cattle dung contained 1-2% nitrogen, 0.7-1% phosphorus and 0.5-1.0% potassium[22-23]. The totality of evidences stand a witness to the fact that BGSE may be employed as a cheap and easily available nutrient source for the commercial production of *Spirulina*, particularly in rural areas.

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