J. Phytol. Res. 7 (2) : 97-102, 1994

INORGANIC NUTRIENT REQUIREMENTS OF *SPIRULINA SUBSALSA* OERST EX GOMONT FOR MASS CULTIVATION

SUNITA CHANDGOTHIA and PUSHPA SRIVASTAVA

Department of Botany, University Rajasthan, Jaipur-302 004, India.

In order to evaluate inorganic nutrient requirement of *Spirulina subsalsa* ten media having different chemical composition and pH were employed, Kratz and Myer's medium showed best results. The pH 8.2-8.5 and the presence of NO_3^{-2}, PO_4^{-2}, Cl^{-1}, K^{+1}, Na^{+1} ions proved to be essential for the growth of the present alga. Micronutrients of Kratz and Myer's medium were replaced by various dilutions of soil extract and addition of NaCl 0.2%/1 showed better growth than the original medium.

Keywords : Inorganic media; NaCl; Optical density; pH; *Spirulina subsalsa.*

Introduction

The culturing of algae in general and cyanobacteria in particular have been the subject of a number of biological investigations for their significant role as photosynthetic gas exchanger for human space travel in treatment of sewage, disposal of radioactive wastes and as hydrogen gas exchanger. Effect of different elements on various algae like univalent cations[1], nitrogen sources[2], cations,[3] ammonium and nitrate[4], sodium, molybdenum and phosphorus[5], iron and nitrogen sources6, have been studied. In present experiment an attempt has been made for developing a suitable medium with minimum input and maximum yield of *S.subsalsa.*

Materials and Methods

Spirulina subsalsa was collected from Jyoti Nagar, Jaipur from damp soil mixed with a number of species of *Oscillatoria* and diatoms and unialgal cultures were obtained by the dilution method. Inorganic media suggested for the various cyanobacteria have been employed to evaluate inorganic nutrient requirement i.e. Kratz and Myer's[7], Zarrouk's[8] , Allen and Arnon improvised[9], Allen and Arnon[9], Fogg's improvised[10], Fogg's[10], bold's soil solution No. 4[11], CFTRI[12], Chu-10[13], Kratz and Myer's medium was found to be most suitable for optimum growth of the alga. In improvised media, soil extract has been used in place of A-5 micronutrients. 20% to 80% soil extract was diluted with Kartz and Myer's medium and in another experiment different concentrations of NaCl i.e. 0.2%-1.0% were added to the medium with a gap of 0.2% to replace A-5 micronutrients.

Table 1. Chemical composition of various media.

Chemical	KM pH8.5	Zr pH10.2	AAS pH7.34	AA pH7.31	FS pH5.75	FAI pH8.0	F pH7.55	B-IV pH7.5	Cftri pH10.0	C-10 pH7.65
Ca CI_2	-	+	+	+	+	+	+	-	-	+
Ca $(No_3)_2$ H_2O	+	-	—	-	-	-	-	-	-	+
Cu $So_4.7H_2O$	-	-	-	+	-	-	+	-	-	+
Citric Acid	-	-	-	-	-	-	-	-	-	-
Fe $So_4.7H_2O$	-	+	-	-	-	-	-	-	-	-
Fe-EDTA	-	-	-	-	+	+	+	-	-	-
Ferric citrate	-	-	-	-	-	-	-	-	-	+
$Fe_2(SO_4)_3 6H_2O$	+	-	-	-	-	-	-	-	-	-
H_3BO_3	-	-	-	+	-	-	+	-	-	+
K_2SO_4	-	+	-	-	-		-	-	-	-
K_2HPO_4	+	+	+	+	-	-	-	-	-	+
KH_2PO_4	-	-	-	-	+	+	+	-	-	-
KNO_3	+	-	-	-	-	-	-	+	-	+
$MgSo_4.7H_2O$	+	+	+	+	+	+	+	-	+	+
$MnCl_2$	-	-	-	+	-	-	+	-	-	+
MoO_3	-	-	-	+	-	-	+	-	-	+
Nacl	-	+	+	+	-	-	-	-	-	-
$NaNO_3$	-	-	-	-	-	-	-	-	-	-
NPK	-	-	-	-	-	-	-	-	+	-
Na_2Co_3	-	-	-	-	-	-	-	-	-	+
Na_2Sio_3	-	-	-	-	-	-	-	-	-	-
$NaHCO_3$	-	+	-	-	-	-	-	-	+	-
Na Citrate	+	-	-	-	-	-	-	-	-	-
Super PO_4	-	-	-	-	-	-	-	-	+	-
$ZnSO_4$	-	-	-	+	-	-	+	-	-	+

Key for Media

KM = Kratz and Myer, Zr= Zarrouk, AAs= Allen & Arnon added soil extract

AA= Allen and Arnon, FS = Fogg's medium added soil extract, F= Fogg's medium

B-IV= Bolds -IV medium, C-10= Chu-10 medium.

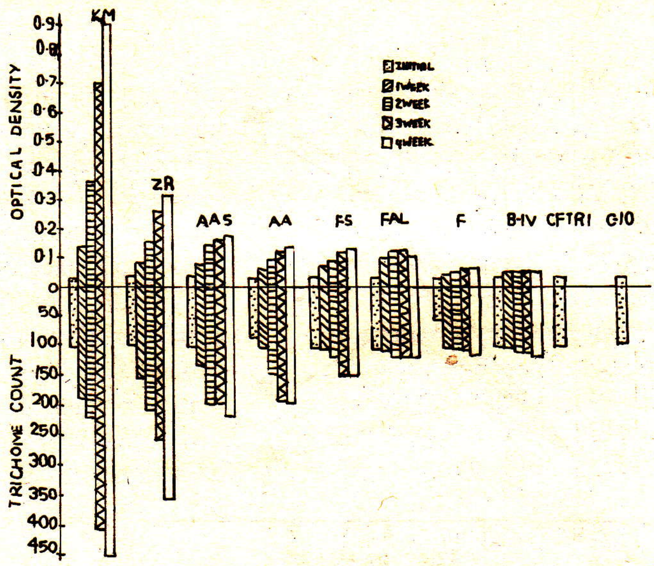

Fig 1. Effect of varied inorganic media on growth of *S. subasla* i.e. KM=Kratz & Myer, Zr=Zarrouk, AAS = Allen & Arnon, added soil extract, AA = Allen & Arnon, FS = Fogg's solution added soil extract, FAL = Fogg's medium adjusted p^H, F = Fogg's medium, B-IV = Bold's No. 4 Solution, C+1O=Chu no. 10 solution.

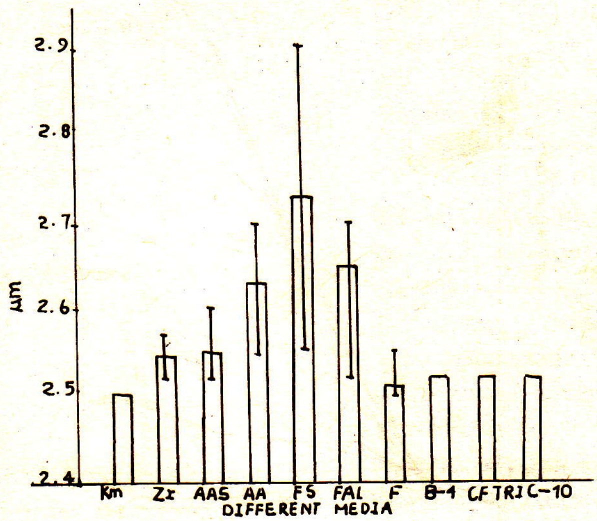

Fig 2. Breadth of *S. subsalsa*(Inμmeter) under varied inorganic media.

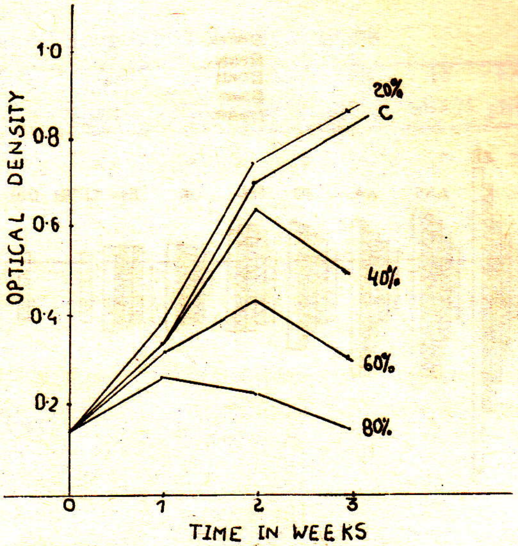

Fig 3. Effect of different concentrations of Soil extract on growth of *S. subsalsa*. C = Kratz & Myer's medium.

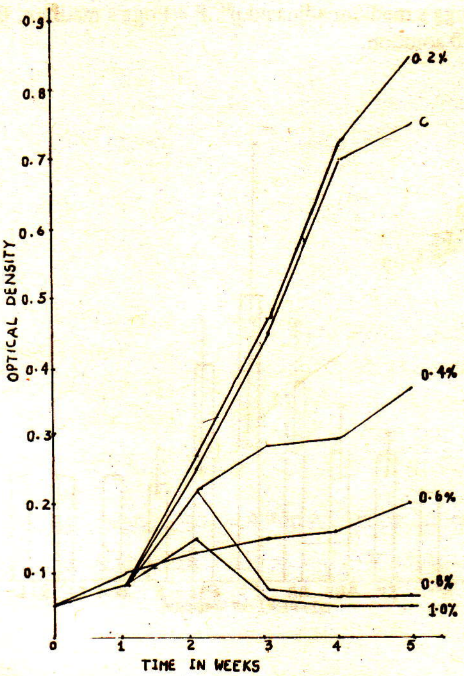

Fig 4. Effect of different concentrations of NaCl on growth of *S. subsalsa*.

A set of two test tubes with 10ml of medium was added with exponentially growing 2ml of centrifuged algal culture. Experiment was run in three replicates and their average values have been taken into account. Growth was followed through optical density and trichome counts. Optical density was recorded by photochemcolorimeter at 600nm and trichome counts were made in slides prepared by using a uniform drop of homogenised cultures. Culture samples were subjected to natural day (128 lux) and dark periods at 35 ± 3°C and shaken twice a day to prevent clumping.

Results and discussions

Fig.1 clearly indicates that the best growth of *S.subsalsa* has been in Kratz and Myer's medium. This was followed by Zarrouk's medium, imporovised Allen and Arnon's medium, Fogg's medium with adjusted pH, Fogg's medium and Bold's soil solution No.4.CFTRI and Chu-10 medium could not support the growth at all. Different growth patterns were recorded in different media (Fig.1).Trichome count also supported the optical density records. Highest number of trichomes were scored in Kratz & Myer's medium, it was nine times enhancement and in the rest of the media the number was concurrent with optical density records (Fig.1). The suggested breadth of the trichomes was 2.5 μm, which was also maintained in Kratz and Myer's medium. The variation in breadth of trichomes in different media has been shown in Fig.2. Totality of evidences have clearly indicated that more than any other factor chemical composition of the medium has influenced the growth of this cyanobacterium. Kratz and Myer's medium had double source of nitrogen i.e. Ca $(NO_3)^2$ 0.025gm/1 and KNO_3 1gm/1, and Zarrouk's medium which was the second best medium had nitrate as $NaNO_3$ (1.5 gm/1). These data draw their support from earlier studies where nitrate as a source of nitrogen had influenced the growth[2,4,14]. Here double source of nitrates as nitrogen in Kratz and Myer's medium seemed to be more effective than single source of NaNO3 in Zararok's medium. However minute quantities of nitrate i.e. 0.5 gm/1 in Bold's IV solution and 0.04 gm/1 in Chu-10 solution could not support the growth of *S.subsalsa*. Nitrogen being essential for protein synthesis was needed by the alga for its growth and protein synthesis which is as high as 55%.The source of phosphates was K_2HPO_4. Kratz and Myer's and Zarrouk had 1gm/1 and 0.5 gm/1, salts respectively. Growth was also proportionate to the phosphate contents. Zarrouk's medium where quantity of PO_4 was just half, growth was also half to that in Kartz and Myer's medium. Phosphates as a source of phosphorus was additional requirement for the substantial growth of this species of *Spirulina*, since phosphorylated compounds were found essential for metabolic activities[15].

$MgSO_4$ as a source of magnessium was common in most of the media. Since magnessium is a constituent of chlorophyll, the algal species have an absolute requirement for this element, which contribute towards brilliant blue green colouration[16] Kratz and Myer's medium is the only medium in which iron was present as $Fe2(SO4)3\ 6H_2O$. This trace element has instricated the growth rate. Iron being a constituent of many enzymes and of cytochromes and certain porphyrins whose deficiency is directly related with retarted growth, which in turn was correlated with reduction in photosynthesis [17,18].

Kratz and Myer's medium without A_5 solution was added with soil extract yielded interesting results 20% extract added to 80% medium proved to be the best. It was followed by 40, 60 & 80% in declining order (Fig.3). The soil extract presumably contained micronutrients of A5 composition.

Besides soil extract in yet another experiment A_5 micronutrients of this medium were replaced by different concentrations of NaCl 0.2% yielded highest growth indicating that NaCl can compensate to micronutrients of A_5[5]. It also revealed the feasibility of adaptation of the alga to fresh as well as salt water.

The pH of various media was an influencing factor which ranged from 5.5 to 10.2 Kratz and Myer's medium with pH 8.5 proved most suitable for the alga, denoting its preference for alkaline medium, but it deserves a special mention that Zr and CFTRI medium with high pH 10.2 and 10 respectively could not support the optimum growth.

With this baseline data it may be concluded that nitrates, phosphates, magnessium, iron are most suitable for the biomass production of this commercial alga. Addition of 20% soil extract or 0.2% NaCl can substitute to the costly A_5 nutrients of Kratz and Myer's medium cutting cost input of 20% for its mass cultivation.

Acknowledgements

Authors are thankful of DST (state) for financial assistance, Shri N.Thajuddin (National facility for marine cyanobactiria, Bharthidasan University, Tiruchirapalli, (India) for rendering the indentification of alga and Head Deptt. of Botany, U.O.R. for facilities.

References

1. Evans HJ and Sorger 1966, *Pl. Physiol.* **17**47
2. Singh HN and Srivastava BS 1968, *Can. J. Microbiol* **.14** 341
3. Gerloff GC and Fishbeck KA 1969, *J.Phycol.* 107
4. Thomas WH 1970, *Limnol. ocesonger* **15** 386
5. Kumar D and Kumar HD 1989, *Phykos* **28** (1 & 2) 254
6. Kerry A and Lauden beck DE and Trick CG 1988, *J.Phycol* **.24** 566
7. Kratz WA and Myer J 1965, *Amer .J. Bot.* **42** 282
8. Zarrouk C 1966, thesis, Paris (Provided by Repley Fox)
9. Allen MB and Aron DI 1955, *Pl. Physiol.* **30** 366
10. Fogg GE 1949, *Ann. Bot.* **13** 241
11. Bold HC 1949, *Bull. Torrey Bot. Club* **76** 101
12. Venkataraman LV 1978, *Arch. Hydrobiol,. Ergebn, .Limnol.* **11** 199
13. Chu SP 1942, *J. Ecol.* **30** 284
14. Agius C and Jaccarini V 1962, *Hydrobiol.* **87** 86
15. Kandler O 1960, *Ann .Rev. Pl. Physiol.* **11** 37
16. Rodhe W 1948, *Symb. bot.* upsatiences **10** 1
17. Pirson A, Tichey C and Wirhelmi G 1952, *Planta* **40** 199
18. Walker J B 1954, *Arch. Biochem. Biophys.* **53** 1