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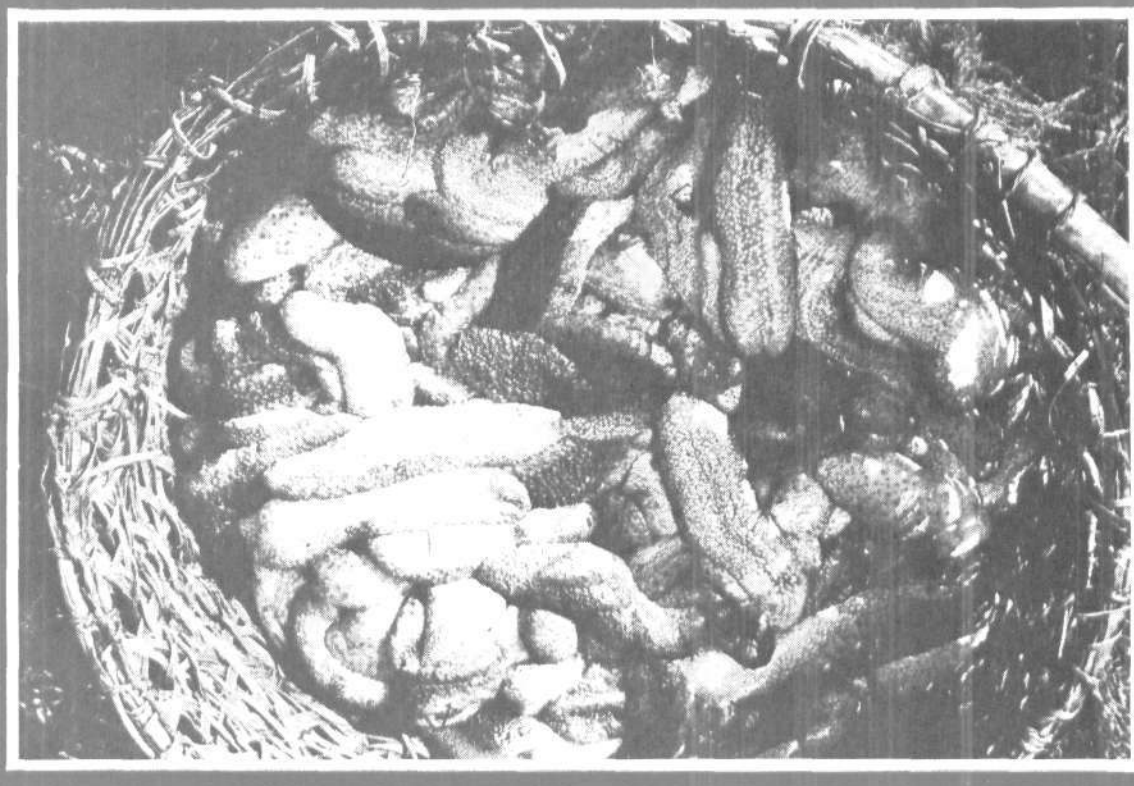

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DEVELOPMENT OF NOVEL TECHNIQUES TO MAINTAIN *CHLORELLA* SPP. STOCK CULTURE IN ARTIFICIAL SEAWATER

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Introduction

Chlorella spp. are used as feed to culture almost all species of zooplankton. Besides they form the important feed in the finfish/shell fish culture systems. Culture media such as Miquel's medium and Convey medium are conventionally used to maintain the stock culture of *Chlorella* spp. For the outdoor mass culture, water is enriched with groundnut oilcake, urea and super phosphate.

Inspite of the development of various techniques, constraints were experienced while maintaining the stock culture of *Chlorella* spp. in the conventional media such as.

1. Contamination with ciliates, 2. difficulty in maintaining stock culture for more than 15 days, 3. long duration (few hours or even days) for acclimatisation to the outdoor conditions after removal from the AC room and 4. labour and cost intensive. With a view to overcome these constraints a new technique has been developed for the stock culture of *Chlorella* spp. without using any of the conventional media. The salient results of the experiments conducted and the merits are presented in this communication.

Preparation of artificial seawater

Artificial seawater (ASWA) was prepared in two stages. In the first stage, solution 'A' was prepared by dissolving the following chemicals in 1 litre of rain or distilled water: (1) Gypsum 10 g, (2) sodium bicarbonate 10 g, (3) potassium bromide 1 g, (4) magnesium chloride 10 g, (5) sodium sulphate 20 g, (6) potassium chloride 2 g, (7) boric acid 0.25 g, (8) calcium chloride 5 g, (9) strontium nitrate 1 g, (10) zinc acetate 0.5 g, (11) potassium phosphate 0.5 g, (12) lithium chloride 0.2 g, (13) aluminium sulphate 2 g, (14) sodium thiosulphate 1 g, (15) potassium sulphate 0.5 g and (16) EDTA 10 g.

In the second stage, 20 kg of cleaned common salt (crystals) was dissolved in 500 litres of freshwater and to this, 800 ml of solution 'A' was added and thus a stock solution of half a ton volume was prepared. The contamination, if any, was removed by passing the water through a bio-filter and aerated. The desired salinity was obtained by adjusting the quantity of salt added. The pH was enhanced by adding sodium carbonate or sodium bicarbonate.

Preparation of culture medium

Fifty grams of each sample of raw rice (RR), boiled rice (BR), wheat (W), ragi (R), peanut (PN), barley (B) and channa (C) were steam cooked separately in 750 ml of fresh water, filtered and out of these 500 ml extract of each component was kept in separate containers to be used as the experimental culture medium. Weighed quantities of groundnut oilcake (125 g), urea (5 g) and suprephosphate (2.5 g) were soaked in 1 of freshwater for a duration of 3 hours and filtered using organdie cloth. Out of this extract 40 ml was taken and used as the control culture medium (GOC).

Experiments

Four liters each of ASWA was taken in eight glass containers of 5 litre capacity. Similarly four litres each of sea water was taken in another set of 8 containers. Forty ml of extracts of RR, BR, W, R, B, C, PN and GOC were added in each of the eight containers with ASWA and the process was repeated in the other set of 8 containers containing seawater. In both the sets 200 ml of *Chlorella* spp. was innoculated and kept in wooden racks, providing with light and aeration. Light intensity was maintained between 250 and 4,600 lux, temperature 20°C and 31°C and salinity 28 and 40 ppt. The pH fluctuated from 8.2 to 9.2. Initial cell concentration was 10.2 lakh/ml and the cell concentration was monitored at every 3 to 5 days interval.

Results

The cell concentration reached the maximum of 280 lakh/ml in the container with PN medium, 240.8 lakh/ml in C medium, 240.0 lakh/ml in RR medium, 144.0 lakh/ml in R medium and 120.0 lakh/ml in by medium in 23 days (Table 1).

TABLE 1 *Growth rate of* Chlorella *cell concentration in artificial seawater (lakh/ml)*

Medium	Observation dates						
	27.7.96	30.7.96	2.8.96	5.8.96	8.8.96	13.8.96	18.8.96
GOC	10.2	17.2	34.1	44.0	95.6	152.0	180.0
RR	10.2	21.6	40.3	99.6	170.0	224.0	240.0
BR	10.2	13.6	20.6	38.8	71.6	160.0	170.0
R	10.2	12.4	18.4	50.4	74.0	128.0	144.0
B	10.2	48.0	66.6	92.8	184.0	209.0	210.0
C	10.2	15.6	25.2	64.4	98.8	200.0	240.8
W	10.2	14.8	23.0	36.0	54.0	'85.0	120.0
PN	10.2	33.4	58.4	87.2	181.0	276.0	280.0

Subsequently the cell concentration was in the stationary phase for 12 days followed by death phase except in B medium (210 lakh/ml). In the similar set with sea water, the cell concentration was less (Table 2). The cell count presented here pertains only to growth phase (for 23 days).

TABLE 2 *Growth rate of* Chlorella *cell concentration in sea water (lakh/ml)*

Medium	Observation dates						
	27.7.96	30.7.96	2.8.96	5.8.96	8.8.96	13.8.96	18.8.96
GOC	10.2	15.6	28.6	32.4	60.0	114.0	135.0
RR	10.2	12.8	24.0	33.2	42.0	93.0	114.0
BR	10.2	19.2	28.0	41.9	61.5	134.0	150.0
R	10.2	09.6	25.5	50.4	88.8	168.0	185.0
B	10.2	09.6	19.5	49.6	72.1	104.0	125.0
C	10.2	11.2	21.2	54.4	100.0	204.0	210.8
W	10.2	09.6	15.0	22.7	43.0	81.7	130.0
PN	10.2	09.4	18.4	47.6	81.2	132.0	194.0

After that the cell concentration was almost constant for 12 days followed by the declining phase. Data collected for the stationary phase and the declining phase, are not included in this paper.

Chemical composition of culture medium

The chemical composition of culture medium was analysed and is given in Fig. 1. The highest cell concentration of *Chlorella* (280.0 lakh/ml) in PN medium had the nitrate level at 5.45 mg/1, phosphate 6.60 mg/1, silicate 59.0 mg/1 and nitrite 0.544 mg/1. The RR medium ranked third with the cell concentration of 240.0 lakh/ml having 4.87, 5.0, 42.5 and 0.55 mg/1 of nitrite, phosphate, silicate and nitrite respectively. The C medium ranked second with the cell concentration of 240.8 lakh/ml having the nitrate 19.75 mg/1, phosphate 3.60 mg/1, silicate 85.0 mg/1 and nitrate of 1.10mg/1.

Observations indicate that the cell concentration reached the maximum within 23 days, the declining phase started after 35 days compared to the 15 days period in conventional media.No ciliate infestation was found in ASWA enriched media. The results obtained here are the outcome of preliminary experiments. Further experiments to prove the possibility of using PN and C media in ASWA for outdoor culture of *Chlorella* are being conducted.