MASS CULTURE OF SPIRULINA USING LOW-COST NUTRIENTS

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SUMMARY: Two species of Spirulina were cultivated in outdoor ponds using low-cost substitutes for some of the recommended nutrients. In particular, bone-meal and biogas effluent were found to be very effective for the growth of these species.

INTRODUCTION: Monoculture of an algal species is an expensive process if we use conventional techniques. This Centre has been active in promoting the use of low-cost methods for the mass culture of Spirulina in rural habitats (Seshadri and Thomas, 1978). Some of the developments that have helped in lowering the cost were: 1) cheap methods of pond construction, 2) cheap agitation procedures, 3) harvesting every alternate day, and 4) solar drying of the algal mat. However, the cost of the medium (Zarrouk, 1966) remains very high. This study reports on the mass culture of a species of Spirulina (received from Indian Agricultural Research Institute, New Delhi and classified as Spirulina platensis) and a locally isolated species of Spirulina referred to as Spirulina (j), (Jeeji Bai, 1978), using low-cost substitutes for the phosphorous, calcium nitrogen salts in the traditional medium.

A. Spirulina IARI - Experimental Methods

1. Bone-meal substitute: Spirulina IARI was cultivated in open algal ponds of 1 m^2 area containing 100 l medium at 10 cm depth. In pond A, 50% Zarrouk's medium was used as control and in pond B, 50% Zarrouk's medium omitting the phosphate and calcium salts but with added bone-meal was used. The trace elements in the medium were restricted to the A_5M (50%); none of the B_6M

constituents were added. EDTA was not included in either of the media. (Normal Zarrouk's medium without EDTA and B_6 micro-nutrients is referred to as modified Zarrouk's, "Zm"). Bonemeal was enclosed in a fine mesh nylon bag and suspended in the medium, to avoid removal of the particles during algal harvesting. The same cultural conditions were provided in both the ponds, such as inoculum quantity, pH, rate of intermittent agitation by hand paddles, light intensity, temperature, etc. Initial optical density of the culture was 0.05-0.15 at 480 nm. Initial pH in both cases was 8.5 and reached 10.5 within five days. The average cultural temperature varied between 26°C at 0800 h, 37°C at 1200 h and 33°C at 1600 h. The lux readings were averaged at 22,000/08.00 h, 85,000/12.00 h and 34,000/ 16.00 h. Coconut thatch covers for the ponds were used between 1100 h and 1500 h for the first week, to prevent photooxidation. Harvesting was done on alternate days (when the culture optical density reached 0.7 - 0.8 at 480 nm) by pouring the algal slurry over cotton cloth filters. Chemicals were replenished periodically.

2. Biogas effluent supplement: Spirulina IARI was cultivated in 2 m^2 ponds containing 200 l medium at 10 cm depth. Three sets of nutrient media were tried: a) Pond A: Zm as initial dose, b) Pond B: 1/2 Zm + 5% volume/volume unfiltered biogas effluent as initial dose and c) Pond C: 1/3 Zm + 5% volume/volume biogas effluent as initial dose. Other cultural conditions were similar to the one with bonemeal substitute. The harvesting was on every alternate day. Small amounts of bicarbonate, phosphate and nitrate were added to the pure synthetic medium culture after harvest, and also occasionally to other cultures (once in 2-3 weeks, depending on the culture performance). Biogas effluent at 1% level was added to pond B and C after every harvest.

B. Spirulina (j): Experimental Methods

1. Biogas effluent supplement: Spirulina (j), a locally isolated species, was grown in ponds of 10 m^2 area containing 3000 l medium at 30 cm depth. Two sets of experiments were carried out. In pond A, 50% Zm medium was used; in pond B,

50% Zm medium was supplemented with 5% volume/volume biogas effluent as initial dose. Agitation was essentially by hand paddle, and intermittently. The ponds were never covered. The pH value ranged from 9.0 initially to 10.5 and above, five days after inoculation. Light intensity (lux) varied between 28,000/08.00 h, 78,000/12.00 h and 30,000/16.00 h. The average culture temperatures were 31°C at 08.00 h, 37°C at 12.00 h and 35°C at 16.00 h. Harvesting was done on alternate days. A special phenomenon observed in these ponds was that the algae floated as mats in the early hours, facilitating easy harvest by simply scooping the algal mat with cotton filters. A bicarbonate-phosphate-nitrate boost was given to pond A after each harvest whereas in pond B only biogas effluent was added, at 1% level after each harvest.

RESULTS AND DISCUSSION: Harvesting was carried out on alternate days; only half the culture quantity was harvested, so that the remaining culture acted as the inoculum to provide a continuous cultivation system. These open pond sytems have been running continuously for six to eight months and it is worthwhile to add that the starting inoculum for other experiments have been taken from these ponds. In other words, the pond cultures have been consistently healthy, though there were infrequent cases of contamination by diatoms and flagellates.

No strong correlations could be made between depth of the culture and harvest. In open ponds of 10 cm depth, the harvesting was done by pouring the slurry over cotton cloth filters for both Spirulina IARI and Spirulina (j), whereas by increasing the culture depth in the case of Spirulina (j), harvesting could be easily carried out by scooping the floating algal mat. However, the increased depth also necessitated increased nutrient input as the initial dose. We have yet to reach a compromise where algal mat formation can be consistently achieved with a culture depth less than 30 cm.

The bonemeal substituted experiment using Spirulina IARI gave as good a harvest as the 50% Zm medium. The alternate day harvest in 50% Zm medium varied from 12 $gm/m^2/2$ days to 23 $gm/m^2/2$ days with an average harvest of 10.1 gm/m^2 day. In the bonemeal substituted medium the alternate day harvest varied from 14-20 $gm/m^2/2$ days with an average harvest of 9.8 gm/m^2 day for a period of 60 days.

Spirulina IARI utilizing biogas effluent as supplement to 1/2 Zm medium gave consistently better harvests compared with the utilization of full Zm medium. The average harvest, over a period of 45 days, was 8.86 gm/m^2 day in full Zm medium, 10.88 gm/m^2 day in 1/2 Zm medium supplemented with biogas effluent and 9.41 gm/m^2 day in 1/3 Zm medium supplemented with biogas effluent.

For Spirulina (j) the average harvest in the biogas effluent supplemented 50% Zm medium was 12.39 gm/m^2 day as compared to 10.3 gm/m^2 day recorded in 50% Zm medium.

It appears that the useful characteristic of algal mat formation found with Spirulina (j) is favoured by: 1) the presence of gas vacuoles in the alga, 2) the greater culture depth used, and 3) the reduction of light penetration due to the addition of biogas effluent to the medium. Such algal mat formation was not often observed in ponds of 10 cm depth.

The economics of Spirulina cultivation in a medium supplemented with biogas effluent have been worked out (Seshadri, 1978). Protein analysis carried out at the National Dairy Research Institute, Bangalore, India showed 40.11% crude protein in Spirulina IARI and 59% crude protein in Spirulina (j). Microbiological analysis of the processed algae, and feeding trials, have been started.

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