

A SIMPLIFIED TECHNIQUE FOR MASS CULTURE OF UNICELLULAR ALGAE

Phool Begum Zahid, Tajwar Sultana and Wajid Hussain

Department of Botany, University of Karachi, Karachi 32, Pakistan

(Received January 8, 1980)

Algae have a bright future as a potential source of food and fodder. They may be introduced as direct sources of protein for humans. One method for production of such proteins is through cultivation of microscopic green and blue-green algae. For this purpose a laboratory scale mass culture apparatus was established and the suitability of *Scenedesmus quadricauda*, *Scenedesmus dimorphus* and *Monoraphidium contortum* as sources of good quality protein was studied.

INTRODUCTION

Search for alternate sources of edible protein is currently a topic of world concern [1-5]. According to the Nutrition Survey of Pakistan [6], 60% of rural and 40% of urban population have deficient level of plasma albumen which indicates the wide spread prevalence of subclinical protein deficiency. This situation is due in part, to an inadequate supply of high grade protein.

To meet the increasing demand of proteinous foods, scientists throughout the world have been searching for sources not generally used for human consumption. Amongst these, fish protein concentrate (FPC), leaf protein concentrate (LPC), oil seed cake proteins, single cell proteins etc., have been thoroughly investigated as protein supplement. Experiments on algae as a source of protein for animals and to some extent for human, have also been undertaken by a number of workers [3,7]. Algal protein cattle fed exhibited 50% higher yield of beef protein than cattle that grazed on grass.

It is therefore, possible that algae might represent a potential source of feed and fodder in an ever increasing economically important feed market [8].

Algae give relatively higher yields of biomass for an equivalent illuminated area due to more economic utilisation of sunlight. They have trace element composition suitable for fertilizers. An additional suitable characteristic of algae is that they can be cultivated on a large scale for harvesting of the protein available in the biomass [9].

Considering algae a potential source of good quality protein, attempts have been made to develop a simplified technique for their mass culture. Present communication deals with this study.

MATERIALS AND METHODS

1. *Selection of Algal Strains.* Species of *Scenedesmus* and *Monoraphidium* e.g., *S. quadricauda* var *Longispina*, *S. dimorphus* and *M. Contortum* were selected for mass culture in the laboratory and for outdoor cultivation. These algae are eurythermic for Sind region, especially Karachi and grow all year round where temperature ranges from 15-40° [10].

2. *Description and Working of the Mass-Culture Apparatus.* The mass culture apparatus used for the cultivation of algae is shown in Fig. 1. This is a portable unit 96 cm long and 85 cm broad and has a plain glass surface with a slope of 40 degrees which is supported by a steel frame.

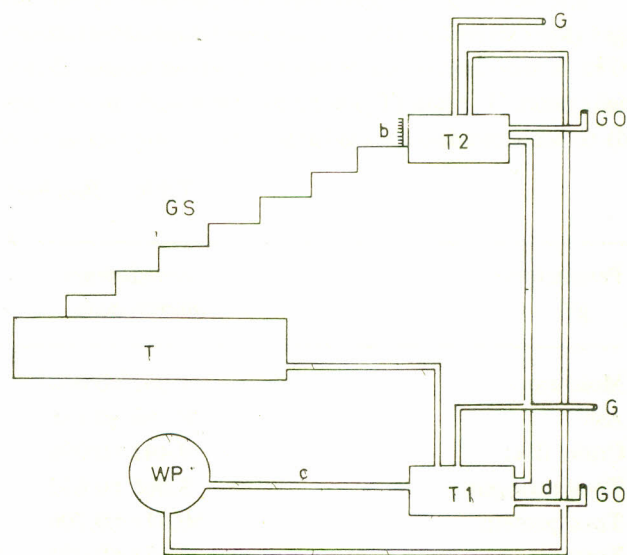


Fig. 1. Diagrammatic sketch of mass culture unit: G- Gas; GO- gas out let; GS- Glass surface; T- Tray; WP- Water pump; a- Valve; b- Multiple tap system; c- Discharge pipe; d- Exit pipe.

The surface is fitted with transverse glass plates (GS), each of which is 2.5 cm broad and 85 cm long. These plates are positioned 4 cm apart, one above the other, to create intensive turbulence of algal suspension which flows down the surface in a layer of 1 cm thickness.

The uni-algal suspension was circulated over the surface (GS) by a water pump (WP) during the day, and was stored in the glass tanks (T_1 and T_2) at night to reduce heat loss. The algal suspension was cultivated in a nutrient solution [11] aerated with 4% CO_2 in air.

To initiate a test run, a massive inoculum was introduced in the culture unit in order to establish a suitable cell suspension. In 30 litres of culture solution the quantity of algae needed for the inoculum was 2 litres (equivalent to 30 g dry wt).

To operate the circulation system, valve (a) of the water pump was first opened and a little culture medium was poured into it. In this manner the circulation pump was set into operation. The pump withdraws the algal suspension from Tank T_1 and discharges it into Tank T_2 . From there the suspension goes to the glass stepped surface (GS) through a multiple tap system (b) which is horizontally placed at the top surface of slanting glass steps. The excess or overflow of the suspension discharge back again into tank No. T_1 through the pipe (c). The flowing continues with a turbulent motion through the glass steps (GS) and collects in a tray (T) for a moment and from there the suspension flows again into Tank T_1 for recirculation. The process of recirculation is completed in half an hour. The suspension flows down the surface of glass steps in a layer of 0.5 cm at a velocity of 35 cm/sec.

The mass culture apparatus was operated in the day light (light intensity 6000 lux at room temperature) usually 10 hr in the summer and 8 hr in the winter season. During night tanks (T_1) and (T_2) were provided with an exit pipe (d) to accommodate gas exchange. The culture was agitated

by gas bubbling during storage in tank T_1 and T_2 to maintain cultural conditions like an open shallow pond. During cloudy weather the illumination was furnished by fluorescent tubes around the culture unit.

3. *Harvesting.* The methods of harvesting of algal biomass were adopted from Freeman [12], Golueke [13] and Levin [14]. The suspension was centrifuged at 1000 r.p.m. for half an hour. The effluent from the centrifuge contained about 5% dry mass, equivalent to 10 mg biomass/litre.

4. *Preparation of Algal Powder.* The effluent obtained above was washed three times with deionized distilled water in order to remove salts absorbed at the cell surface. The residue was dried at 80° in an oven for 2 hr and the powder was stored in bottles at room temperature.

Chemical Composition of the Biomass.

Ash was determined by Tribold and Avrand method [15] and protein by the micro Kjeldhal's distillation method [16]. Fat was extracted in soxhlet with ether and the oil was dried over sodium sulphate.

Amino acid profile of the biomass was determined by the method of Geoffrey [17] using two dimensional thin layer chromatography.

DISCUSSION OF RESULTS

Chemical composition of the biomass of the strains of *Scenedesmus* and *Monoraphidium* is shown in Table 1. It will be seen that under the reported conditions of cultivation the biomass of the three strains contained as high as 50% high quality protein. The protein on TLC showed the presence of all essential amino acids especially lysine in which the Pakistan diet is deficient. These results agree well with those reported in literature [18].

Table 1. Biochemical composition of algae.

| Parameters g% | <i>Scenedesmus quardicauda</i> | <i>Scenedesmus demorphus</i> | <i>Monoraphidium cortortum</i> |
|--------------------|------------------------------------|----------------------------------|------------------------------------|
| Moisture | 11.042 \pm 0.058 [#] | 10.641 \pm 0.151 | 12.291 \pm 0.231 |
| Ash | 10.763 \pm 0.186 | 8.406 \pm 0.137 | 8.856 \pm 0.231 |
| Crude fiber | 9.441 \pm 0.036 | 8.781 \pm 0.066 | 9.266 \pm 0.101 |
| Total nitrogen | 8.066 \pm 0.032 | 7.875 \pm 0.019 | 6.856 \pm 0.091 |
| Total protein | 50.417 \pm 0.206 | 49.023 \pm 0.222 | 42.854 \pm 0.564 |
| Total fat | 9.571 \pm 0.055 | 10.212 \pm 0.066 | 12.437 \pm 0.121 |
| Total carbohydrate | 8.765 \pm 0.273 | 12.746 \pm 0.204 | 16.706 \pm 0.316 |

* Mean. # Standard error of Mean.

From the results thus obtained it can be concluded that the three strains of algae belonging to the species *Scenedesmus* and *Monoraphidium* can be cultivated under controlled conditions and achieve the required level of protein within a short period.

Acknowledgement. The authors gratefully acknowledge financial assistance of Pakistan Science Foundation, Islamabad (Project-No. S-KU/BIO (54)).

REFERENCES

1. R.W. Krance, *Am. J. Bot.*, **49**, 425 (1962).
2. L. Bjorkman *et al.*, *Acta polytechn. Ser. Chem.*, **4**, (1955).
3. H. Tamiya, *Proc. Symp. Algology (New Delhi)*, Indian Council Agricult. Research, p. 379 (1959).
4. FAO Nutrition report series No. 37 Rome (1965) FAO. p. 51 & 61 (1957).
5. FAO Nutritional Studies No: 10 (1957).
6. Nutrition Survey of Pakistan, A report issued by the Directorate of Nutrition Survey and Research, Ministry of Health Labour and Family Planning, Health Division, Government of Pakistan, June (1970).
7. H. Nakamura, *Japan Nutrient Association*, p. 1 (1961).
8. H. Tamiya, *Proc. World Symp. Appl. Solar Energy Phoenix Arizona No: 1-5*. Published by Stanford Res. Inst. Menlo Park Calif. (1956).
9. J. Bartos, *Acta Univ. Carol. Biol. Supple.* (1966).
10. P.B. Zahid, *Pakistan J. Sci. Ind. Res.*, **24**, (1981) (in Press).
11. H.C. Bold., *Bot. Rev.*, **8**, 69 (1942).
12. R.R. Freeman, *Biotechnol. Bioeng.*, **6**, 87 (1964).
13. C.G. Golueke and W. Oswald, *J. Water Poll. Control Fed.*, **37**, 471 (1965).
14. G.V. Levin, *et al.*, *Appl. Microbiol.*, **10**, 169 (1962).
15. H.O. Tribold and L.W. Arrand, *Food Composition and Analysis* (Nostrand Company Inc. Princeton. New Jersey, 1963), p. 14.
16. P.B. Hawk, *et al.*, *Practical Physiological Chemistry* (McGraw Hill Co., New York, 1964), thirteenth edition, p. 560.
17. F. Geoffrey, *Recent Advances in Clinical Pathology* (Little Brown Co., Boston 1968).
18. E. Tamura, *et al.*, *Ann. Rep. Nat. Inst. Nutr. Tokho*, Japan (1958), p. 20.