ENZYMATIC STUDIES ON SCHISTOSOMA MANSONI

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Contents

I N T R O D U C T I O N

In recent years the possibility of utilization of unicellular fresh water algae as new source of food supply has been investigated in many institutions in America, Germany, France and Britain. These investigations include the development of mass culturing of unicellular algae, the study of the growth and chemical composition of these organisms as influenced by the variation of the different physiological and environmental conditions as well as the nutritive value of the algal products.

These studies have shown, however, that unicellular algae are remarkably flexible in the sense that their composition can be altered at will and thus by careful control of the culturing conditions a wide variation in the relative amounts of the chemical constituents especially proteins and fats, can be attained.

Laboratory experiments on the mass culturing of autotrophic micro-organisms were carried out in the botanical Institute of Gottingen university (professor A.A. Pascher). The aim of such experiments was mainly the study of the commercial quality of these algal organisms for the production of fats as well as vitamins. These organisms were cultured in nutritive solution in glass tubes 5 x 25 - 30 cm. and 10 x 100 cm. provided with an air stream fortified with

... Co_2 and illuminated with water - cooled - 500 watt - ... yielding up to 10,000 lux.

Aach, 1952; however used culture vessels illuminated from inside. He used 5 - 15 cm. wide cylinders. Quartz tubes were put inside and the culture solutions were introduced in the space between the glass cylinders.

In Essen the carbon Biological research station, 1953; (Dr. Gummert, Dr. Meffert and Dr. Strattman) have been highly interested in the mass culturing of algae which may provide a possibility of the biological utilization of the large quantities of Co_2 of the waste gases available in the industrial Rohr district.

Since 1950, they have studied the unsterile large scale culturing of the green algae, Chlorella and Scenedesmus. Two methods of culturing were applied in Essen. The open air cultures and the green house cultures. The open air cultures were carried out in culture trenches nine meters long, 1 m. wide and 20 - 24 cm. deep at the low ends. They were lined with plastic material. Gas mixture for aeration was led to the cultures in iron tubes having many small holes and protected with inert coating.

In America the mass culturing of algae was intensively studied and pilot plants for culturing of these

organisms were installed . For the preparation of ... cultures for the large scale experiments one litre ...lenmeyer flasks containing 500 ml. of the Stward... ...dium were inoculated with small quantity of the ... from the slant cultures. These flasks were set in ... ...ining rack illuminated from below by 2 fluorescent ...bes and 2% Co_2 in air mixture was bubbled through ... ...ture.

To produce enough cultures for inoculation ... large growth units a second intermediate step was ... ... cultures in vertical cylinders,4 inches in diameter ... feet long,were made.

The large scale operations were carried out in ... scale units. A unit was composed mainly of large ...h - walled plastic tubes (polyethylene) to form ...annels with simultaneous flow of cultures.

The polyethylene tubings seemed to provide a ...itable conbination of light transmisson, chemical and ...ysical stability under exposed conditions, freedom ...om toxicity and physical strength.

Tamiya, 1954; compared closed circulation system ...milar to that run by Arthur D. Little in America and the

open trench system similar in principle to that run in Essen, Germany, and gave a detailed report on the advantages and disadvantages of both systems and concluded that the open trench cultures appeared to be superior to the closed circulation cultures in its apparently lower cost and simplicity of equipments and operations although it is rater inefficient in the CO_2 utilization. He finally constructed a new type of the open trench system which proved to give a better yield.

Tamiya and his co-workers of the Tokugawa institute for biological research were also highly interested in the physiology and biochemistry of unicelluler algae and their mass culture. In his laboratory experiments Tamiya used either culture flasks or special culture chambers. The culture chamber described by Tamiya was an oblung flat flask with uniform inner thickness and total capacity of 600 ml.

In England M.J. Geoghegan, 1951; used cylinders and aspirator bottles for the culturing of unicelluler algae in the laboratory, while in his outdoor experiments he used plastic tanks.

The green algae, especially Chlorella and Scenedesmus had been used for a long time by many investigators as experimental tools in the study of physiological processes. More recently, the chemical constitution of these organisms became of interest to many authors especially after the

... of the mass culturing of these organisms.

From the results of some detailed investigations carried on few algal species especially Chlorella ... Scenedesmus it has been established that the chemical composition of algae depends to a large extent upon the biological and environmental conditions under which ... is cultured. Thus Spoehr and Milner, 1949; ... studied the influence of various environmental factors ... on the chemical composition of Chlorella. ... CO2 concentration, ... nutrients, more particularly ... light intensity and temperature. ... have ... the biochemical composition of ... under the conditions under which they grow.

Spoehr and Milner, 1949; have ... other marked variations in chemical composition ... related to environmental changes or whether ... alteration of a strain of the organism ... under special conditions and from which ... They have concluded that the chemical ... Chlorella - their experimental organism - ... by environmental conditions ... selective growth of ... strains.

Milner, 1950; ... the biochemical composition ... different algal species including ... and ...

[illegible], has demonstrated that this composition varies in response to change in environmental culture conditions. This variation is much more significant in the case of Chlorella pyrenoidosa.

Similar results were, however, obtained by Redfield, 1949 ; on growing different algae under different environmental conditions.

Fowden, 1952; has also isolated bulk protein samples representative of the whole cell protein from Chlorella cultures of different ages and the amino acid composition of these protein samples has been determined chromatographically. It has demonstrated that the amino acid composition of the cell proteins remains virtually constant with increasing age, although the percentage of proteins within the cells markedly decreased.

Meffert , 1952 - 1955; has found that the chemical composition of her experimental organisms, namely Scenelesmus obliquus and Chlorella pyrenoidosa, when grown in large scale unsterile cultures varies but very slightly in response to change of climatic conditions during the different months of the year and also from year to year. It has been recorded by Meffert the protein content also varies between 50 and 60% of the dry weight, the fat from 15 and 20% while the carbohydrate content remains

...ent or less constant.

Though many authors have shown that the proportion of the chemical composition of the algae may ... extremely wide limits depending on the physiological ... environmental conditions under which the ... grow rather than on genetically determined ... of metabolism, (Hoteman & Hedfield, 1954; Spoehr & Milner, 1949; ... ens, 1951; 1953) yet the results reported by ... Collyer concerning the lipid accumulation in ... indicate that nevertheless there are considerable inherent differences in the capacity of algae to accumulate lipids.

Proteins seem to be the most abundant organic constituent of the unicellular green algae with the exception of the high lipid forming organisms (Fogg & ...ibbs, 1942; Mitch, 1948) and the protein content of ... of the algal species investigated was appreciably higher than that of the best vegetable sources of protein ... in animal and human feeding (Fisher, 1953).

Geoghegan, 1951; studied also the physiology, behaviour and chemical constitution of Chlorella vulgaris var. viridis, and demonstrated that the true protein ... of the dried Chlorella material was ... %. He also investigated the amino acid content of the protein.

Fowden, 1951, 1952; has also studied the composition of the algal proteins in 3 different organisms namely Chlorella pyrenoidosa, Ch. vulgaris and the blue green alga Anabena. He has shown that all the essential amino acids occured in his 3 organisms. He also identified and determined the relative quantities of 23 amino acids in the free (in the alcoholic extract) or in the combined form (in the protein hydrolysate). He applied paper chromatography methods .

Fowden also demonstrated that the amino acid composition of the algal proteins remains virtually constant at increasing culture age, although the percentage of protein within the cells steadily decreased. Fowden continued his studies and in a latter work, 1954; he made analysis of 4 different algae to investigate the nature of any differences in composition between algae of all types and also to compare the composition of these algal proteins with those of other plants. These investigations revealed that the total nitrogen and protein contents of the examined algae varied widely. On the other hand the proportions of the total nitrogen present as soluble non - protein nitrogen remained relatively constant for all the 4 species. The results presented by Fowden also showed that the proportions of most of the individual amino acids present in the algal protein fractions of the different species examined were similar.

[illegible] of the amino acids (glycine, phenylalanine, leucine, valine and proline) are present in each of the [illegible] in approximately equal amounts, if allowance is made for normal experimental error. Significant differences between the percentage of certain amino acids (cystine, histidine, lysine, threonine, serine and aspartic acid) are apparent. It is considered that the main differences exist in the amino acids threonine, serine and glutamic acid.

Bowden finally concludes that the composition of the bulk of proteins of algae is similar to that of higher plants and other micro-organisms and with regard to their possible nutritional value the algal proteins are likely to resemble proteins in being deficient in the sulphur containing amino acids.

The studies of the composition of proteins of unicellular algae proceeded also in U.S.A. Thus the food research lab.(washington) found in 1953 that Chlorella pyrenoidosa contained about 44 % protein. The different amino acid composition of this protein were also estimated and 11 different essential amino acids were proved to be present.

In Japan Tamiya and his co-workers, 1951 - 1954; carried intensive studies in physiology and biochemistry of

unicellular algae. They have shown that the protein content of their different experimental organisms lies between 40 % and 50 % of the dry weight and all the essential amino acids were found present in Chlorella ellipsoidae proteins.

In Germany Meffert, 1956; giving a review of the work done in Essen station on large scale production of algae, 1951 - 1955; had shown that the protein content of Scenedesmus and Chlorella varies within limits very slightly from month to month and from year to year. The protein content lies between 50 - 56%. Also Meffert demonstrated that all the essential amino acids are present in Chlorella and Scenedesmus proteins.

Edible seaweeds have been used for centuries. A full account of the use of a number of macroscopic marine algae as food is given, however, by F. chase, 1942; along the coast of Europe some forms, are regularly collected and dried for use as cattle fodder and on the west coast of Ireland and Scottland there is a variety called Dilse which is often sold like peanuts or popcorn. The Japanese also have used certain seaweeds in cooking. In America Chondrus crispus or irish mass is harvested commercially along the new en land coast for use in food products.

Recently more emphasis had been imposed on the investigations concerned with the possible utilization of fresh water algae, especially the unicellular forms, as food resources.

The primitive character of these microscopic organisms gives them a number of advantages over higher plants as a source of food. These advantages can be generally summarized in the following

I. The whole algal plant is nutritional as little of it is converted to indigestible structures.

II. The algal material contains a high protein content (in some amounts sometime to more than 50% of the dry weight) and this is much higher than that found in the edible part of any of the higher plants.

III. The preliminary investigations of many authors indicate that the unicellular algal proteins contain the essential amino acids needed for human nutrition.

IV. The chemical composition of the unicellular algae is more or less flexible and could be changed at will by modification of the culture conditions.

V. The area needed for large scale cultivation of algae is limited and it has been assumed that the total area required for algal culture needed to provide the half of the recommended requirements of proteins is less than a million acres for the present world population. In countries