The large scale culture of algae (*)

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One of the most important problems which faces the world is that of hunger.

The population of the world is increasing continuously and, in addition, the struggle against disease, which in many of its aspects has made great strides forward, is another factor adding to the already large number of human beings thereby further burdening the present food supplies available for daily living needs. In fact it has been calculated that based on the present rates of population increase, the problem of hunger within 20 years' time will take on tragic aspects. Therefore, the interest of scientists has been directed towards the search for new sources of food, both in trying to render fertile land that has been unproductive up till now, and in opening up new sources of food. Many attempts have been made in this second direction and, for instance, a method has been found of extracting proteins from petroleum. But what interests us today is another very important source of proteins, the algae, not only as a source of food, but also for other important purposes.

The algae are particularly interesting in regard to the production of proteins since they are micro-organisms capable of an autotrophic development; in other words they do not need carbohydrates for their growth, since they use CO₂ from air as a source of carbon.

The important amino acids are present in the proteins obtained from algae; moreover the lipidic content of green algae is about 20% of the whole weight and at times, for instance, when the cells grow old or when there exists a nitrogen lack, this figure increases up to 40-70%. Finally, the algae contain many growth factors. In particular pro-vitamin A (expressed as beta-carotene), vitamin B, vitamin C (this last one is formed only in the presence

of light), vitamin K and pro-vitamin D (ergosterol) are present. The growth factors are particularly abundant in the alga *Chlorella* which is the organism most cultivated for food purposes.

Some American authors have calculated that, in theory, algae utilizing only the CO₂ of the air could produce 10 to 20 tons of dry organic material per acre in a few days' time, while from traditional crops several months are necessary to obtain 2 to 10 tons per acre. Of course, the problem of large scale production of algae is a very difficult one, but it is certainly not insoluble. For all these reasons it is quite easily understood why the efforts of many groups of scientists are centered on the study of the growth of algae, including problems of industrial microbiology, microbiological engineering, biochemistry and genetic factors important in the development of new strains more suitable for various needs.

**USE OF ALGAE**

In addition to food production, algae are used for many other purposes. They are utilized for producing particular substances, like carotenoids, chlorophyll, steroids, etc.

They can be employed in symbiosis with bacteria to facilitate the depuration of sewage (in fact they assure a good oxygenation of the medium thereby facilitating the degradation activity of aerobic bacteria, which, on the other hand, liberate CO₂ which is photosynthesized by the algae). An interesting example of utilization of these organisms for fertilizing poor soils is the cultivation of some blue-green nitrogen fixing algae in rice fields.

But what is really a striking aspect of the use of algae is their utilization in closed ecologic systems like space crafts. Experiments run by NASA have already demonstrated the possibility of a man living in a space craft for many days with a culture of *Chlorella*; the micro-organism releases oxygen and acts as a food source while the astronaut returns the algae the substances and the CO₂ necessary for their growth (1). To produce the human requirement of two pounds (600 litres) of oxygen per day, the culture must yield about 500 grams of dry cells each day. If a steady state concentration of 2.5 grams per litre produces 10 grams per litre per day, about 50 litres of culture (167 pounds) are necessary to sustain one man. An experimental one-man system at the School of Aerospace Medicine uses 80 to 100 litres of culture, 2 to 6 grams of algae per litre, in 10 tanks. Each tank is one meter long, half a meter high and one centimeter thick. The weight of the system including accessories is about 1000 pounds per man (450 kgs) with a power requirement of 21 Kw per man.

Finally, the algae are also used for desalting marine water: in this case they are utilized as amphoteric ion-exchange-resins. Experiments
for this purpose are going on in many laboratories and the results so far obtained show that this aspect of algae utilization will soon become a reality.

In conclusion it can be said that algae are one of the most interesting organisms studied to-day and the contribution they can give to human life can be considered almost as important as the discovery of antibiotics. We are going now to review the systems more widely used for the cultivation of microscopic green algae on a large scale.

METHODS OF MASS-CULTURE

The methods thus far proposed by several workers for the mass-culture of algae differ somewhat in principle. Among these the following two appeared most feasible and were subjected to pilot-plant investigations (5):

1. the «closed circulation method», in which the culture is circulated either in a clear plastic tube or in a shallow concrete trench covered with a plastic top;

2. the «open bubbling method», in which the culture contained in an open shallow trench is constantly aerated with CO₂-enriched air.

In the closed-circulation system the algae culture is circulated in a flat tube made of vinyl sheeting (Area of illuminated surface : 15 square meters; total volume of culture : 800-1000 liters; rate of flow of culture: 35-50 cm per second in the plastic tube; CO₂-enriched air is supplied at a rate of 25-30 liters per minute per ton of culture). In the open bubbling system, the ponds are provided with a number of ridges (triangular prism in form) at the bottom and, from plastic pipes placed in the hollows between the ridges, CO₂-enriched air is constantly bubbled into the culture at a rate of about 250 liters per minute per ton of culture (Area of illuminated surfaces : 2.5-5.5 square meters; total volume of culture : 200-500 liters; distance between the pipes : 40-60 cm). The ridges between the bubbling pipes have the double purpose of increasing the ratio of illuminated surface to volume of culture, and of making the agitation of culture as effective as possible.

The advantages and disadvantages found in operating these two types of culture can be summarized as follows:

a) Durability of equipment. — While the open system is quite durable and requires almost no attention during its operation, the closed system is subject to various operational problems. The vinyl sheetings employed became brittle within several months, and small holes formed, which disrupted the operation of the unit. Moreover, the plastic tube
containing the culture solution was not stable in strong winds, the stress so induced caused it to break at its point of attachment.

b) Temperature of culture solution. — In experiments run by Tamiya (7) in Japan in which the temperature of the culture solution was not artificially regulated, the highest values were obtained in the afternoon during the month of August and the lowest values in the early morning hours during the month of January. The maximum and minimum temperatures attained throughout the year were respectively 38°C and -3°C in the open system, and above 50°C and 0°C in the closed system. To prevent over-heating of algae cells during the summer months, the closed system must be specially equipped for cooling of the culture.

c) Dust, rain, and contamination. — While in the closed system the culture is protected from dust and rain, this is not the case in the open system. Water loss by evaporation during the dry seasons, to which the open system is particularly vulnerable, is not a serious problem in some countries such as Japan. Moderate flooding of the system because of rain did not appreciably affect the growth of algae. In this case the increase of the culture solution is easily controlled by removal of the extra water at the time of harvest. Dust accumulation in the open system is dependent upon the local climate and terrain conditions. Concerning the possibility of contamination of the culture by other organisms, no essential difference was found between the open and closed systems.

d) Precipitation of algae cells. — There is a tendency towards precipitation of cells to the bottom of the culture chamber with increase in the population density of the algae. This creates a difficulty in the harvesting of the algae cells, and also constitutes a serious cause of contamination by other micro-organisms. In this respect the closed system presents here more disadvantages than the open system. The precipitation of cells on to the bottom of the plastic tube cannot be eliminated even when the flow rate is raised to 50 cm per second. Moreover, a large quantity of algae cells adhere onto the upper (inner) surface of the tube, and because they are partly dried, a problem in their removal is encountered. In the open system, on the other hand, precipitation can be avoided or lessened by increasing the rate of bubbling (and by increasing the number of aeration pipes). It should, however, be noted that the tendency towards precipitation and aggregation of the algae cells varies a great deal depending upon the algae strain used. In some cases this cannot be eliminated even though the aeration rate (in the open system) is raised to as much as 500 liters per minute per ton of culture solution.

e) Economy of power and CO₂-pre-vision. — As is evident, the open bubbling system requires about 5 to 10 times more CO₂-enriched air than
the closed circulation system. The ratio of the power needed for the supplying of this gas to the open and closed systems is estimated to be about 15:1. However, the open system can dispense with the power needed for the circulation of the culture, which constitutes the more significant part of the energy needed for operating the closed circulation system. With these points in mind, the total power needed for the open bubbling system is estimated to be less than 1/5 of that required by the same quantity of culture run by the closed circulation system. In this figure, it should be noticed that no account is taken of the power needed for the cooling of the culture, which, as stated previously, is necessary for the closed system but not for the efficient operation of open system.

1) Relative cost of construction. — According to an estimation of the Arthur D. Little, Corporation, the cost of construction for the growth area of the closed circulation system is about $18,400 per acre (including the cooling system, but excluding the system for harvesting, main system of gas and nutrient supply, and other miscellaneous items such as, the shelter for operators and instruments, etc.). If the open bubbling system is constructed with concrete, the corresponding cost is estimated to be about $38,000 per acre. This cost could, however, be considerably lowered if, in the construction of the ridges, a special type of roller is used for the hardening process and if the mounds are covered with plastic sheeting instead of concrete.

A new culture method, based on an open circulation system with a device for intermittent sweeping, was developed by Japanese workers (9). It consists of a round pond 5 meters in diameter and 20 cm in depth, with a small round island situated at its center. The culture (2000 liters in volume) is circulated (by a pump of 1/2 h.p.) from one corner of the pond and through a pipe placed underground, to two arms which can be rotated and which extend from the center to the periphery of the pond. The arms are perforated with many holes (3 cm apart) which are directed somewhat diagonally downward. These arms are submerged in the culture solution, the clearance between the arms and the bottom of the pond is 4 cm. By the reaction of water flux from the holes, the arms rotate slowly (approximately 180° per minute) and circulate the solution evenly in the pond. Simultaneously this action stirs and sweeps the algae cells in the solution. Near the outlet from the pump a small tube for feeding CO₂-enriched air is inserted into the circulating pipe, thus the culture is evenly supplied with CO₂ during culture.

The advantages of this culture system can be stated as follows:

1) a small amount of energy is needed for the circulation of the culture and the supplying of the carbon dioxide;
2) the precipitation of the algal cells on to the bottom of the culture pond can be satisfactorily prevented;

3) there is no need for any cooling equipment;

4) mixing of the whole culture, which is necessary on such occasions as seeding with fresh algae cells, and the regulation of composition or acidity of the culture solution, can be easily achieved. The only care necessary is in the regulation of the water following a heavy rain, and the occasional removal of accumulated dust.

The possibility of utilizing the traditional system of cultivation of micro-organisms in a submerged culture, in which it is possible to produce dry weights up to thirty times higher than those obtained by the cultures in ponds, cannot be excluded.

Recently some experiments have been run in a new type of fermentor (Fig. 1). Some of these experiments performed in the "Istituto Superiore di Sanità" have given very promising yields. Some strains of algae have reached a dry weight of 0.4-0.5%.

The factors which are important in obtaining a high cellular concentration per unit of surface (this is the datum which gives the real

efficiency of photosynthetic conversion) both in the growth and production stages are:

a) composition of the culture medium;
b) concentration of the CO₂ in the medium;
c) depth and turbidity of the culture.

a) In regard to the composition of the culture medium, great variations exist as regards the mineral requirements of algae. Preference is for nitric nitrogen instead of ammonium nitrogen. The micro-elements which are important for their growth are: Fe, Zn, Ca, Mn, Cu and Mo. The presence of sugars is not necessary:

b) The concentration of CO₂ in the culture medium is strictly correlated with the efficiency of the aeration system of the culture. In fact, methods of enriching the culture with CO₂ are still unsatisfactory and much work remains to be done in this field.

c) The open system of culture utilizes the sun light, obviously intensity varies according to the time of year and to the daily meteorologic conditions. Therefore, the yield of these cultures depends not only on the temperature but to a great extent upon the percentage of utilization of the sun light. This percentage is not only a function of the saturation limit of photosynthesis, but also of the correct choice of culture conditions.

The growth of the culture can be followed by the usual microbiological methods, that is, by the determination of the dry weight directly per unit of volume and indirectly through measurement of the volume of the precipitate after centrifugation, the number of cells per unit of volume, and the optical density.

ECONOMIC ASPECTS

The average annual production obtained with the open circulation culture method (Japanese method) is about 12.5 grams of dry substance per square metre per day, or 125 grams per cubic metre of culture.

This figure is considerably higher than the amount obtained with the open bubbling system which yields 7.6 grams per square metre per day. It is notably lower than the figure of 21.5 grams per square metre per day reported by American authors for the closed cycle system.

Concerning production costs, both Japanese and American workers report a figure of about 25 cents (one quarter of a dollar) per kg of Chlorella for pure protein (the protein content of Chlorella is 50% of the dry weight) which amounts to 50 cents per kg of protein. Unfortunately, this value is at present non-competitive with that given for other proteins such as the ara-

chis protein (25 cents per kg) or yeast protein (30 cents per kg). But proposed improvements in the method of production, particularly that of open bubbling, should make this source of protein truly competitive with yeast derived protein, and with soya-bean protein which is at present the cheapest available.

In any case, the use of algae as a food source has been fairly well studied particularly in Japan. In the United States some attempts have been made, but it seems that in regards to the taste requirements of Americans, the algae are not very palatable. In Japan, on the other hand, cakes, macaroni and even some quite good ice-creams made from algae derived proteins are already available.

BASIC RESEARCH ON ALGAE

Some experiments involving the laboratory cultivation of algae were run in our Institute, with the purpose of using these organisms as a tool for the study of particular metabolic reactions of the living cell. The species Dietyococcus cinnabarinus, of the family Chlorophyceae, quite different from the family Chlorella, was utilized.

The first observations on carotenoid production by Dietyococcus cinnabarinus were made by Wenzinger, Haag and, more recently, Kol. Based on experiments carried out on static cultures and for periods of a few months' growth, these authors reported that when sugars were present in the medium the cultures changed from a green to red colour. This colour change they attributed to the prevalence of carotenoids in the chlorophyll formed.

With these investigations in mind, work in our laboratory was carried out to investigate the behaviour of Dietyococcus cinnabarinus and the eventual carotenoid production in submerged cultures using different sugars, with and without addition of carbon dioxide, and under varying conditions of temperature and light.

The details of these experiments and the discussion of the results have been published (3,4). It is noteworthy to mention that in the absence of sugars the cultures remained green, while in the presence of sugars, namely glucose, the cultures changed colour gradually form green to yellow, pink and to an intense orange colour by the end of the fermentation, which indicated the accumulation of carotenoids.

In fact, six carotenoid pigments were isolated from the culture grown in the presence of sugars. They were then identified respectively as: beta-carotene, echinenone, 3,4-dioxo-beta-carotene, canthaxanthin, neoxanthin and astacin. It should be emphasized that the second pigment, echinenone, does not seem to have been previously demonstrated in the Chlorophyceae.

In addition to the studies of sugar metabolism, other investigations were carried out in regard to proteins and fatty acids.

Electron microscopic photographs showed that in the absence of sugars, chloroplasts are quite evident with their characteristic lamellar form, while in the presence of sugars, the chloroplasts are reduced in number and in their place drops of lipids containing carotenoids appear.

In conclusion, the experiments just mentioned demonstrate once again the usefulness of algae, not only as a promising means in the struggle for the well-being of humanity, but also as a very convenient tool in the investigation of the living cell, which to-day represents one of the most important challenges in scientific research.

REFERENCES

(2) TAMIYA, H. Growing Chlorella for Food and Feed (Conference). The Tokugawa Institute for Biological Research, University of Tokio 1955.
Aspetti sanitari ed economici delle micotossicosi (*)

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Ricordate le attività biologiche dei funghi che risultano utili alla vita dell’uomo sia sul piano sanitario sia su quello economico e produttivo, l’O. sottolinea che altrettanto importanti sono tuttavia le attività dannose dei funghi: alcuni risultano patogeni per l’organismo che essi infettano dando origine a diverse forme di micosi; altri, contaminando i prodotti alimentari, non solo ne determinano un deterioramento che li rende inutilizzabili, ma possono anche trasformarli, coi prodotti tossici del loro metabolismo, in fonti di intossicazioni collettive (micotossicosi dell’uomo e dell’animale da allevamento).

I funghi che contaminano le sostanze alimentari sono gli stessi che si trovano nel terreno e nell’aria. I primi invadono le piante e i semi in via di sviluppo, gli altri contaminano le derrate alimentari quando già sono state raccolte: si tratta di 10-18 specie di Aspergillus e di Penicillium che vivono come saprofiti su una larga varietà di residui vegetali ed animali ed in particolare di semi e di derivati di questi. La contaminazione avviene sempre in condizioni ambientali adatte allo sviluppo dei funghi (umidità del 75-90%, temperatura 25°-35°C). Le perdite economiche derivanti dalla contaminazione dei materiali alimentari non sono trascurabili: si calcola che l’1-2% delle messi annuali venga reso inutilizzabile ai fini sia della semina, sia dell’impiego alimentare.

Le micotossicosi sono sostenute dai prodotti tossici elaborati da Fungi imperfecti, da Ascomiceti e da alcune specie di Basidiomiceti (Tab. 1). Esse possono classificarsi in base ai substrati di crescita di questi funghi:

a) vegetali in fase di crescita (Claviceps purpurea: ergotismo);

b) grano e sementi (Aspergillus flavus: aflatossicosi; Fusarium sporotrichioides: fusariotossicosi);

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<thead>
<tr>
<th>MUFFA</th>
<th>TOSSINA</th>
<th>EFFETTO</th>
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<tbody>
<tr>
<td><strong>1) Aspergillus ochraceus</strong></td>
<td>1) Ocratossina A</td>
<td>— Mortale per bovini, ovini, ratti, topi ed anatroccoli.</td>
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<tr>
<td></td>
<td>2) Ocratossina B</td>
<td>— ?</td>
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<tr>
<td></td>
<td>3) Ocratossina C</td>
<td>— ?</td>
</tr>
<tr>
<td><strong>2) Aspergillus wentii</strong></td>
<td>Sconosciuta</td>
<td>— Mortale per ovini, conigli, anatre e pollame.</td>
</tr>
<tr>
<td><strong>3) Aspergillus amstelodami</strong></td>
<td>Sconosciuta</td>
<td>— Mortale per conigli. Inibisce la crescita nelle anatre e nel pollame.</td>
</tr>
<tr>
<td><strong>4) Aspergillus clavatus</strong></td>
<td>Sconosciuta</td>
<td>— Sindrome emorragica del pollame. Ipercheratosi nei bovini.</td>
</tr>
<tr>
<td><strong>5) Aspergillus oryzae</strong></td>
<td>Sconosciuta</td>
<td>— Mortale per ratti. Attiva su topi.</td>
</tr>
<tr>
<td><strong>6) Aspergillus avenaceus</strong></td>
<td></td>
<td>— 100% di mortalità nelle anatre, nei topi e nei ratti.</td>
</tr>
<tr>
<td><strong>7) Aspergillus flavipes</strong></td>
<td></td>
<td>— 100% di mortalità nelle anatre e nei topi. Alta mortalità nei ratti.</td>
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<tr>
<td><strong>8) Aspergillus nidulans</strong></td>
<td></td>
<td>— Mortale per anatroccoli e pollame. Attiva su bovini, suini, ratti e topi. Alta mente cancerogena.</td>
</tr>
<tr>
<td><strong>9) Aspergillus niveus</strong></td>
<td></td>
<td>— Produce cirrasi ed epatoma nei topi. D.L.50 per ratti e topi = 22 mg/Kg.</td>
</tr>
<tr>
<td><strong>10) Aspergillus carneus</strong></td>
<td>Sconosciuta</td>
<td>— D.L.50 per ratti e topi = 6,5 mg/Kg. Non cancerogeno.</td>
</tr>
<tr>
<td><strong>11) Aspergillus flavus</strong></td>
<td>Aflatossina</td>
<td>— Mortale per anatroccoli e pollame. Attiva su bovini, suini, ratti e topi. Alta mente cancerogena.</td>
</tr>
<tr>
<td><strong>12) Penicillium puberulum</strong></td>
<td>B₁ B₂ G₁ G₂</td>
<td>— Produce cirrasi ed epatoma nei topi. D.L.50 per ratti e topi = 22 mg/Kg.</td>
</tr>
<tr>
<td><strong>13) Penicillium islandicum</strong></td>
<td>1) Luteoschirina</td>
<td>— D.L.50 per ratti e topi = 6,5 mg/Kg. Non cancerogeno.</td>
</tr>
<tr>
<td></td>
<td>2) Peptide ciclico contenente Cl</td>
<td>— Attiva su ratti e topi.</td>
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<tr>
<td><strong>14) Penicillium citrinum</strong></td>
<td>Nefrotossina</td>
<td>— Paralisi nei ratti e topi.</td>
</tr>
<tr>
<td><strong>15) Penicillium toxicarum</strong></td>
<td>Neurotossina</td>
<td>— Setticemia emorragica nei bovini.</td>
</tr>
<tr>
<td><strong>16) Penicillium cyclopium</strong></td>
<td>Sconosciuta</td>
<td>— Mortale per topi e cavie. Epatite nei cani.</td>
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<tr>
<td><strong>17) Penicillium rubrum</strong></td>
<td>Solubile in acqua</td>
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c) paglia e fieno (*Stachybotrys alternans*: stachibrotiotossicosi);

d) terreno e humus (*Amanita phalloides*: intossicazione da funghi velenosi).

L'A. si sofferma in particolare sull'aflatossicosi che negli anni più recenti è stata oggetto di estese ricerche in conseguenza, soprattutto, dei danni che da essa derivano all'allevamento del bestiame (vedi Tavola Rotonda sulle Micotossine pubblicata in questo stesso volume, parte III–IV, pagina 327).

I provvedimenti da adottare nella lotta contro le micotossicosi devono tendere essenzialmente a prevenire la contaminazione evitando quelle condizioni ambientali, e in particolare l'umidità, che favoriscono lo sviluppo dei funghi sui prodotti agricoli all'atto della raccolta e dell'immagazzina-
mento. Questi provvedimenti non possono andare disgiunti da un controllo che, per quanto riguarda l’accertamento di contaminazioni fungine, si basa su metodi di semplice realizzazione. Più complesso risulta invece l’accertamento di un’attività tossigena nelle specie isolate: esso richiede la coltura in fermentazione dei funghi e l’impiego di metodi biologici atti a mettere in evidenza la presenza di sostanze tossiche nel materiale di coltura (tossicità per via alimentare negli anatroccoli, nei ratti, nei topi e nelle cavie; test cutanei nelle cavie e nei conigli; tossicità per via i. v. e i. p. nel ratto e nel topo; effetti sullo sviluppo di embrioni di pollo e di tessuti coltivati in vitro). L’accertamento di un’attività tossica è il primo passo verso l’isolamento dei principi attivi responsabili dell’azione biologica. Infine un campo totalmente aperto che non può essere trascurato per la sua importanza sul piano economico-sanitario è rappresentato dallo studio e dall’elaborazione di metodi di detossificazione dei materiali contaminati.