Cercetări marine	I.R.C.M.	Nr. 4	41-56	1972

THE INFLUENCE OF ORGANIC MATTER ON THE METABOLISM OF SOME ISOLATED MICROPHYTE ALGAE OF THE BLACK SEA PHYTROPLANKTON

Pia Elena Mihnea

Romanian Institute for Marine

Research Constantza

ABSTRACT

The results obtained on three unicellular algae grown in a medium with organic addition are shown. The influence of organic substances is represented as division rate. In order to avoid the influences of other factors on algae metabolism (viz. microelements and vitamins) we tested the cultivation of algae both in media containing these factors, as well as in media lacking them. The results obtained undoubtedly show the importance of organic substance on the metabolism of algae.

One of the modern problems of oceanologic research is the exploration of the interrelations between environmental factors and organisms. With regard to the danamics of planktonic microphytes, a considerable of data is available, where the environmental factors considered major are : temperature, salinity and biogeneous elements.

REDFIELD, KETCHUM and RICHARDS (1966) showed that the

fertility of the ocean is a consequence of major mineral constituents : nitrogen and phosphorus, while on the other hand, the development of the biologic processes in the ocean is deeply echoed in the cycles of these elements.

More recent observations however (BELSER 1966) show that marine areas with very similar nitrogen and phosphorus concentrations are quite different from the point of view of the amount of phytoplankton.

It is known that the waters along the Californian coast contain large amonts of phosphorus, and that they show a much lesser productivity in comparison with the waters near the British Isles, which sustain a massive development of phytoplankton, although they contain little phosphorus (PROVASOLI 1966).

The problem of the existance of other essential factors for the development of phytoplankton was first set by ALLEN, (1914) and at present we possess a great number of results which indicate without doubt the influence of certain organic substances on the biology of aquatic organisms (DROOP, 1964; PROVASOLI & PINTNER 1953; PROVASOLI & MACLAUGHLIN, 1963 ; PROVASOLI, 1963;PINTNER & PROVASOLI, 1963; SWEENEY, 1954; BELSER, 1966; NORTH & STEPHENS, 1968; NORTH & STEPHENS, 1971).

Dissolved organic matter is represented in the marine environment by carbohydrateds, proteins, amino-acids, amines, peptone - lipides, organic acids, and also by biologically active compounds as vitamins and hormones (GAVRILESCU, 1964; JÖRGENSEN, 1966; PROVASOLI 1966; SAMEJINA and MYERS, 1958; GUILLARD, 1963).

Frequently the detection of these organic compounds is very difficult because the concentrations of some of these are very low This is,however,also the reason why there is only a small numer of data on the amounts and even on the exact appertaining of many of these substances.

The source of these compounds is constituted among others, by the organisms themselves and particularly by the unicellular algae.

All data obtained from tests with algae cultures show the utilization of certain organic products both under illuminated conditions

(GUILLARD, 1963; PROVASOLI, 1963; McLAUGHLIN, 1963; PINTNER PROVASOLI, 1963) as well as under darkness conditions (LEWIN, 1963 ; SAMEJIMA & MYERS, 1958).

Thus the unicellular vegetal organisms require for their vital cycle organogenic mineral substances, microelements and also dissolved organic substances. Which one of these substances is indispensable for the vital cycle of unicellular vegetal organisms and which role is played by each of the chemical entities mentioned, must be established individually for each systematic group or species (PROVASOLI, 1966).

The observations on the nutrition of marine microphytes open wide prospects for the explanation of the phenomena of dynamics and dispersion of aquatic organisms, and for the detection of species or physiologic races indicating pollution, and for the identification of those algae which participate at the purification of polluted marine zones. The results therefore of such observations are widely applicable in practical industrial cultivations of microphyte algae.

The purpose of our work is to establish the requirements of some species of isolated Black Sea chlorophyceous algae, in order to be able to explain both their behaviour in their natural habitat, as well as in order to use them with maximum efficiency for their practical artificial cultivation.

METHODS AND WORKING TECHNIQUES USED

The approach for such investigations is constituted by testing the influence of various concentrations of those pure organic substances, the presence of which was or was not detected within the natural environment.

We opted for the use of B-vitamins and of organic extracts, the complex biochemical composition of which is well known and which we added to the mineral medium. Depending on the mode of reaction of the algae under the influence of the respective organic extract, we will then identify the compound or association of compounds influencing the

development of the culture. The mineral PMS[5] medium is composed of sterilized sea water, fertilized with nitrogen and the complex chelate Na_2 E.D.T.A. Each experiment was doubled by a PMS[5] medium with synthetic sea water in order to have a check on the results obtained. The culture media - either with natural or with synthetic sea water - were prepared at the same date and then stored for two weeks in order to make sure, the electrolytes are uniformly distributed. The extract was prepared in PMS[5] medium, and various aliquotes were added before innoculation.

The "mother" cultures of Chlamydomonas, Tetracoccus and Platymonas algae were washed four times in distilled water before innoculation, for the total removal both of salts and of excretion products in case of their presence in the culture. As far as possible, the innoculation was performed with a similar number of cells per cubic centimetre, in order to enable the results to be compared.

The culture media, thus innolucated were exposed to light - intensity 4000 luces - and dark alternations :8/16 hrs and continous air bubbling.

The response of the algae to the action exerted by the composition of the culture medium is expressed in rate of division $N/N_o = kt$, where N_o = the initial number of cells,

N = number of cells obtained through division,

kT = rate of division in time

In order to establish the preponderance of the action of the mineral or organic substances in the nutrition of the above three species of algae selected, the culture media were prepared as folows :

1. Mineral medium PMS[5] ;

2. Mineral medium PMS[5] with microelements (Fe^{3+} = 10.3 mg/1; Mn^{2+} = 1.45 mg/1; Zn^{2+} = 0.24 mg/1; Co^{2+} = 0.03 mg/1) and vitamins (B_1 = 0.50 mg/1; B_2 = 0.50 mg/1; B_6 = 5 mg/1; B_{12} = 5 gamma/1), plus organic extract (10 ml/1) ;

3. Mineral medium PMS[5] with vitamins (same amounts as for (2.-) and organic extract (10 ml/1);

4. Mineral medium PMS[5] with organic extracs (10 ml/1) ;

5. Mineral medium PMS[5] with organic extracts in various concentrations : A = 5 ml/1, and C = 20 ml/1 ;

6. Sterilized sea water plus organic extract (20 ml/1).

Results

I. Microelements. The determination of the role played by the addition of microelements on the nutrition of unicellular algae was possible through the parallel cultivation of Chlamydomonas algae in type 2 and 3 media. From an examination of fig. 1 we can see that the cell division rate is very similar in both types of medium. Towards the end of the period of observation, the behaviour of both cultures is identical. This fact entitles to the affirmation that the sea water probably contains sufficient amounts of microelements for the nutrition of the algae investigated. The additions in the quantities tested by us apparently influenced in no direction the cell division rate.

The results obtained in this test constituted the base for making all the other variants without microelements.

II. Vitamins. The role of vitamins in the synthesis and cell division process of Chlamydomonas, Platymonas and Tetrecoccus algae was demonstrated by comparing variants 3 and 4.

In case of Chlamydomonas, the development takes place nearly identically in both variants (fig. 2).

For Platymonas, too, very similar rates of division in both variants of medium were obtained.

The rate of growth of the Tetracoccus alga (fig. 4) is characterized by a very high rate of cell division both in the vitamin-additioned medium, as well as in the medium where no vitamins were added. The character of the development of the cultures stays very similar right from the first day, till the 17-th day of the culture. It is to be noted, however, that in case of the variant nr. 3 with vitamin addition, the rate of growth was higher.

If therefore, in the case of Chlamydomonas and Platymonas

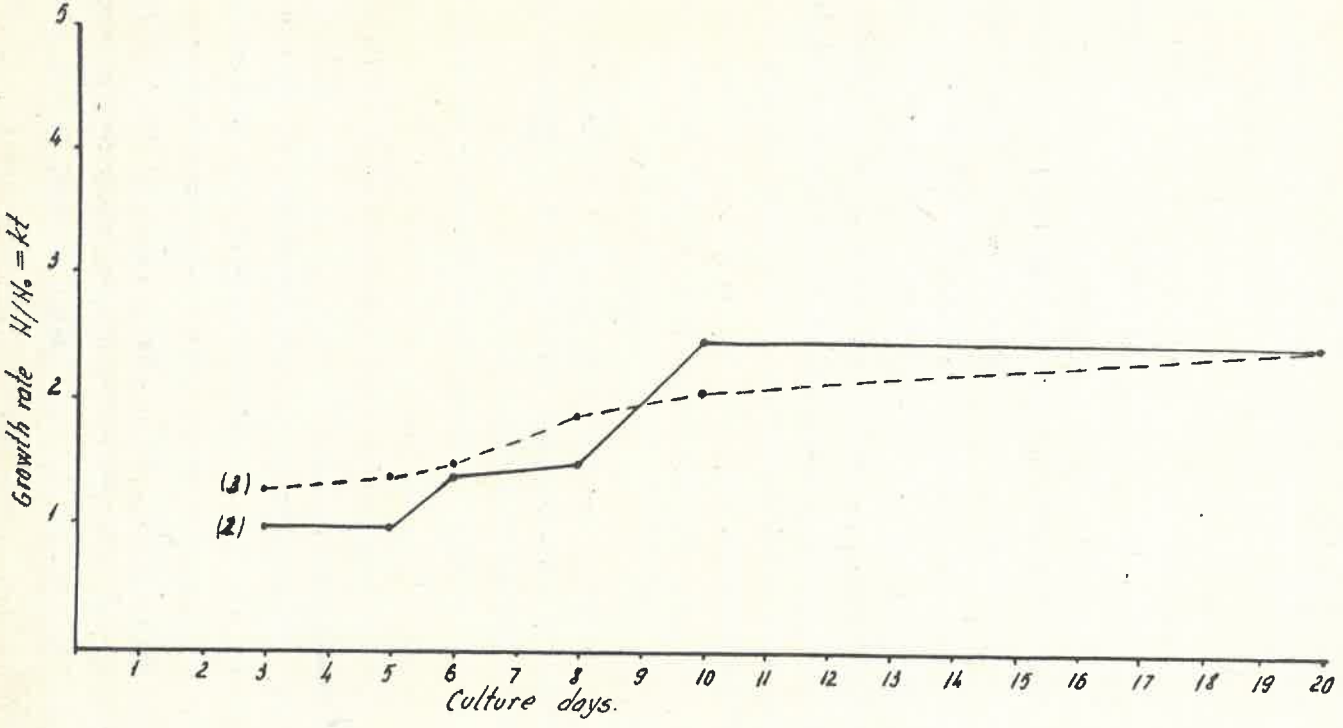


Fig. 1 - Growth rate of Chlamydomonas algae, in PMS[5] medium variants 2 and 3.

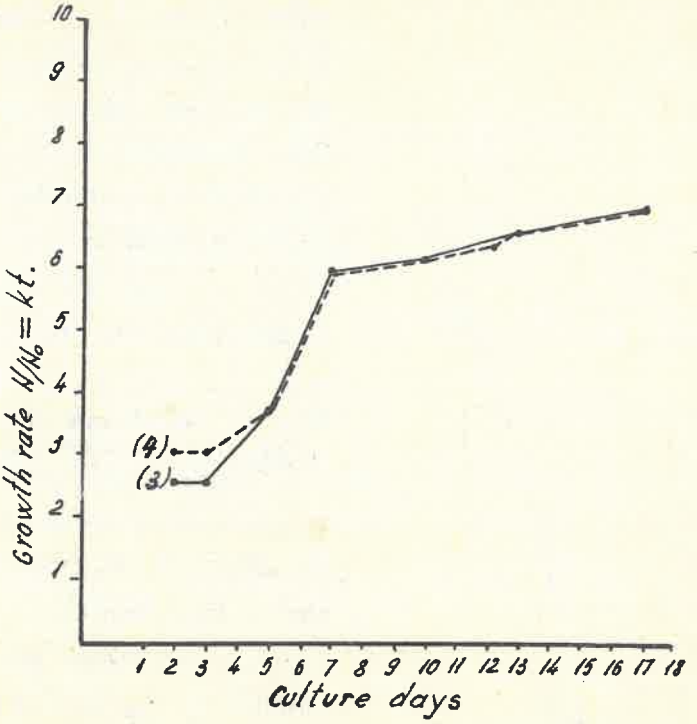


Fig. 2 - Growth rate of Chlamydomonas algae, in PMS[5] medium variants 3 and 4

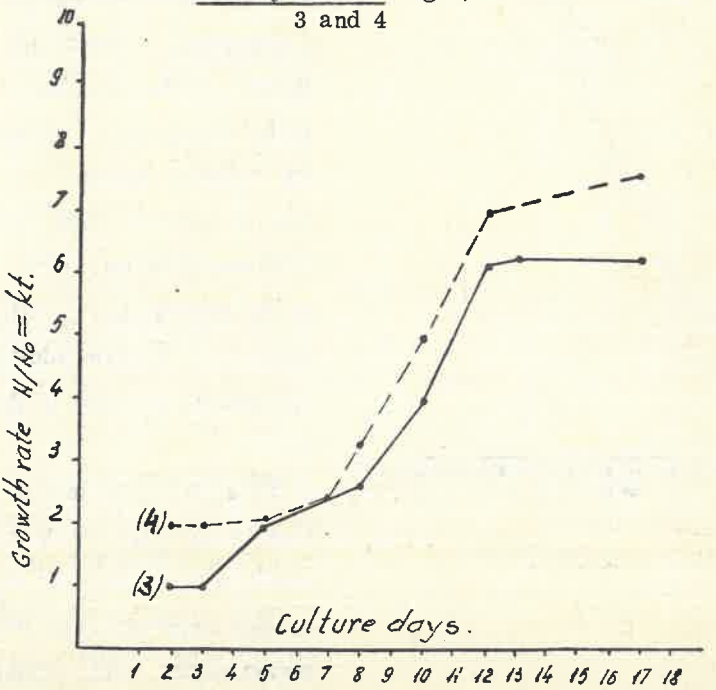
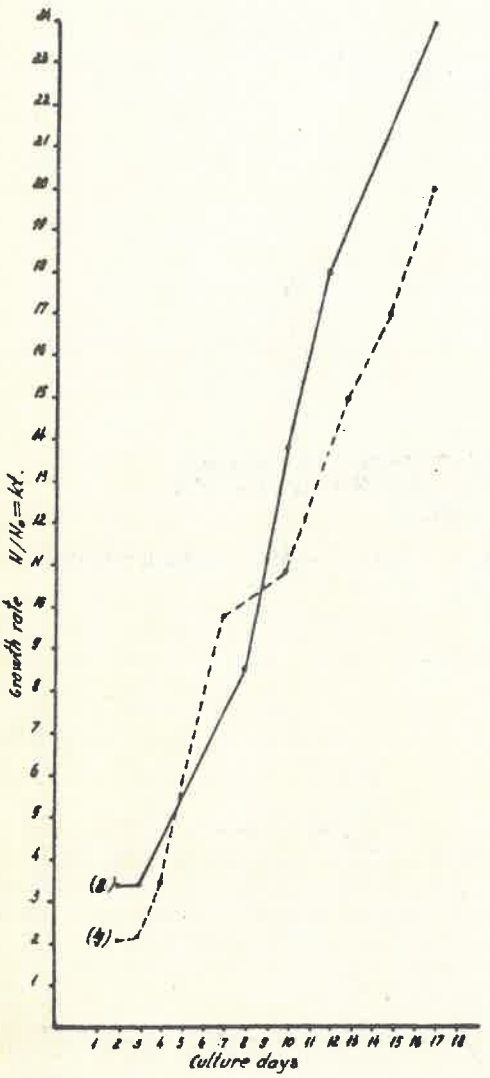


Fig. 3 - Growth rate of Platymonas algae, in PMS[5] medium variants 3 and 4.



algae, it appears that vitamin addition does not influence the growth of the cultures, the presence of vitamins in a Tetracoccus culture does stimulate the metabolic processes. We have however to point out, that Tetracoccus grows very well also in

Fig. 4 - Growth rate of Tetracoccus alga in PMS[5] medium, variants 3 and 4

the absence of vitamins, which enables the affirmation that for the cases investigated by us, the vitamins B_1, B_2, B_6 and B_{12} can just stimulate the development of Tetracoccus, but are no intrinsic factors of the existance of none of the above three species tested.

III. Organic extract. The cultivation of algae in PMS[5] mineral salt-fertilized sea water, as well as in mineral salt fertilized sea water with organic additions (fig. 5, 6 and 7) resulted in the following :

- for each of the tested species, a weak development in the exclusively mineral variant (var. 1) ;
- netly superior rates of cell dedivion in the PMS[5] variant with organic extract addition (var. 5).

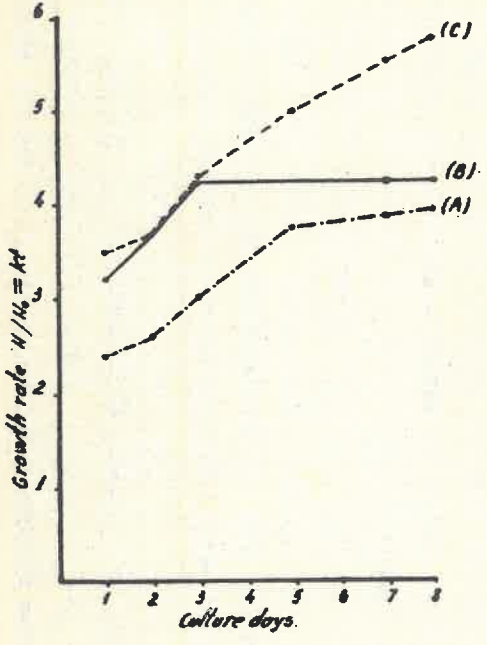


Fig. 5.

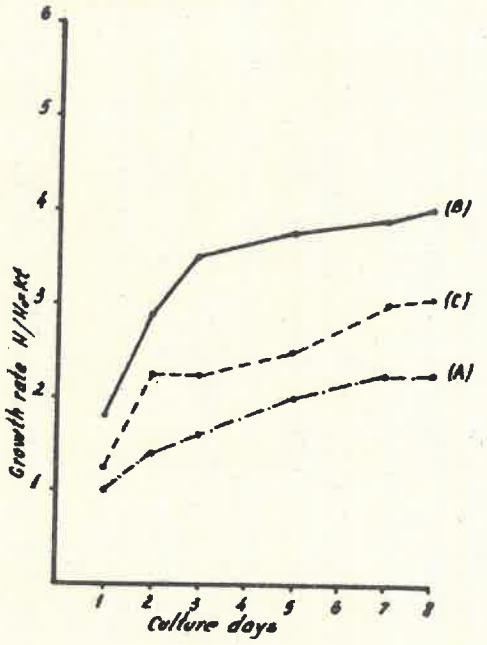


Fig. 6.

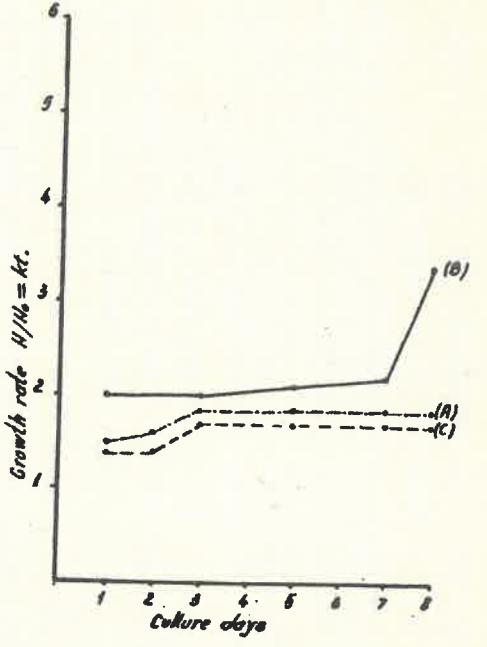


Fig. 7

Fig. 5, 6 and 7 - The influence of organic extrac in different concentrations on the growth rate of the algae Chlamydomonas, (fig. 5), Tetracoccus (fig. 6) and Platymonas (fig. 7) A=5 ml/1; B=10 ml/1; C=20 ml/1

The very large differences between the rates of division demonstrates the role of the dissolved organic substance.

In considering the above results, we investigated the influence of the organic extract concentration on the development of the cultures of the three species of algae. For the ease of the discussion of the results, we designated : A = a concentration of organic extract of 5 ml/l; B = a concentration of 10 ml/l and C = a concentration of 20 ml/l.

For Chlamydomonas, the rate of growth (fig. 5) increases with the increase of the organic extract concentration. Moreover, for concentration A and B, Chlamydomonas, enters the stationary phase after the first days of culture, while for concentration C the exponential phase continues also beyond the eighth day of culture. From fig. 6 it may be seen that for Tetracoccus the optimum concentration is 10 ml of organic extract per litre. Concentrations A and C yield high rates of division but incomparably lower than concentration B. For Platymonas, also concentration B causes optimum stimulation of the culture, although a rate of division of kt = 2 continues until the seventh day of culture, after which the development enters the exponential phase.

For concentrations A and C we found a weak development of the cultures during the first three days, after which the cultures enter the stationary phase.

Thus, not only the presence of the organic substance, but its concentration acts on the metabolic processes of the algae tested.

Taking into account the varied roles which may be played by dissolved organic substances, as : solubilizers for mineral salts, chelates, stimulators or nutritions substances (PROVASOLI, 1963; PROVASOLI & McLAUGHLIN, 1963; LEWIN, 1963; NORTH & STEPHENS, 1969 ; NORTH & STEPHENS, 1971) we introduced into the experiment variant Nr. 6 (sterilized sea water with organic extract addition). At the same time, the development of a culture in variant Nr. 5 (mineral substance - fertilized sea water with organic extract additions) was followed. The respective results are shown in fig. 8, 9 and 10. From an examination of the rates of division obtained in the variant Nr. 6, it may be seen that a

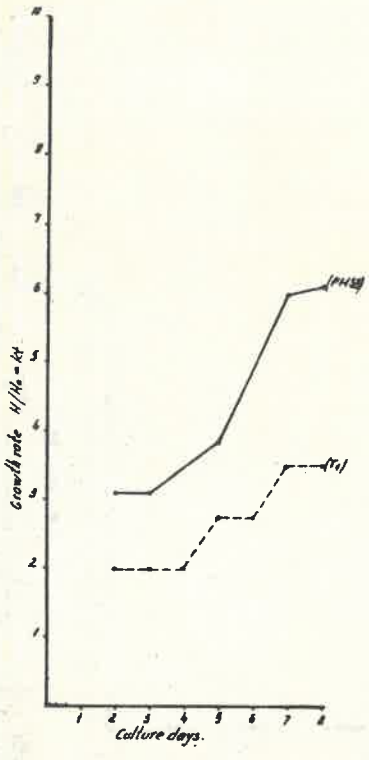


Fig. 8 - Growth rate of Chlamydomonas algae in PMS[5] medium with organic extract addition (-) beside the medium done of sea water and organic extract addition (-----): T_1

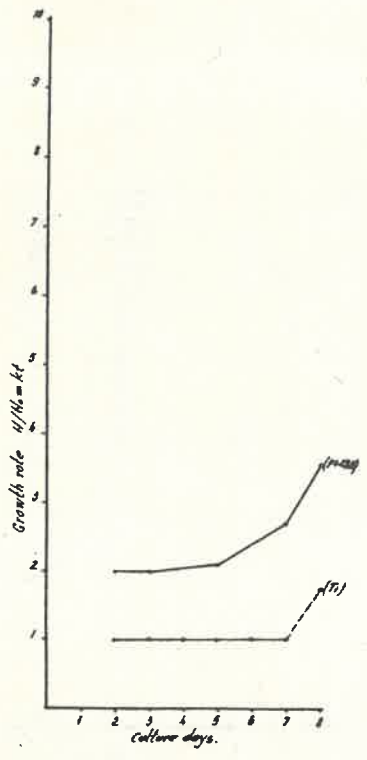


Fig. 9 - Growth rate of Tetracoccus algae in PMS[5], medium with organic extract addition (-), beside the medium done of Sea water and organic extract addition (-----): T_1

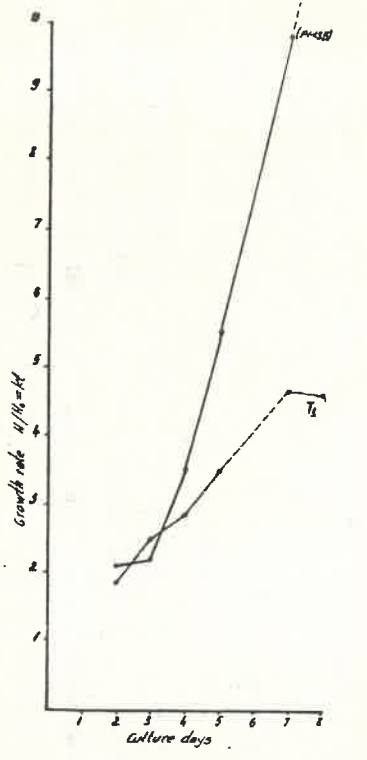


Fig. 10 - Growth rate of Tetracoccus algae in PMS[5], medium with organic extract addition (-), beside the medium done of Sea water and organic extract addition (——): T_1

good development of the three algae is possible, higher rates of division being obtained with Chlamydomonas and Tetracoccus, in comparison with Platymonas. Comparing however the development of the cultures in variant Nr. 6 with that of the cultures grown in variant Nr. 5, it will be seen that the latter medium sustains a much more active development.

The development of the cultures in these two media, demonstrates without doubt the importance of organic substances in the metabolic processes of microphyte organisms.

IV. Development in darkness conditions. In order to determine whether the algae investigated feature heterotrophic abilities, their development under complete darkness conditions was tested in a medium obtained by adding organic extract to sterilized sea water.

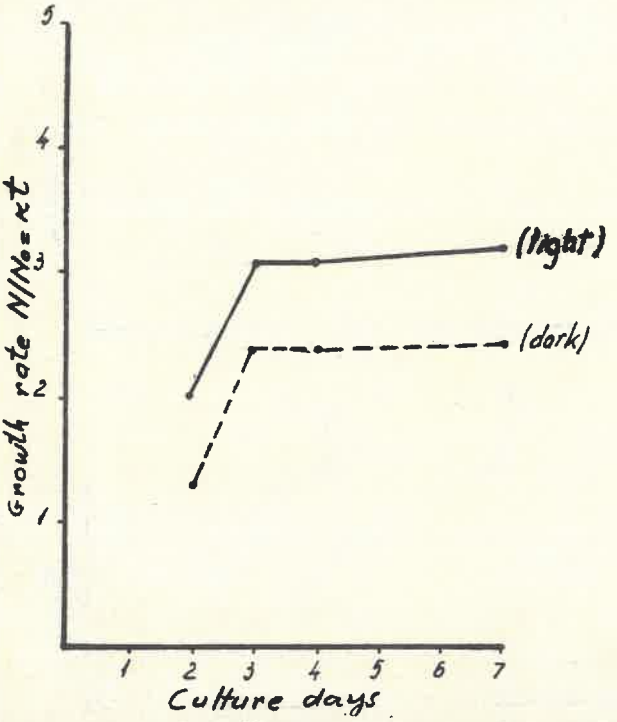


Fig. 11 - Growth rate of Chalmydomonas algae, in Sea water with organic extract (the variant 5), in the light (-) and in the dark (----)

Doubtless results were obtained only with Chlamydomonas (fig. 11). The rate of division in darkness is somewhat lower than the rate of division in day light, the respective plots keeping an absolutely similar

character in both experiments. Therefore we are in a position to state that, at least for Chlamydomonas, dissolved organic substances play the role of nutritions substratum. For the other two species of algae the stimulative action on the cell division processes is certain.

Discussions

From the results presented so far, it is clear that from the wide category of "organic substances" the vitamins can have a slight stimulative role, while the organic extract certainly does influence the rhythm of cell division.

For a considerable number of unicellular algae, the presence of vitamin B_{12} and of the other cobalamines is essential. GUILLARD (1963) showed that out of twenty two species of microphytes, sixteen require vitamin B_{12}, two require thiamine, one requires vitamin B_{12} plus thiamine, and only two are capable to develop without vitamins.

It is known however that marine unicellular algae are among the vitamin producers. PROVASOLI (1966) stresses out the possibility of vitamin synthesis both in axenic and in nonaxenic conditions.

Thus, in our case, the development of the cultures nothwithstanding the addition or lock of vitamins, enables the presumption that these specise are either capable to synthesize their own vitamins, or else they do not require vitamins for their development.

Our present knowledge on the utilization of organic substances shows that species from very different systematic groups, as well as in various habitats can use as source of nitrogen substances like ammonia, ureea, uric acid, aminic acids (GUILLANRD; 1963).

The extract used by us is a complex containing the main aminic acids. Their consumption as nitrogen sources in the presence of light may be interrelated with the zone of origine of these algae : the N-W corner of the Black Sea, and the littoral zone where there is a larger supply of organic matter than in the open sea.

The development of Chlamydomonas in darkness conditions is

a positive proof of its auxotrophy, connected also to the fact that this species of algae develops usually in coastal waters or in the vicinity of tributary river mouths.

The utilization of organic compounds, both under illuminated and as well in darkness conditions, supplies a means for the interpretation of the small amounts in which generally speaking organogenuous mineral substance, and particularly phosphates are present. Through the utilization in an organic state of, for instance, phosphorus-countaining compounds, this element enters in a mineral state only in very minute amounts (PROVASOLI, 1966). Therefore, the reference of the amounts of phytoplsnkton to the dynamics in the mass of water, of phosphorus only, cannot constitute a real reflection of the dynamics of algal communities.

On the other hand, the development of certain species up to "blooming" proportions, can be explained, among other causes, through the supply of organic matter. An illustrative example is the development in large amounts of certainly auxotrophic organisms, as Cyclotella caspia and Exuviaella cordata in the coastal waters, particularly in periods following floods of the Danube river.

The possibility to determine which one of the organic substances and in what amounts, stimulates the development of algae, will supply a means for the interpretation of the ecology of vegetal planktonic organisms.

The problem of the investigation of the abilities of microphyte vegetals, of using organic substance, apparently represents upmost importance, at least from two practical points of wiew :

- the use of algae as factors for the identification of polluted zones and as a means of purification of the zones where domestic waters are discharged into the sea;

- the successful cultivation of microphyte algae in media containing domestic wastes as source for organic substances.

In conclusion, it results clearly that our study must be supplemented by detailed observations on the clear biochemical appertainment of the "Stimulating" or "nutritions" factors. This necessity is

deducted from the results of our experiments, which demonstrate that the algae respond in different ways to the various concentrations of organic substances proving the highly specificity of these organisms.

We also consider necessary to continue our observations on a larger number of species appertaining to various systematic groups.

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