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THE INFLUENCE OF SOME DIFFERENT ORGANIC EXTRACTS ON THE METABOLISM OF THE MARINE *Chlorella sp.* FROM THE BLACK SEA.

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ABSTRACT:

Organic extracts of different origin and various concentrations have influence on the division rate as well as on the cellular products content. Division rate and protein content has increased proportionally to addition of yeast extract in 10 to 150 % concentration. Division rate was stimulated by addition of mussel extract in 10 to 50 % concentration. Protein synthesis is directly proportionally to organic extract concentration. Our experiment has proved that yeast extract has a stronger effect than mussel extract.

A previous work has shown the influence of organic extract on dividion rate of some unicellular marine algae : Chlamydomonas sp., Platymonas impellucida (McLACHLAN and PARKE), and Tetracoccus sp. (4).

The present paper presents the influence of three different organic extracts in different concentrations on the developing in culture conditions of a Chlorella strain as well as some preliminary data about protein synthesis.

METHOD OF WORK

The culture was done in neutral glassware, continuously aerated; the temperature was $19^{o}C^{\pm 2}$. Light (5,ooo lx) and dark periods were 16/8 hours. Starvation of mother culture by maintaining one week in sterilized sea water was used to obtain strong responses from the algae. Thorough rinsing by distiled water before chemical analyses were absolutely obligatory. Culture media composition have been very simple one (Tables 1 - 2). Organic extracts were prepared from mussels, yeast and domestic wastes sludge. The experiments was carried on with separated and mixed extracts.

Table 1

Chemical composition of PMS_5 culture medium (for 1 l sterilized sea water)

Substances	g/l
N as NO_3^-	o.28
N as NH_4^+	o.oo5
Fe^{2+}	o.ol
S as SO_4^{2-}	o.ol2
H_3BO_3	o.15
Na_2 EDTA	o.o825

Table 2

Chemical composition of 46 variants medium (mg/l of sterilized sea water)

Substances	Variants of medium						
	46	46A	46B	46C	46D	46E	46F
N as NO_3^-	14o	21o	35o	456	57o	684	171o
P as PO_4^{3-}	114	114	114	114	114	114	114
Na_2EDTA	5o	5o	5o	5o	5o	5o	5o

RESULTS AND DISSCUTIONS

The heterotrophy of unicellular marine algae was revealed by using of marked organic products (5; 6; 7; 11).

Most papers present results referring to synthesis products in a given step of the biological cycle (Table 3). VAKLINOVA and DENKEVA (14), CHAMPIGNY (2), for exemple, reported a protein content variation in different algae depending on nitrogen source. The authors have emphasized that ammoniacal nitrogen involved a much larger protein synthesis than nitrite and nitrate nutrition. Those authors, as well as others, has shown only the organic products utilization nor the influence of different concentrations of organic extracts on the metabolism, as we did in this paper.

Table 3

The content of proteins in some unicellular marine algae

Algae	Proteins % from dry weight	Authors
Monochrysis lutheri	49	(8)
Skeletonema sp.	37	(8)
Coscinodiscus sp.	17	(8)
Amphydinium carteri	28	(8)
Exuviaella sp.	31	(8)
Dunaliella salina	57	(8)
Scenedesmus sp.	56	(10)
Scenedesmus acuminatus	62,04	(1)
Chlorella pyrenoidosa	52,3	(9)
Chlorella ellipsoidea	37,5-46,7	(1)
Cyclotella caspia	32,5	Our data
Navicula sp.1	33	"
Navicula sp.2	18,6	"
Navicula sp.	47,5	"
Chaetoceros sp.	37,9	"

The developing of algae in PMS_5 media shows an increasing when mussels extract in 10-50 ml ‰ was added (Fig.1). Increasing of the mussel extract addition to 100 ‰ produced a division rate very closed to the control, even slight smaller than that, after the 3rd biological cycle. In concentrations bigger than 150 ‰ the division rate was small, so the curve has slow values beeing much smaller than control. So, mussel extract additions of 10 to 50 ml ‰ are optimum as 100 ml ‰ is the upper

limit for organic extract utilization.

We have to emphasize that bacteria are not involved in that process. The microscopical examination has shown a low and equal number of bacteria for all the tests.

Yeast extract has stimulated the growth culture for all the tested concentrations within 1o to 15o ml %o (Fig.2). We like to emphasize that yeast extract addition gives to growing phase a sudden feature, for the first biological cycle, the maximum values beeing at 3o ml %o and 5o ml %o concentrations(Fig.2,curves 9 and 1o). These concentrations sustain the same division rate during four biological cycles. For all others used concentrations after a first high biological cycle follows a slow growth; 15o ml %o is an exception to this: after four biological cycles the division rate (K) is equal to 14.

3o ml %o yeast extract induced the strongest response; the bigger ones although have stimulated the division giving bigger values than control,were lower than that (Fig.2).

Comparing the effects of mussel and yeast extracts we notice distinctly superiority of the last one (Figs.3 - 6). The curves show a similar pattern of culture developing for both organic extracts. This proves that the effect of both extracts is on the metabolism of the formative substances, the range of influence beeing different.

Although, smaller concentrations than 1o ml %o additions from each extract has produced a low response, the mixing of mussels, yeast and sludge extracts has given a marked response. The addition of 5 ml %o mussels, 2,5 %o yeast and 2,5 ml %o sludge extract has produced a stimulation of growing for almost all variants of 46 media (46 media is one of mineral media that gives a good growth of this alga) (Table 2 and Figs.7 - 9).

In the same time with tests for influence of organic extracts on division rate were analised the proteins dynamics.The Fig.1o illustrates the correlation between organic extract concentration and proteins synthesis. It points clear a direct relation between the organic extract concentration and proteins concent; it has revealed higher values for yeast extract than others.

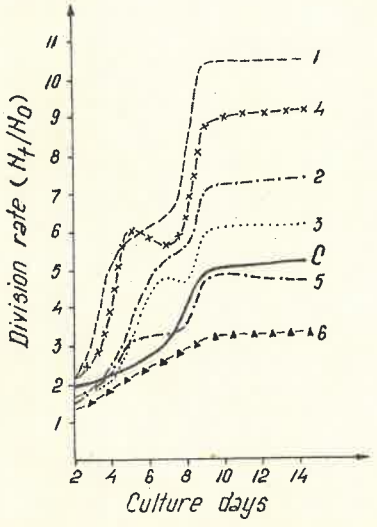

Fig.1. The developing of Chlorella sp. culture in PMS_5 media with mussel extract addition. Extract concentration: 1=10‰; 2=20‰; 3=30‰; 4=50‰; 5=100‰; 6=150‰; C = control (PMS_5).

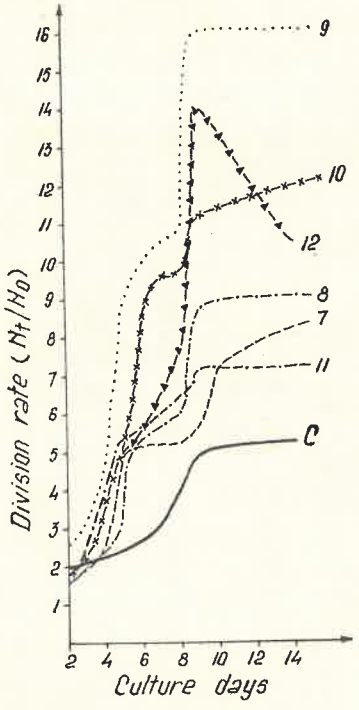

Fig.2. The developing of Chlorella sp. culture in PMS_5 media with yeast extract addition. Extract concentration: 7=10‰; 8=20‰; 9=30‰; 10=100‰; 11=110‰; 12=150‰; C = control (PMS_5).

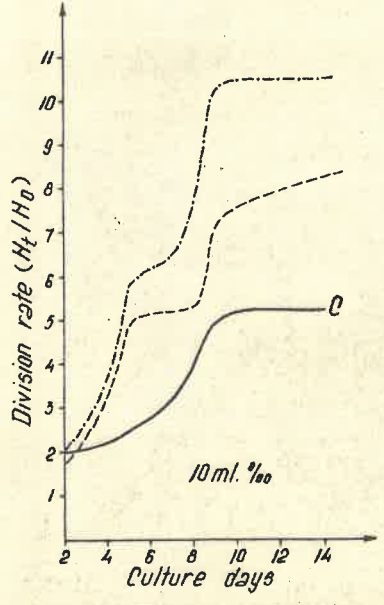

Fig.3. The developing of Chlorella sp. culture in PMS_5 media with mussel(-.-) and yeast (---) extract addition in 1o ml‰ against control (C).

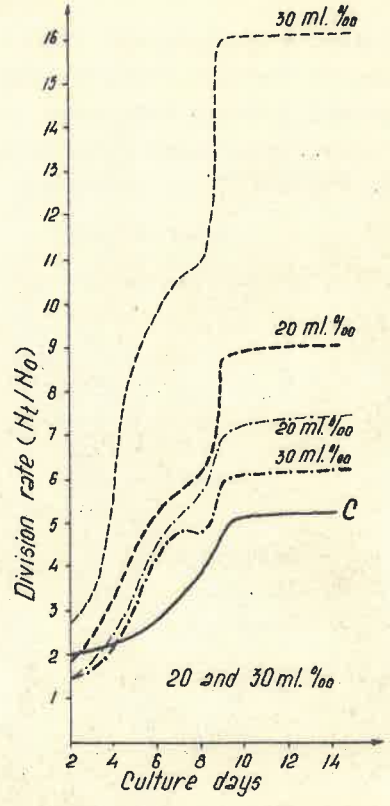

Fig.4. The developing of Chlorella sp. culture in PMS_5 media with mussel(-.-) and yeast (---) extract addition in 2o, 3o ml‰ against control (C).

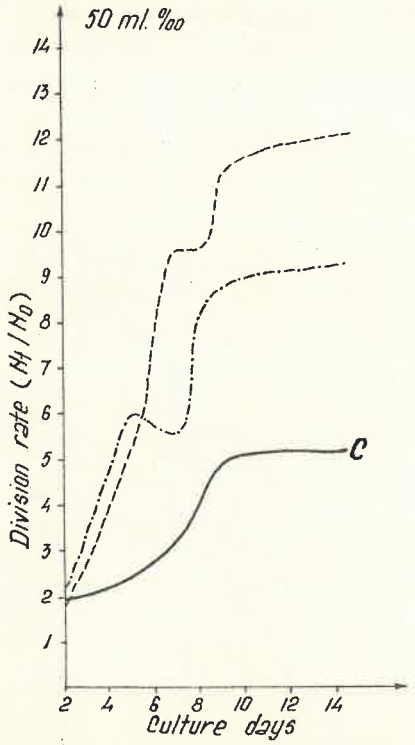

Fig.5. The developing of Chlorella sp. culture in PMS_5 media with mussel (-.-) and yeast (---) extract addition in 5o ml ‰ against control (C).

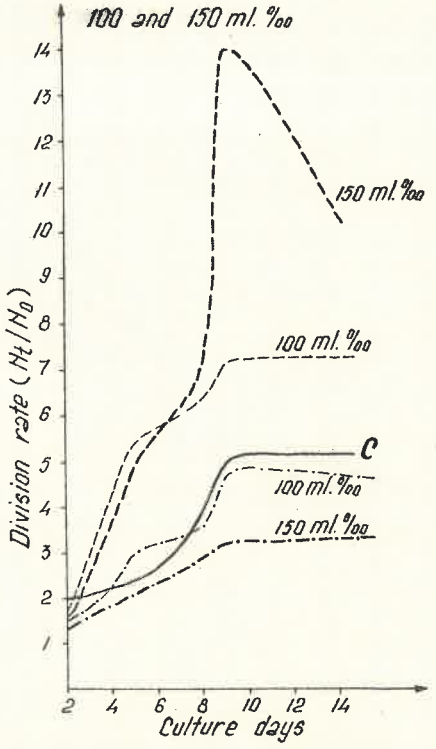

Fig.6. The developing oh Chlorella sp. culture in PMS_5 media with mussel (-.-) and yeast (---) extract addition in loo and 15o ml ‰ against control (C).

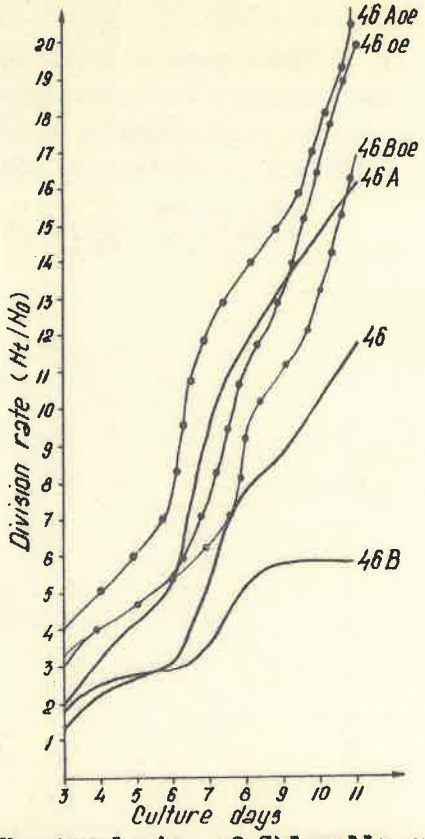

Fig.7. The developing of Chlorella sp. culture in four variants of 46 media (____no organic addition; o-o-o with organic addition).

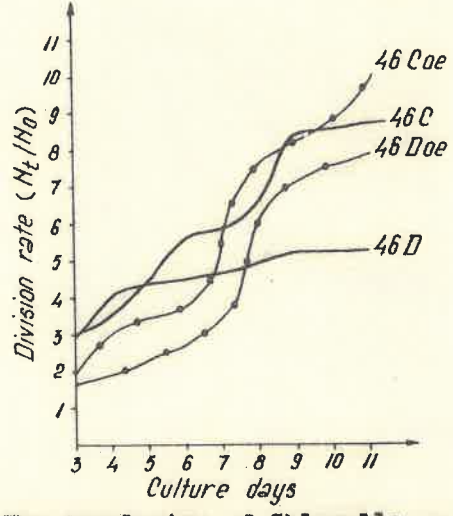

Fig.8. The developing of Chlorella sp. culture in four variants of 46 media (____no organic addition; o-o-o with organic addition).

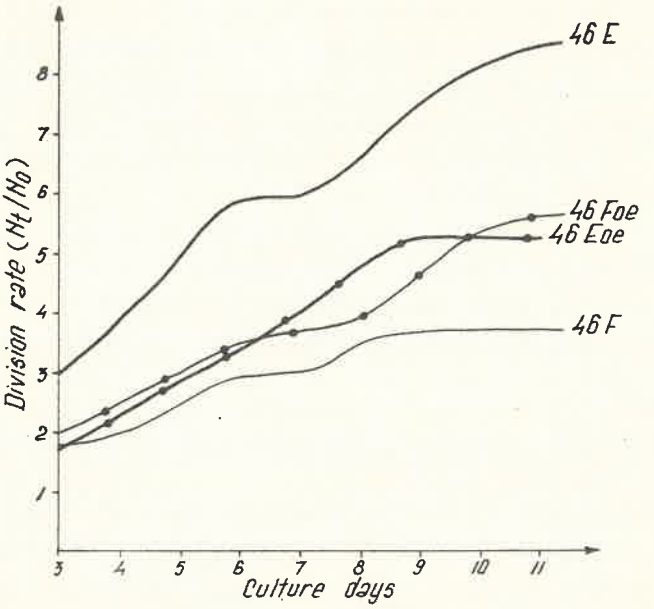

Fig.9. The developing of Chlorella sp. culture in 46 E and 46 F media (———) no organis addition, (o-o-o) with organis addition.

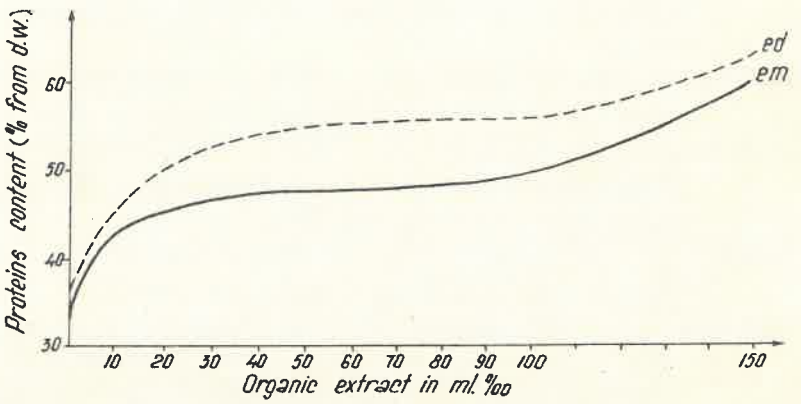

Fig.1o. Proteins content of Chlorella sp. culture in PMS_5 medium depending on organic extract concentrations; 1 = yeast extract; 2 = mussel extract.

Take into account the division rate (Figs.1 - 2 and 7 - 9) and proteins content (Fig.1o) the followings are noticed:

- the division rate and the proteins content are directly proportional to yeast extract concentration;
- mussel extract in concentration to 5o ml %o stimulated the division rate;
- all the experimented mussel extract concentrations have increased the protein synthesis;
- the proteins synthesis pattern was the same for both extracts although the values for mussel extract were smaller.

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