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THE INFLUENCE OF N/P RATIO ON THE SYNTHESIS AND GROWTH PROCESSES OF *Chlorella sp.* ALGA, ISOLATED FROM THE BLACK SEA.

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ABSTRACT:

Preliminary results on the influence of N/P ratio in marine unicellular algae Chlorella sp. are presented. The authors correlate the culture growth stadium with mainly cellular compounds synthesis.

The studying of nitrogenous and phosphorus effect on the cultures or natural communities developing (3; 4; 5; 9; 13; 15) was approached in most cases as an aspect of absorption of different forms of these trophic ions. The effect acted by nitrogenous or phosphorus compounds are limited generally, only to division rate process.

There is a small number of data on the protein dynamics. DAUMAS (2) has studied free and proteic amino acids variation during Phaeodactylum tricornutum BOHLIN diatom growth and founded a closed interrelation between developing stadies of the cultures and amino acids synthesis.

According to THOMAS and DAUMAS (14) Dunaliella salina TEOD. presents also, a parallelism of protein and carbohydrates synthesis with culture developing stage.

In the same time, there are very few data about the influence of N/P ratio, requested in nutrition, on the cellular compounds synthesis.

This paper presents the first results on the influence of N/P ratio both on the Chlorella sp. growth and protein synthesis.

METHODS

Chlorella sp. was chosen because from the previous studies, nutritional peculiarities were known.

The cultures were done in cylindrical shape neutral glassware of 1,000 ml capacity.

Cells were suspended in medium by continuous aer bubling.

The temperature was 19± 2°C, and the illumination (5,000 lx) and dark periods alternated 16 to 8 hours.

Results presented in this paper are the average of three consecutive experiments.

The mother culture was grown one week in 46 medium (see the paper about the influence of organic extracts on Chlorella sp. growth, in this volume) lacked by N, all others conditions being absolutely identically to those of the future cultivations.

Algae were thoroughly washed with sterilized see or distiled water before insemination as well as before every analysis.

RESULTS AND DISSCUTIONS

From existent knowledge, usual quantities of the two nutrient anions N and P there are on the 1o/1 ratio for the most culture media. For instance, HUMPHREY (7) has grown Nitzschia closterium (Ehr.) W.Sm., Gymnodinium sp. and Skeletonema costatum (Grev.)Cl. in nutrient solution in which the two ions were in the mentioned ratio. The same ratio is recomanded for optimal growth of Microcystis sp. (1) Cylindrotheca closterium var.californica (Merschk) REIMANN & LEWIN (8) and Dunaliella salina (14).

The experiments of enriching sea water done by MENZEL and coworkers (1963) shown that 1o/1 ratio allowed a fast growing of the big majority of the phytoplankton constituents.

There are also some evidence that prove a different optimal ratio of N/P.

Taking into account the quantities in which the N and P compounds are founded in the culture media given by different authors, according to experimented species of algae, the N/P ratio can also be: 4/1 for Gonyaulax polyedra STEIN (12), 15/1 for some phytoplanktonic algae (13) and the same 15/1 for Phaeodactylum tricornutum (2). PARSON, STEPHENS and STRICKLAND (11) founded for eleven species of unicellular algae that N/P ratio can range between 5·5/1 to 17·4/1)+.

In our experiment the variants of medium were adjusted for N/P rations between 1/1 - 15/1 (Table 1).

Table 1

The variants of medium tested for N/P ratio

Variants	46	46A	46B	46C	46D	46E	46F
N/P ratio	1/1	2/1	3/1	4/1	5/1	6/1	15/1

Chlorella sp. has grown well in all variants but showed significant differences for each of them (Fig.1,A).

The lag phase was not longer than one life cycle. Exponential phase has became after one or three days. Along first days have been remarked the lowest values of division rate)++ k=4 and k=3,6 for 46D and 46F variants, corresponding to 5/1 and 15/1 N/P ratio, respectively. During this time in the others, the division rate has reached following values: 8 for 46A and 46C, 8·5 for 46 and 17 for 46B.

The exponential phase was going till the 7^{th} or 8^{th} day of experience and the differences have became more evident, like followed.

The ratio 2/1 (46A variant) sustained a division rate closed to 1/1 ratio (Fig.1,A).

)+All the ratios were calculated by us after the respectiv culture media.

)++From $k=k_t/k_o$, where k_t is the number of cells/ml at a given time and k_o is the initial number of cells/ml.

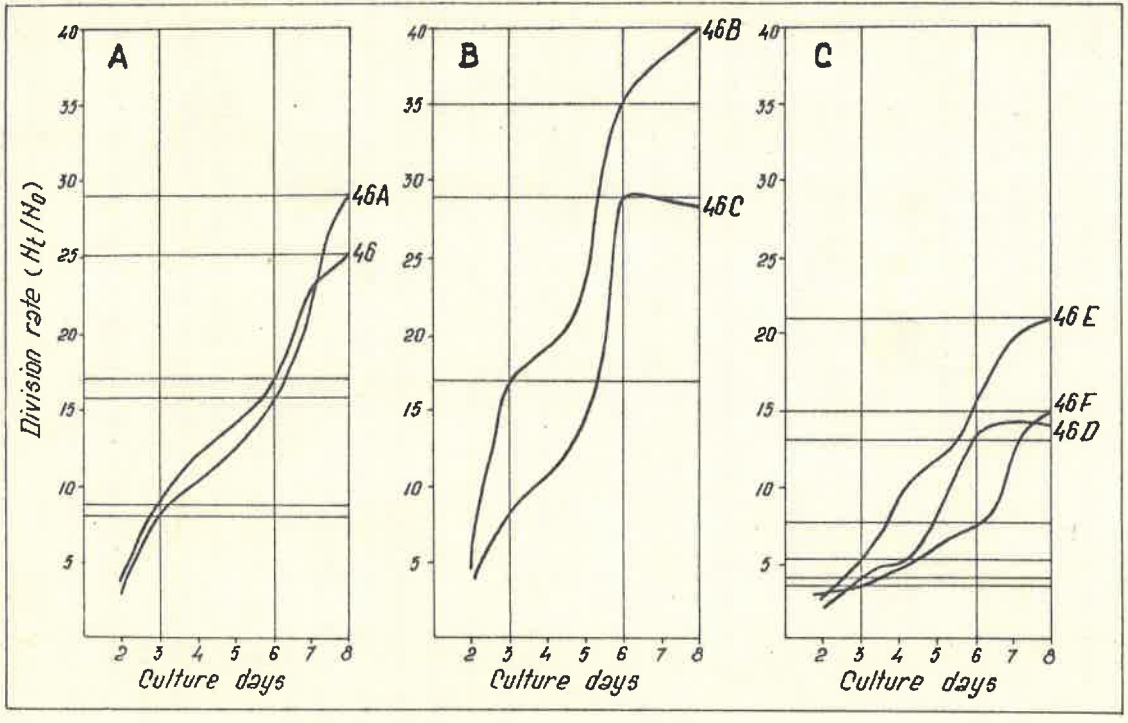

Fig.1. The division rate of Chlorella sp. grown in variants of 46 medium

The only exception consisted in the fact that after eight days of culture Chlorella sp. still kept the exponential phase in this variant while in 1/1 anionic ratio variant the culture entered in stationary phase. After eight days of culture k was 25 for 46 variant, and 29 for 46A variant. It is possible that in 46 variant the exhaustion of N would involved the cessation of cell division.

The 3/1 ratio led the highest division rate (Fig.1,B). The shape of the curve, in this case, shows that the cells are maintaining an active division along all the experiment. After eight days the division rate was forty.

The increasing of quantities of nitrogenous ions to 4/1 (46C variant) induced a lower exponential phase till the forth day of culture (Fig.1,B) in compareson to 3/1 ratio. After the fifth day the culture has adapted itself to that concentration. It develops a fast division and the yield of cells becoming double during a life cycle; that activity stoped suddenly and the culture passed into the stationary phase. The maximum k value of 28.5 was reached in the sixth day of experiment.

The 46D, 46E and 46F variants, in which the ions ratio was even bigger to the 5/1, 6/1 and 15/1, have induced a division rate closed to the 1/1 variant or lower than that, with the tendency to come into the stationary phase after seven days of culture (Fig.1,C). The division rate corresponding to those ionic ratios, ranged between 13 to 21.

Though, the optium ratio for the metabolism of this alga is 3/1, that stimulates a higher division rate than other ones.

The protein content was analysed during some experiments for twelve days, in the sixth, nineth and twelveth day.

The protein synthesis shows a closed relation with the growth stage. The protein amount hase came over the initial value for all the variants; that increase took place in the first days of culture and have reached the highest value in the nineth day (Fig.2). Although, the protein level is high, it droped slightly in the twelveth day or was maintained constantly. The increasing of the protein content corresponded to the exponential

phase.

Our data are in accordance with DAUMAS (2) for Phaeodactylum tricornutum and THOMAS and DAUMAS (14) for Dunaliella salina.

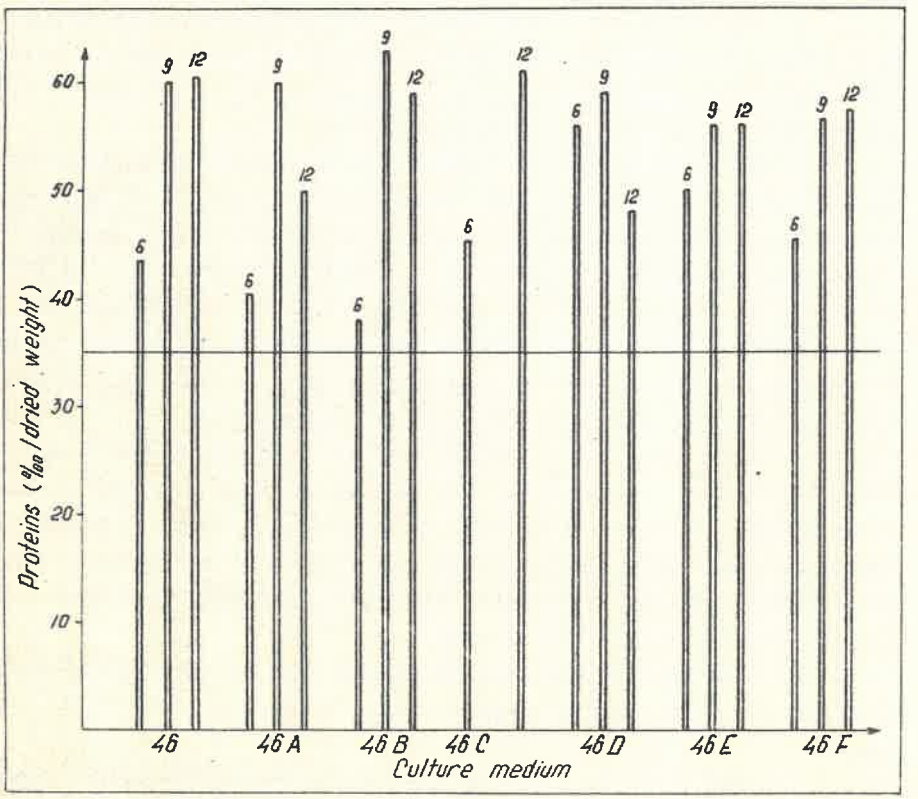

Fig.2. The dynamics of protein content of Chlorella sp. grown in 46 medium variants. The values correspond to the sixth, ninth, and twelvth day of experience. The mother culture has had an initial protein content of 35 % (it is represented by horizontal line).

The stagnation of protein synthesis or their decreasing overlap with the beginning of the stationary phase (Fig.1).

For the particular case of the 46D, 46E and 46F media, where the amount of protein is bigger in compareson with the division rate, we suppose that the synthetised protein should be a constitutive and not a formative one. On the other hand, it is possible that nitrogenous should be accumulated inside the cells till certain level when it is not metabolised to compounds requested by biological cycle.

We presume also, that the maintaining of the total protein to the quite high level in all the variants even in the stationary phase of the cultures, should be explained by the accumulation of the constitutive proteins in that stage.

It is noticed that the highest values for these compounds were obtained for 46B and 46C variants (ratio: 3/1 and 4/1).

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