

MASS PRODUCTION OF UNICELLULAR ALGAE IN OUTDOOR CULTURES BY UTILIZING WASTE WATER AS A TROPHIC SOURCE

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ABSTRACT:

Results of the utilization of treated waste water in culturing Chaetoceros simplex var. calcitrans Pauls, and Nitzschia closterium (Ehr.) W.Sm. in outdoor experiments are here presented. The $N-NH_4$ and $P-PO_4$ concentrations in waste water - sea water mixtures and the proper time for harvesting are concerned, as well as a cheap and very convenient device for culturing.

The study of the natural phytoplanktonic communities in the zones affected by waste water inflows has shown a dependence of the primary production upon the increase in concentration of the principal trophic elements: N and P. This masterminded the utilization of waste water as a trophic source in culturing phytoplanktonic species with a view to provide the food for the filter feeding organisms of economic importance (3, 7, 8, 9, 12, 13, 14, 15, 16).

This work aims to establishing the mixing ratio of sea water/waste water which favours the division and synthesis processes of the unicellular algae Chaetoceros simplex var. calcitrans

and Nitzschia closterium, as cultured in large scale and outdoor conditions.

METHODS AND PARTICULAR CULTIVATING CONDITIONS

The algae Chaetoceros simplex var. calcitrans and Nitzschia closterium were inoculated in sea water previously fertilized with waste water, ensuring of their uniform suspension being done by continuous bubbling with air. The algae were grown in the mass culture system.

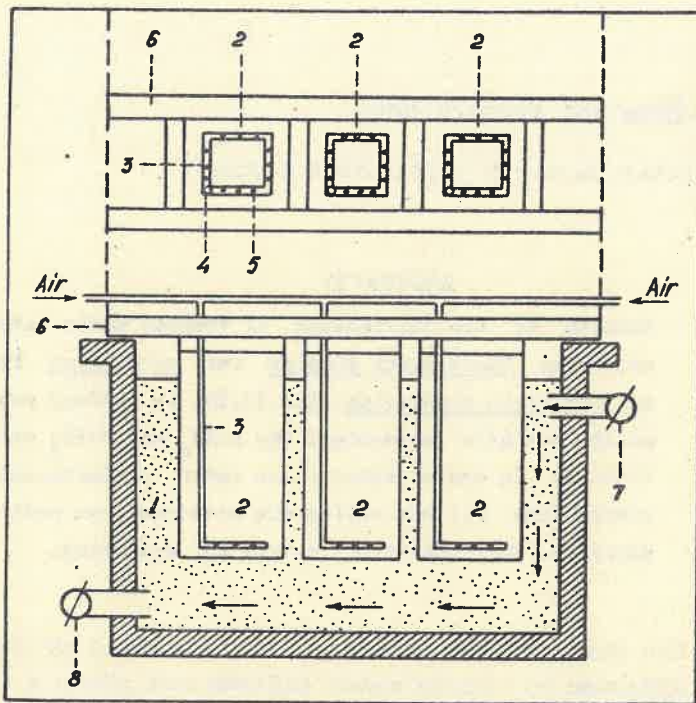


Fig. 1 - Schematic drawing of the mass cultivating system, used in outdoor conditions for Chaetoceros simplex var. calcitrans and Nitzschia closterium experiments.
1: swimming-pool; 2: polyethylene sacks; 3: bubbling pipes; 4: air input in the terminal quadrangular part of the bubbling pipe, situated on the sack bottom; 5: air output; 6: wooden frame that sustains the polyethylene sacks; 7: sea water inflow tap; 8: sea water out flow tap.

The experimental variants were 4/1, 2/1 and 1/1 mixtures of sea water/waste water. These proportions were settled after laboratory testing of a great number of mixing ration influence on the growth of the studied algae.

The culturing installation consists of 60-liter polyethylene sacks fixed on a wooden frame; so jointed, the sacks are suspended in water basins, thus ensuring both decrease of their specific weight and preservation of a constant temperature of the cultures (Fig.1). The water in the basins is changed whenever temperature rises, or it is allowed to circulate at a daily flow rate of 50%.

The cultures of the two algae developed under thermic conditions averaging about 20-21°C and a mean light intensity of 50,000 lx.

The experiments were carried on during two consecutive years, in the period from June to September; there was a duplicate for each variant of study. The results discussed in the following are average values of all the experiments.

Study was carried out on cellular density - by hemacytometrical counting; dynamics of the main trophic anions, as N-NH₄, P-PO₄ or Si; organic matter - through oxidizability with KMnO₄ - and salinity, after GENOVESE and MAGAZZU (5).

Determination of total proteins was performed by the method of KJEIDAHL, as modified (1), and the technique of DUBOIS (2) was used for carbohydrates.

The sewage water used was taken from one of the treating plant of the town of Constanța; it showed variations in the content of the researched trophic elements.

RESULTS AND DISCUSSIONS

The increase in the amount of cells in both algae can be positively correlated with the increase of waste water quantity (Figs.2 and 3), which demonstrates both a strong affinity of these organisms toward a medium rich in trophic substances, and their resistance to the salinity decreasing, as related to its ordinary value in natural circumstances (Tabs.1 and 2).

The exponential stage of both studied algae starts one day after inoculation and comes to a peak after 3 to 7 days' cul-

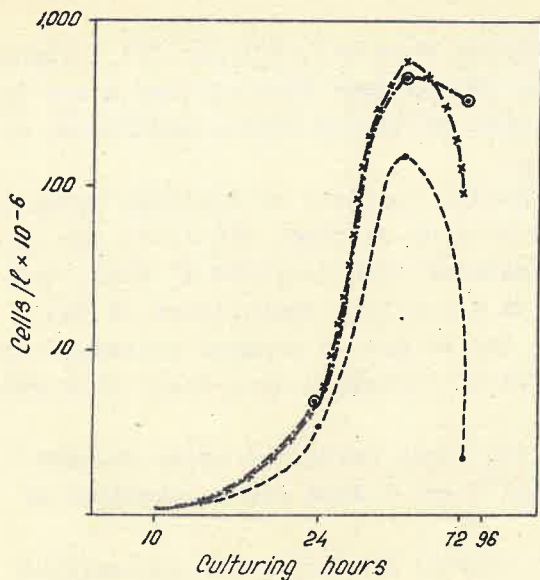


Fig.2 - Logarithmic drawing of Chaetoceros complex var. calcitrans growth in sea water/sewage water mixture ----- 4/1; x - x - x - 2/1; -.-.- 1/1.

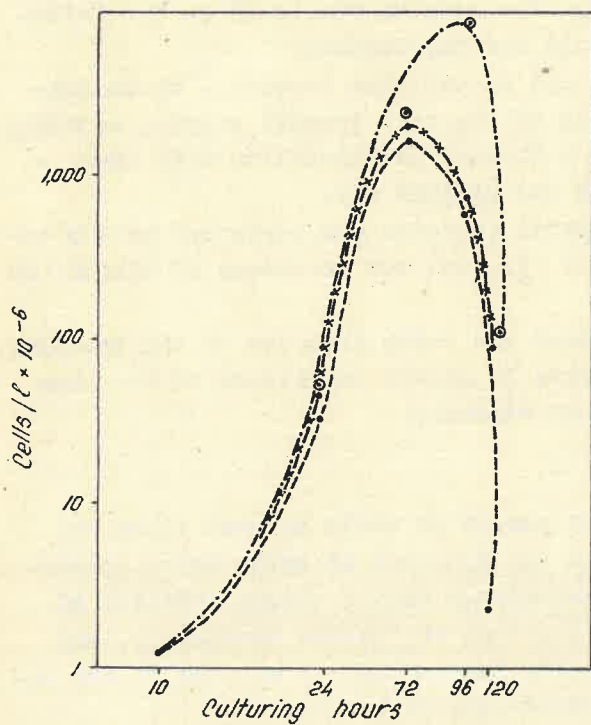


Fig.3 - Logarithmic drawing of Nitzschia closterium growth in sea water/sewage water mixture ----- 4/1; x - x - x - 2/1; -.-.-.- 1/1.

Table 1

The modification of the medium (as chemical composition) implied by
Chaetoceros simplex var. calcitrans culturing

| Ratio: sea water/ sewage water | P-PO ₄ (µg/l) | | | N-NH ₄ (µg/l) | | | Si(µg/l) | | | Salinity (g‰) | | |
|-----------------------------------|--------------------------|-----|-----|--------------------------|-----|-----|----------|------|------|---------------|-------|------|
| | 4:1 | 2:1 | 1:1 | 4:1 | 2:1 | 1:1 | 4:1 | 2:1 | 1:1 | 4:1 | 2:1 | 1:1 |
| Culturing day | | | | | | | | | | | | |
| 1 | 113 | 152 | 213 | 285 | 496 | 580 | 936 | 1212 | 1476 | 12,35 | 10,84 | 8,06 |
| 6 | 0 | 0 | 250 | 107 | 248 | 340 | 678 | 1025 | 1344 | 12,50 | 10,20 | 8,22 |
| 11 | 31 | 70 | 145 | 0 | 0 | 0 | 552 | 654 | 708 | 12,53 | 10,50 | 9,30 |

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Table 2

The modification of the medium (as chemical composition) implied by
Nitzschia closterium culturing

| Ratio: sea water/ sewage water | P-PO ₄ (µg/l) | | | N-NH ₄ (µg/l) | | | Si(µg/l) | | | Salinity (g‰) | | |
|-----------------------------------|--------------------------|------|-------|--------------------------|-----|-----|----------|-----|-----|---------------|-------|-------|
| | 4:1 | 2:1 | 1:1 | 4:1 | 2:1 | 1:1 | 4:1 | 2:1 | 1:1 | 4:1 | 2:1 | 1:1 |
| Culturing day | | | | | | | | | | | | |
| 1 | 540 | 1020 | 1248 | 62 | 88 | 98 | 438 | 564 | 607 | 12,40 | 11,37 | 10,34 |
| 4 | 138 | 138 | 139.2 | 0 | 0 | 0 | 173 | 294 | 216 | 12,90 | 11,62 | 10,86 |
| 11 | 54 | 138 | 84 | 0 | 0 | 0 | 261 | 384 | 384 | 12,92 | 11,89 | 11,12 |

turing. The active cellular division is followed by the stationary stage or by the decline of the population. These stages point out some distinct particularities to each of the studied species as a function of the proportion of sea water/sewage water mixture.

Consequently, the division of Chaetoceros simplex var. calcitrans is partially interrupted after 3 days of culturing, the rate of this process being inversely proportional to the additional sewage water volume. Determination of $N-NH_4$ and $P-PO_4$ showed a sudden fall of these trophic anions during the exponential stage (Tab.1). $N-NH_4$ is completely exhausted after 6 days of culturing. According to GOLDMAN et al. (6), the growth of Chaetoceros simplex var. calcitrans is linear in media containing a total amount of N that exceed $210 \mu g/l$. Although only $N-NH_4$ was determined in the presented experiments, the resultant values surpassed the mentioned concentration, their magnitude being directly dependent on the additional sewage water. $N-NH_4$ exhaustion out of the medium coincides with the moment of slowing down of the division process, or even with its transition to the stationary phase.

On the other hand, though in great quantity, $P-PO_4$ is absorbed from the medium down to the analytical zero after 5 days' culturing. It appears in the medium in the tenth day of culturing, phenomenon that is explained both by death of a percentage of the population and by its excretion from the living cells that stored $P-PO_4$ as a reserve (4).

The increase in concentration of this element, however, does not re-establish the physiological processes of the species.

Accordingly, the reduction of $N-NH_4$ and probably, of the other forms of nitrogen, as well as of $P-PO_4$, entail modifying of the cellular division rate; as a result, the medium should necessarily contain N and P to a certain concentration per cell, to maintain an active exponential stage.

From the high amount in which had been present, only 50% of Si was consumed in cellular multiplication, so that, this biogenous element should not be taken into consideration when one estimates the limitation in the growth of Chaetoceros in media fertilized by sewage water.

As concerning Nitzschia closterium, much more limited needs were observed for concentration of trophic compounds (Tab.2)

While working with different charges of sewage water on the experimented species, the water mixtures in which Nitzschia was cultured were casually less concentrated in $N-NH_4$: this implying its exhaustion after 3 days' culturing. As for the 4/1 and 2/1 mixtures, the population goes into decline; it must be specified that the amplitude of this phenomenon depends, too, on the volume of additional waste water: for 4/1, the decline comprises about 90% of the population, whereas in 2/1 mixtures only 38% of it affected. In 1/1 mixing variant, division occurs for 4 more days, the decline intervening only after a week's culturing.

P- PO_4 and Si dynamics takes a course similar to the one observed in Chaetoceros.

By comparing the growth of the two species, the following differences still can be remarked.

The growth efficiency of Nitzschia closterium is clearly superior to that of Chaetoceros simplex var. calcitrans; for approximately equal values of P and Si and much smaller values of $N-NH_4$, Nitzschia closterium yields biomasses 10 to 100 times greater than those yielded by Chaetoceros simplex var. calcitrans (Figs. 2 and 3).

Chaetoceros simplex var. calcitrans shows a strong avidity in the absorption of the nutrient elements, reducing not only the nitrogen, but all the studied elements.

During the growth, both in populations of Chaetoceros simplex var. calcitrans and in those of Nitzschia closterium, the evolution of several species of unicellular algae was noticed - all of them had been in the sea water at the beginning of the experiments. Amphora sp., Nitzschia tenuirostris Mer., Platymonas impellucida McLachlan & Parke, Ankistrodesmus falcatus Corda were reported in the cultures of Chaetoceros simplex var. calcitrans; Platymonas impellucida, Ankistrodesmus falcatus and Scenedesmus sp. were noted in the cultures of Nitzschia closterium. In both cases, the species contaminating the communities develop gradually with the regression of the cultured species; that is why, in the culture of Chaetoceros simplex var. calcitrans, the proportion of these organisms becomes significant only after 7 days of culturing, in contradistinction with the culture of Nitzschia closterium, when their percentage is scarcely important after 11 days of

Table 3

Chaetoceros simplex var. calcitrans and Nitzschia closterium developing as
report to their contaminant algae (% from total number of cells/l)

| Culturing day | Ratio: sea water/ sewage water | Chaetoceros simplex var. calcitrans | Contaminant algae | Nitzschia closterium | Contaminant algae |
|------------------|-----------------------------------|--|----------------------|-------------------------|----------------------|
| 1 | 4/1 | 95.71 | 4.28 | 82.04 | 17.95 |
| | 2/1 | 100.00 | 0 | 100.00 | 0 |
| | 1/1 | 95.55 | 4.44 | 100.00 | 0 |
| 2 | 4/1 | 91.06 | 8.94 | 96.26 | 3.73 |
| | 2/1 | 99.25 | 0.75 | 97.54 | 2.46 |
| | 1/1 | 99.00 | 0.99 | 98.16 | 1.84 |
| 7 | 4/1 | 7.17 | 82.37 | 93.52 | 6.48 |
| | 2/1 | 53.22 | 46.77 | 88.95 | 11.05 |
| | 1/1 | 85.02 | 15.97 | 76.97 | 23.03 |
| 10 | 4/1 | 0 | 100.00 | 18.03 | 81.97 |
| | 2/1 | 0 | 100.00 | 30.80 | 69.20 |
| | 1/1 | 0 | 100.00 | 36.52 | 63.48 |

culturing (Tab.3).

In the exponential stage, the organisms contaminating the cultures represent very small percent of the total biomass. Their preponderant growth along with the decline of both cultural species has a certain signification: either they have limited exigences to concentration of trophic substances as determined in our experiments, or their growth is impeded by some or one of the products of the sewage water, which Chaetoceros simplex var. calcitrans as well as Nitzschia closterium take out from the medium along their growing. One must not exclude, of course, the possible relations between the members of a dense community and the unnumeros contaminated species existing in the sea water at the beginning of the experiment.

However, taking into account that those same contaminated species are involved in all our experiments and whichever of them can constitute a good trophic base for juvenile and adult bivalves, we consider that they do not denature the nutrient value of the suspension of both algae in the course of their active division stage.

The analysis of proteins and total sugars reveals an increase of these products during the exponential stages. The preponderance of proteins outlines the persistence of an active metabolism; consequently, at least in the initial 8 days of experiments, the culture has the ability to resume division concurrently with completion of the trophic support. On the other hand, a positive correlation was noticed between the quantity of additional waste water and the percentage of proteins and sugars. The highest values are obtained for sea water/waste water mixture = 1/1.

While analysing the cell content, too, it is to be noticed that the growth of other species in the cultures does not diminish their trophic value, the percents of proteins and sugars maintaining always at more high values as related to the initial^{x/} values, both in Chaetoceros cultures and in the cultures of the alga Nitzschia. However the relation between the purity of the

x/ It means the amount of proteins and sugars determined in the algae before their suspension in sea water with additional sewage water.

Table 4

Proteins and sugars content of Chaetoceros simplex var. calcitrans and Nitzschia closterium cultivated in outdoor conditions with sea water/sewage water mixture during eleven days of experiment

| Alga | Ratio: sea water/ sewage water | Cellular compounds | | | | | |
|--|---|--------------------------|----------|-----------|------------------------|----------|-----------|
| | | Proteins (g % from d.w.) | | | Sugars (g % from d.w.) | | |
| | | Initial | 8-th day | 11-th day | Initial | 8-th day | 11-th day |
| Chaetoceros simplex var. calcitrans | 4/1 | | 27.9 | 29.60 | | 8.85 | 23.5 |
| | 2/1 | 21.87 | 28.55 | 36.90 | 5.07 | 4.95 | 11.9 |
| | 1/1 | | 32.75 | 40.15 | | 4.80 | 17.0 |
| Nitzschia closterium | 4/1 | | 30.85 | 25.80 | | 5.00 | 19.00 |
| | 2/1 | 16.25 | 36.85 | 29.55 | 10.00 | 7.80 | 16.38 |
| | 1/1 | | 42.40 | 28.85 | | 9.50 | 16.45 |

culture and the values of proteins and sugars must be emphasized. When contamination percentage is low, meaning that the biomass is mostly due to the species followed by the experiment, the highest values of these cellular products is to be found (Tables 3 and 4).

Consequently, the high nutrient content of the two algae is obvious. The results have pointed out that, in contradiction with some previous statements (10), Nitzschia closterium may be taken into account as a substantial alga from the point of view of the quantities of sugars and proteins which it can reach. But there is a condition that its harvesting should be done in the exponential stage. What must be verified for a certainty is the efficiency of its utilization, as well as the proportion in which it is to be added beside other algae into the diet of the filter-feeding organisms.

While growing, the algae showed a diminishing tendency of nutrient compounds, from their culturing medium or even of their reduction to the analytical zero (Tabs.1 and 2).

In Chaetoceros simplex var. calcitrans, the removing of $N-NH_4$ from the medium by the end of the exponential stage, i.e. the 6th culturing day, takes percentage values of 62,46%, 50% and 41.40% - in the rising order of the proportions in which the sewage water is added - while the removing of $P-PO_4$ reaches the analytical zero. In the same species, Si is diminishing with 8.90 to 27.57%.

Nitzschia closterium utilizes, after only 4 days of culturing, 74.4%, 86.47% and 88.84% of $P-PO_4$ - in the rising order of the proportions of the additional sewage water; $N-NH_4$ is exhausted and Si is absorbed from 47.87 to 64.35%.

Microscopic observations showed that the bacteria-charge regresses as the algal community tends to a peak of the exponential stage.

Coming back to the essential purpose of this paper - the establishing of the optimum values of sea water/waste water mixture that favour the development and synthesis of the studied algae, some important aspects are to be pointed out. As it comes out from chemical determinations, the waste water content is extremely variable and is influenced at least by the treating stage and the "age" of the waste water. In consequence, it is not the

value of the ratio of sea water/waste water that counts (9 10; 11), but the concentration of trophic produces as related to a given density of the algae which we intend to culture. By relating the initial concentration of $N-NH_4$ and $P-PO_4$ to the number of cells inoculated in both species, it came out that, for 1,000 cells/l, $P-PO_4$ must reach a concentration of 0.2 to 0.5 $\mu\text{g/l}$ in both cases and $N-NH_4$ must reach 0.13 and 0.0016 $\mu\text{g/l}$ for Chaetoceros simplex var. calcitrans and for Nitzschia closterium, respectively.

Therefore, chemical determination of essential nutrient produces of waste water is requested and subsequently, adjustment of mixing percentages to favour optimum division and synthesis.

CONCLUSIONS

1. In mass culture conditions, using sewage water as a trophic support, the species Chaetoceros simplex var. calcitrans and Nitzschia closterium reach a maximum density after 3 to 7 days of culturing.

2. In the course of their exponential stage, the two algae diminish the concentration of the principal trophic elements or reduce them to the analytical zero and, at the same time, inhibit the growth of bacteria; in this way, algal suspensions are resulted, in a liquid medium from which at least N and P were totally removed and Si - partially.

3. The dynamics of the principal synthesis products has shown that the proposed support for culturing the algae Chaetoceros simplex var. calcitrans and Nitzschia closterium has a stimulating effect on the proteins and sugars content, especially during the active division stage.

4. In utilizing sewage water, it is important to start from its content of trophic elements, then to establishing the proportion of the mixture of sea water/waste water, which is to be adjusted so that, for 1,000 cells/l, $P-PO_4$ concentration should be 0,2 to 0,5 $\mu\text{g/l}$ with both species and $N-NH_4$ concentration should be 0.13 and 0.0016 $\mu\text{g/l}$ with Chaetoceros simplex and Nitzschia closterium, respectively.

5. The development of other algae in the experimental cultures reaches significant values only by the end of the expo-

mental stage, with both Chaetoceros simplex var. calcitrens and Nitzschia closterium.

6. The analysis of the cellular density as well as of the removing dynamics of the nutrients from medium, the proportion of the bacterian or algal contamination and the cellular content, showed that the optimum harvesting interval for the algal suspension is of 3 days.

7. The installation presented in this work provides a sufficient volume of algal suspensions, offering the advantage of being very cheap and handy; it can be multiplied in a great number, as it is required.

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