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EFFECT OF NITRATE CONCENTRATION ON PHOTOSYNTHETIC ACTIVITY OF UNICELLULAR ALGAE CULTURES

1. INTRODUCTION

The photosynthetic activity of an algal culture is controlled by a large number of different factors of which the most important are light and salt content of nutrient solution, which determines the physiological condition of the algae and the CO_2 concentration available by each single alga cell.

Environmental pollution by nitrate ions enlarges the concentrations of NO_3^- in water reservoirs. This phenomenon may effect the unicellular green algae and other microorganisms living under such conditions. It was shown that the concentration of nitrate ions in drinking water is in the range of 10 - 30 mg/l, but there is evidence that the absolute NO_3^- concentration is increased several times, especially after chemical fertilisation of the soil [4]. Changes of NO_3^- concentrations in water are quite easy to explain by increased chemical fertilisation of the soil, from which they are collected in various water supplies and finally brought to open water systems. The increased nitrate concentration is accompained by increased bacterial contamination [3]. On the other hand, it is known from electron microscope examination of algae living under conditions of increased NO_3^- concentration, that significant changes in the subcellular organelles do not occur. Nitrogen deficiency can also produce major changes in the metabolic pathways of various substances present in algae cells. Unicellular green algae and other microorganisms living in open water systems are necessary in the food chain of living organisms. Experiments were therefore undertaken to

examine some general living conditions of chosen unicellular green algae when cultivated in conditions of increased or in sufficient concentration of nitrogen in the culture medium. In the experiments described, the available CO_2 concentration was limited by the natural occurrence of CO_2 in the air determined directly above the extensive cultivated algae suspension and therefore also, the light administrated during cultivation was kept at a relatively low level of intensity. It is known that for a culture which is illuminated with a high intensity, a relatively high CO_2 concentration is necessary to obtain the optimal photosynthetic activity, while this critical CO_2 concentration is much lower when the culture is illuminated at a much lower light intensity.

2. MATERIAL AND METHODS

Unicellular green algae cells of the Chlorella strain No. 366 were taken for the described experiments. The cells were obtained from the Algae Collection of the Institute of Zootechnics (Zator Division) in 1966 [2] and from that time cultivated at the Institute of Chemistry and Physics under conditions described by Lefévre [5], modified by Jankowski [2]. The medium was enriched with microelements as described by Vladimirova and Semenenko [9]. The basic medium was composed as follows in mole/dm^3: KNO_3 — $4.94 \cdot 10^{-3}$; $Ca(NO_3)_2$ — $3.04 \cdot 10^{-3}$; $MgSO_4 \cdot 7H_2O$ — $6.08 \cdot 10^{-4}$; citric acid — $1.10 \cdot 10^{-5}$; KH_2PO_4 — $1.47 \cdot 10^{-3}$; Fe-citrate — $1.00 \cdot 10^{-5}$ and 1 cm^3 of microelement solution prepared in mole per dm^3 was added: H_3BO_3 — $4.62 \cdot 10^{-3}$; $MnCl_2$ — $9.28 \cdot 10^{-3}$; $ZnSO_4$ — $7.76 \cdot 10^{-4}$; MoO_3 — $1.22 \cdot 10^{-3}$; NH_4NO_3 — $1.96 \cdot 10^{-3}$.

Three experimental media were prepared on the basic medium solution. The first one where nitrogen was eliminated and where KNO_3 was replaced by KCl ($4.94 \cdot 10^{-3}$ mole/dm^3) and $Ca(NO_3)_2$ by $CaCl_2 \cdot 2H_2O$ ($3.04 \cdot 10^{-3}$ mole/dm^3). The presence of NH_4^+ at a concentration of about $1.0 \cdot 10^{-9}$ mole per dm^3 was neglegible and therefore without any influence on the algal growth.

The second and the third experimental media were prepared from the basic medium, where KNO_3 and $Ca(NO_3)_2$ were added in 100 per cent and 200 per cent excess respectively. All mineral media were autoclaved for 30 min. at 2 atm. and 120°C, and used directly after preparation in the described experiments.

Luminescent tube lamps and bulbs with a light intensity of 2000 lx in both cases were used for cultivation and micromanometric measurements of the photosynthetic activity. An algal suspension from a 10-days-old culture kept on basic medium under extensive condition was added to the experimental flasks. To avoid algological and bacterial contamination the algae cultures were checked microscopically and

microbiologically. The age distribution of the algae was stable during the experiments and synchronisation effects were carefully avoided. Direct counting of the number of algal cells per 1 mm^3 were carried out microscopically using a Bürker chamber. Determination of the RQ coefficient i.e. production of CO_2 in µl to consumption of oxygen in µl was obtained by micromanometric measurements on a Warburg apparatus type WA 0130. After shaking to avoid sedimentation of algal cells, aliquots of 2 cm^3 were taken under sterile conditions and placed in the Warburg flask where for oxygen measurements the inner reaction vessels melted to the bottom were filled by 0.2 ml of 20% KOH solution. The CO_2 was measured directly without KOH. Control thermobarometers filled with distilled water in identical volumes were used to take exact measurements at 20°C, for 60 minutes and shaking velocity equal to 90 per min. Identical measurements were carried out in the dark and at an illumination of 2000 lx.

3. RESULTS AND DISCUSSION

It was proved by microscopic observation that the age distribution of the Chlorella cells examined was stable during the experiments. The increase of algal biomass measured microscopically by direct counting is shown in Table 1.

As shown in the table, the highest concentration of cells per cm^3 was achieved in culture where the nitrogen concentration was 200 per

Table 1. The increase of algal biomass during cultivation at various nitrate concentrations
Tab. 1. Przyrost biomasy glonów podczas hodowli w środowisku o różnym stężeniu azotanów

Day of culture Dzień hodowli	Number of cells Liczba komórek [mln/cm^3]			
	basic medium podłoże podstawowe	without nitrogen bez azotu	100% excess of nitrate 100% zwiększona dawka azotanów	200% excess of nitrate 200% zwiększona dawka azotanów
1	0.59	0.48	0.58	0.66
14	1.31	0.85	1.61	1.11
22	7.29	1.03	6.46	7.66
28	8.11	3.59	12.81	13.85
35	9.12	2.25	14.24	15.94
45	12.63	0.81	15.57	17.38
53	9.61	0.74	13.58	14.90
63	7.60	0.26	11.70	13.43

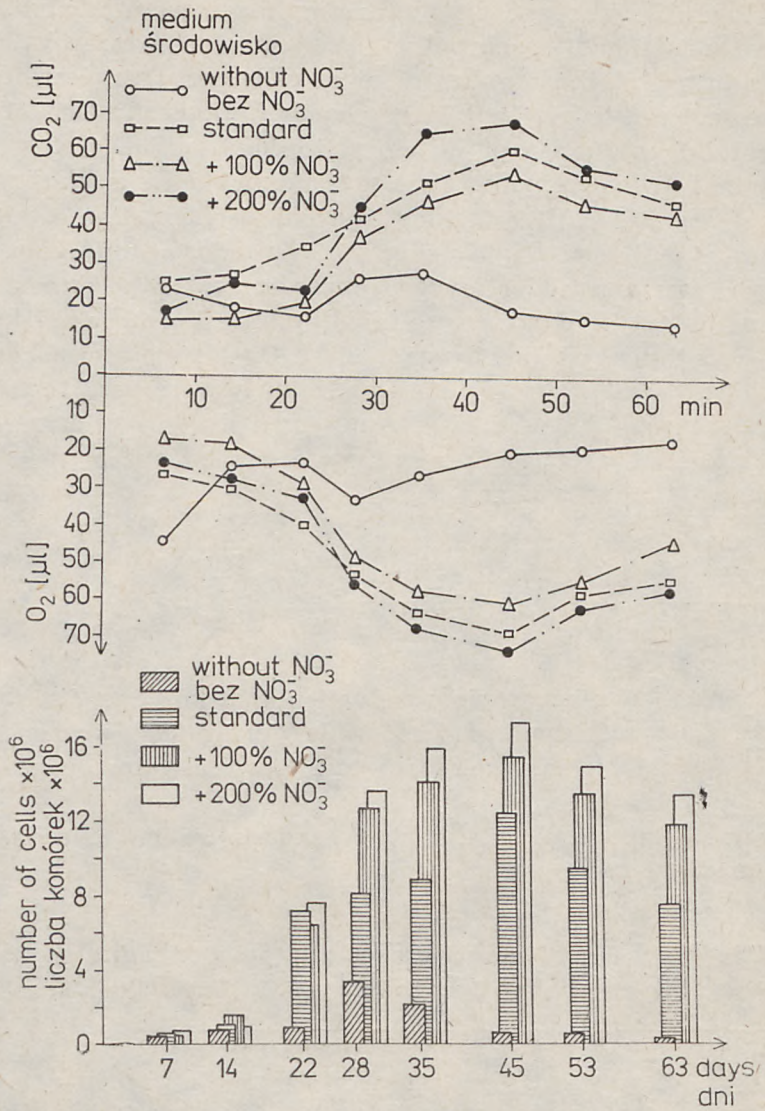

Fig. 1. Changes of CO_2 and O_2 levels during cultivation of Chlorella 366 cells on media at various nitrate concentrations, measured in the dark and the biomass production during the experiment

Rys. 1. Zmiany poziomu CO_2 i O_2 podczas hodowli komórek Chlorella 366 w środowisku o różnych stężeniach azotanów, mierzone w ciemności, oraz produkcja biomasy w czasie eksperymentu

cent higher than in the basic medium. The lowest concentration of biomass was obtained when nitrogen was completely eliminated from the basic medium, attaining only about 5 per cent of the comparable biomass.

The data obtained are graphically illustrated by oxygen consumption and CO_2 production when examined micromanometrically by the use

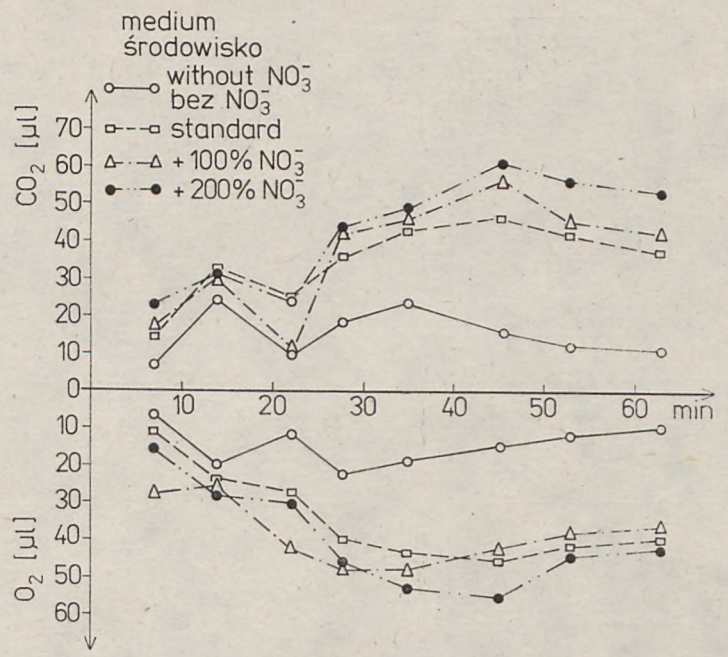

Fig. 2. Changes of CO_2 and O_2 levels during cultivation of Chlorella 366 cells on media at various nitrate concentrations measured by illumination

Rys. 2. Zmiany poziomu CO_2 i O_2 podczas hodowli komórek Chlorella 366 w środowiskach o różnych stężeniach azotanów, mierzone w świetle

of Warburg apparatus in the dark (Fig. 1) and during illumination of 2000 lx (Fig. 2).

The oxygen consumption of algal cells cultivated in basic medium are rather low at the beginning of cultivation, showing an increase up to about 45 days of cultivation and further a significant decrease. This phenomenon strongly depends on the number of algal cells present in the culture. The oxygen consumption expressed in µl per 10^6 cells also shows an increase up to 20 - 30 days of cultivation reaching a plateau for the period of about 2 months. This suggests that the oxygen consumption does not depend only on the number of cells, but also on their general metabolic activity. The CO_2 production is closely related to O_2 consumption. The increase of nitrate in the basic medium by 100 or 200 per cent is not reflected by increased oxygen consumption or CO_2 production. Similarly, the oxygen consumption and CO_2 production calculated per 10^6 cells shows an increase up to 20th day of cultivation reaching a plateau. Large differences as compared with the basic medium and media enriched by nitrate are observed when nitrogen from the basic medium is eliminated. The oxygen consumption as well as the CO_2 production is markedly decreased. These data are not comparable when calculated per constant number of cells present in the

Table 2. Changes of O_2 consumption and CO_2 production per 10^6 Chlorella cells examined during illumination and in the dark

Tab. 2. Zmiany w pobieraniu O_2 i produkcji CO_2 przez komórki Chlorella (liczba komórek 10^6) hodowane w świetle i w ciemności

Day of cultivation Dzień hodowli		µl O_2				µl CO_2				RQ			
		basic medium podłoże podstawowe	without nitrogen bez azotu	100% excess of nitrate 100% zwiększona dawka azotanów	200% excess of nitrate 200% zwiększona dawka azotanów	basic medium podłoże podstawowe	without nitrogen bez azotu	100% excess of nitrate 100% zwiększona dawka azotanów	200% excess of nitrate 200% zwiększona dawka azotanów	basic medium podłoże podstawowe	without nitrogen bez azotu	100% excess of nitrate 100% zwiększona dawka azotanów	200% excess of nitrate 200% zwiększona dawka azotanów
7	i	10.15	8.35	22.08	12.91	12.79	5.57	14.44	14.80	1.26	0.67	0.66	1.15
	d	22.40	46.07	13.71	18.49	21.47	25.29	13.23	12.24	0.96	0.55	0.97	0.65
14	i	9.38	12.65	7.78	12.79	12.45	15.12	9.77	16.58	1.33	1.20	1.26	1.29
	d	11.53	13.74	5.66	13.47	10.17	10.60	5.13	11.67	0.88	0.78	0.89	0.87
22	i	2.02	6.40	1.83	1.89	1.89	5.41	1.46	1.82	0.94	0.85	0.80	0.96
	d	2.74	11.09	2.25	1.95	2.05	7.66	1.67	1.51	0.75	0.70	0.74	0.77
28	i	3.57	2.90	1.67	1.68	3.36	2.73	1.76	1.65	0.94	0.94	1.05	0.98
	d	5.34	3.50	1.93	2.04	3.61	4.16	1.53	1.68	0.68	1.19	0.79	0.82
35	i	2.50	4.13	1.63	1.52	2.57	4.60	1.67	1.56	1.02	1.11	1.02	1.03
	d	3.80	2.10	1.93	2.10	3.20	5.50	1.71	2.07	0,84	0.99	0.87	0.99
45	i	1.80	9.05	1.51	1.65	1.90	11.16	1.66	1.81	1.06	1.23	1.09	1.09
	d	2.70	12.30	1.86	2.05	2.45	13.24	1.81	2.03	0.90	1.07	0.94	0.99
53	i	2.11	8.51	1.55	1.69	2.17	10.68	1.69	2.01	1.03	1.25	1.09	1.19
	d	2.98	13.10	1.85	2.01	2.81	12.10	1.80	1.87	0.94	0.92	0.97	0.93
63	i	2.56	16.60	1.59	1.77	2.51	21.95	1.72	2.05	0.99	1.32	1.08	1.15
	d	3.45	30.96	1.81	2.12	3.10	32.73	1.90	2.08	0.89	1.06	1.04	0.99

i — illumination 2000 lx (oświetlenie 2000 lx); d — dark (ciemność)

culture. Even after 60 days of cultivation the examined cells actively participate in O_2 consumption and CO_2 production on levels similar to cells growing on basic medium. One has to take into consideration that this kind of cells, when examined microscopically, are almost colourless and two to three times larger in diameter as compared with cells cultivated on basic medium. These cells, when examined by electron microscopy, show a high reduction of subcellular organella and especially the chloroplast with large accumulation of storage material being mainly large drops of lipids [8].

When the Warburg apparatus is illuminated for photosynthetic measurements, the oxygen consumption and CO_2 production are decreased as compared with measurements carried out in the dark. As in the dark, an increase of O_2 and CO_2 values was observed up to the 45th day of cultivation and again a drop was demonstrated as the cultivation period was prolonged. When calculated per 10^6 cells an increase was observed during the first 20 - 30 days of cultivation. Later an almost constant O_2 and CO_2 value was obtained. At increased concentration of nitrate the maximum is reached after 26 - 35 days. Differences in O_2 and CO_2 values were observed only when both mechanisms, the O_2 consumption and the photosynthetic oxygen reduction, were analyzed together.

The measure of general biological activity of the examined photosynthetic green algal cells is significant when the CO_2/O_2 ratio is compared. This is shown in Table 2, where data on O_2 and CO_2 expressed in µl and the calculated RQ value are presented for algal cells cultivated on basic medium, media enriched by nitrate and on medium with eliminated source of nitrogen. The increase of the RQ value during active photosynthesis reflects the metabolic process in the cells. Algae cultivated on increased nitrate concentration or on media without nitrogen, need a prolonged time of adaptation as compared with cells cultivated on basic medium L_{5m}.

It was shown by Rabinovich that the RQ value for Chlorella pyrenoidosa was 1.02 [7]. When cultivated under different conditions the same strain showed RQ values of 0.9 - 1.25 [1].

Various sources of nitrogen in cultivation medium also affect the RQ value [6]. Warburg demonstrated that Chlorella cells are characterized by RQ value equal to 0.7 - 1.3 [10]. Almost identical data were obtained in this paper where, during 63 days of cultivation, values of from 0.75 to 1.32 were obtained. Basing on the above data it is evident that environmental pollution by nitrate iones affects the general biological activity of unicellular green algae cells and evokes changes in the cell division cycles, as well as in the general metabolism which, under the condition described is oriented towards accumulation of storage material.

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WPŁYW STĘŻENIA AZOTANÓW NA AKTYWNOŚĆ FOTOSYNTETYCZNĄ JEDNOKOMÓRKOWYCH GLONÓW

Streszczenie

Stosując mikromanometryczne metody pomiaru O_2 i CO_2 w ciemności i przy oświetleniu komórek Chlorella 366 hodowanych na pożywkach ze zwiększoną i zmniejszoną ilością azotanów, porównywano współczynniki oddechowe (RQ). Obserwowano wzrost wartości RQ w procesie fotosyntezy, wskazujący na przyśpieszenie procesów metabolicznych. Komórki hodowane na podłożach ze zwiększonym stężeniem azotu lub bez jego udziału wskazują potrzebę wydłużonego czasu adaptacji w porównaniu z komórkami rosnącymi na standardowej pożywce L_{5m}.

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