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nfluence of reducing and oxidizing compounds and of the redox potential of the medium on the biomass of *Scenedesmus quadricauda* (Turp.) Breb.

STEFAN GUMIŃSKI, KRYSTYNA JURAJDA, EWA TATKOWSKA

Department of Plant Physiology, Institute of Botany, Wrocław University, Kanonia 6/8, 50-328 Wrocław, Poland

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Abstract

The influence was investigated of several concentrations of the reducing agents: cysteine, glutathione, ascorbic acid, pyrocatechol and of the oxidizing agents: $KMnO_4$, $K_2Cr_2O_7$ and H_2O_2 on the total dry weight increment and that of protein with reference to redox potential changes of the medium in *Scenedesmus quadricauda* cultures. The culture was run in a photothermostat. It was found that the reducing compounds had as a rule a stimulating influence under 24-h illumination, whereas the oxidizing agents gave the same effect when a period of 7-h darkness was applied within 24 h.

Key words: redox potential, redox compounds, Scenedesmus quadricauda.

INTRODUCTION

In the natural environment of algae there are various substances which decide of what is known as the oxidability of the solution and which show various activities in oxidation and reduction reactions. The degree of exidability (permanganate value) is frequently studied by hydrobiologists, but the redox potential of the solution in which algae live is in general unknown. On the basis of the results of numerous studies on the influence of reucing or oxidizing properties of the environment on bacteria (Hewitt 1950, Rodina 1968) and the results of similar two papers concerning higher plants (Machold 1967, Ślesak 1975), it was decided to start investigations on a green alga.

In the first step of study the influence of the given substances on dry mass production of the alga and protein content in it under 24-h illumination and under a photoperiod L:D=17:7 was investigated and the changes

of the medium redox potential were followed in the liquid medium in the course of culture development.

MATERIAL AND METHODS

The experiments were performed with *Scenedesmus quadricauda* (Turp.) Bréb, a microbiologically pure strain of which was received from the Store of Algological Cultures of the Czechoslovak Academy of Sciences in Prague.

For stock cultures modified Uspenski medium was used composed of: $Ca(NO_3)_2 \cdot 4H_2O$ — 0.207, KNO_3 — 0.025, KH_2PO_4 — 0.025, $MgSO_4 \cdot 7H_2O$ — 0.05, K_2CO_3 — 0.035, $Fe_2(SO_4)_3 \cdot nH_2O$ — 0.002 ($g \cdot dm^{-3}$ bidistilled water) with the microelements ($mg \cdot dm^{-3}$): H_3BO_3 — 0.6, $MnCl_2 \cdot 4H_2O$ — 0.4, $ZnSO_4 \cdot 7H_2O$ — 0.05, $CuSO_4 \cdot 5H_2O$ — 0.05, $H_2MnO_4 \cdot H_2O$ — 0.02. The microelements were used according to Czopek (1963) and the macroelements after Uspenski (1927). Every two weeks 5 cm^3 of the algal suspension were inoculated into 100 cm^3 of fresh medium.

Inoculation of the experimental cultures was done according to the following procedure: 20 cm^3 of the suspension from a two-week stock culture was introduced into 600 cm^3 of medium in a flask of 1 dm^3 capacity. Before inoculation the respective reducing or oxidising compound was added to the medium in gradually increased concentrations, as shown in the tables. Then the algal suspension was poured into Erlenmayer flasks of 100 cm^3 capacity, 25 cm^3 into each.

Preliminary investigations on the usefulness of Pringsheim, Tamiya, Lefevre and Benson media, recommended by Jankowski (1964) for *Scenedesmus quadricauda* gave worse results than the above described medium. Therefore, the latter was chosen for the experiments proper, especially as the Uspenski medium had already been used in our laboratory with success (Gumiński 1950, Kyć 1970). All the experiments were carried out under sterile conditions. The cultures were kept in a photothermostated room. On the basis of earlier papers (Kyć 1970, Jurajda 1974) a light intensity of about 7000 lux was applied (ca. 10 $W \cdot m^{-2}$, fluorescent tubes "Flora") and temperature was maintained at 24°C. In the first experiment the cultures were exposed to light for 24 h (L:D=24:0), and in further ones for 17 h (L:D=17:7).

For examining the course of the culture growth curve of the alga the culture was observed up to the disappearance of the green colour and at several day intervals the dry weight and protein content were determined in the biomass. This allowed to establish the time of culture in the experiments proper.

The following reducing compounds were used: cysteine, reduced glutathione, ascorbic acid and pyrocatechol, and the following oxidizing

agents: potassium permanganate, potassium dichromate and hydrogen peroxide solution. A number of different concentrations of these substances was used and their influence on biomass increment was tested. The concentrations are listed in the tables. In successive experiments the initial redox potential of the medium was decreased or increased in relation to the control combination by applying a suitable concentration (see details in tables). Moreover, changes in redox potential of the medium occurring with time were studied. The pH of the medium was adjusted before starting the experiment to pH 6.9. The redox potential was measured with the use of a platinum electrode in reference to calomel ones (Rodina 1968, Ślesak 1975). Before measurement the platinum electrode was checked on Michaelis buffer according to the above quoted authors.

The amount of simultaneous replications for each experimental combination was established so that at each time of dry mass and protein determination during continuation of the culture, material could be examined from at least three replications (from 3 flasks).

After a specified time of culture (see tables) material was collected from three flasks in the form of stirred algal suspension, 20 cm^3 from each, filtered through a "Synpor" No. 5 membrane filter, the sediment was washed with 0.01 N HCl, then with distilled water and dried at 65°C with the filter to constant weight and the dry weight of algae was determined.

For protein determination 5 cm^3 of suspension was taken from each of two further flasks together with the remains from the flasks used for dry weight determination. The three samples thus obtained (each of 5 cm^3) were poured into test tubes and centrifuged at 2000×g for 20 min. The supernatant was decanted and the sediment was treated with hot 10 per cent TCA, this giving after 20 min a protein sediment. After dilution with TCA to 5 per cent the material was centrifuged, the sediment was dissolved in hot 1 N NaOH and protein was determined by the method of Lowry (Mejbaum-Katzenellenbogen and Mochnacka 1968). The procedure was based on the paper by Buczek et al. (1975).

RESULTS

INFLUENCE OF REDUCING AGENTS UNDER CONTINOUS LIGHT

Dry weight increment in the control culture was rapid up to the 12th day of culture, then it diminished and so did the protein content in dry mass. All the three reducing commounds caused an increased

Table 1

Influence of cysteine, ascorbic acid and reduced glutatione on dry weight increment and protein content under continuous light in *Scenedesmus* cultures. Means from 3 replications

Reducing agents, M	6 Days of growth			12 Days of growth			18 Days of growth		
	d.wt., mg per flask	protein, mg · per flask	protein, mg · g^{-1} d.wt.	d.wt., mg per flask	protein, mg per flask	protein, mg $\cdot g^{-1}$d.wt.	d.wt., mg per flask	protein, mg per flask	protein, mg $\cdot g^{-1}$d.wt.
Control	3.43±0.04	0.325	94.75	20.79±2.04	2.025	97.39	21.94±1.08	1.725	78.62
Cysteine 10^{-8}	2.37±0.99	0.375	158.22	16.40±2.11	1.975	120.42	20.05±0.94	1.877	93.51
Cysteine 10^{-7}	5.45±1.02	0.775	142.20	18.60±2.04	1.950	104.83	22.83±1.13	1.740	76.21
Cysteine 10^{-6}	5.41±0.93	0.450	83.17	14.05±1.06	1.950	138.79	21.70±0.96	1.717	79.14
Cysteine 5×10^{-6}	5.91±0.65	0.550	93.06	22.75±1.24	2.225	97.80	27.62±1.12	1.775	64.26
Ascorbic acid 10^{-8}	5.08±0.48	0.575	113.12	20.62±0.76	1.900	92.14	26.45±0.88	1.800	68.05
Ascorbic acid 10^{-7}	5.62±0.51	0.845	150.35	20.29±1.06	1.928	94.87	25.83±1.14	1.737	67.26
Ascorbic acid 10^{-6}	4.54±0.62	0.537	118.30	14.83±0.94	1.700	114.63	28.18±1.04	1.675	59.43
Ascorbic acid 5×10^{-6}	4.12±0.47	0.845	205.00	18.54±1.05	1.850	99.77	22.41±0.96	1.670	74.52
Control	5.41±0.97	1.075	198.70	13.70±2.04	1.262	92.15	23.02±0.96	2.252	97.82
Glutathione 10^{-8}	3.16±0.68	0.812	257.12	9.27±1.14	0.975	105.17	22.66±1.07	2.125	93.77
Glutathione 10^{-7}	3.54±1.12	0.820	231.61	15.55±1.34	1.263	81.15	23.83±0.97	2.122	88.84
Glutathione 10^{-6}	3.66±0.68	0.900	245.91	16.45±0.98	1.550	94.22	23.00±0.76	2.225	96.73
Glutathione 5×10^{-6}	7.08±0.74	1.250	176.50	21.08±1.16	1.580	74.95	24.37±0.991	2.400	98.48

d.wt. = dry weight

Table 2

Influence of cysteine, reduced glutathione, pyrocatechol and ascorbic acid on dry weight increment and protein content in *Scenedesmus quadricauda* cultures under photoperiod L: D=17: 7. Means from 5 replications

Combinations	Initial potential, mV	5 Days of growth			10 Days of growth			15 Days of growth			20 Days of growth		
		mg d. wt. per flask	protein, mg per flask	protein, $mg \cdot g^{-1}$ d.wt.	mg d.wt. per flask	mg protein per flask	protein, $mg \cdot g^{-1}$ d.wt.	mg d.wt. per flask	mg protein per flask	protein, $mg \cdot g^{-1}$ d.wt.	mg d.wt. per flask	mg protein, per flask	protein, $mg \cdot g^{-1}$ d.wt.
Control	212	3.61±0.16	0.475	131.57	8.27±0.49	1.987	240.32	17.05±0.94	2.594	152.12	24.61±1.14	2.250	91.42
Cysteine 10^{-6} M	129	3.49±0.19	0.425	121.77	5.66±0.38	2.062	346.39	17.77±1.02	2.375	133.65	27.25±1.38	2.525	92.66
Cysteine 10^{-5} M	118	3.19±0.23	0.475	148.90	7.13±0.29	2.700	378.25	17.44±1.14	2.250	126.83	26.38±1.42	2.270	86.05
Glutathione 10^{-5} M	124	3.60±0.21	0.376	101.60	6.66±0.19	2.131	320.00	19.79±0.91	3.187	161.00	26.00±1.18	2.400	92.30
Glutathione 10^{-4} M	112	3.85±0.09	0.425	118.71	6.86±0.32	2.812	409.98	18.75±1.03	3.125	166.60	26.39±1.54	2.550	96.62
Pyracatechol 10^{-4} M	90	no growth	—	—	3.12±0.09	0.875	280.44	10.41±1.14	1.968	188.90	16.13±1.36	2.410	147.85
Ascorbic acid 10^{-5} M	90	3.18±0.11	0.250	78.61	5.08±0.32	1.815	357.27	11.25±0.92	2.593	230.55	12.77±1.17	1.950	152.70

d.wt. = dry weight

Table 4

Influence of depression of redox potential by means of various reducing agents on dry mass increment and protein content under photoperiod L: D = 17: 7. Means from 3 replications

Combinations	Initial potential, mV	After 5 days of growth			After 10 days of growth			After 15 days of growth			After 20 days of growth		
		d.wt., mg per flask	protein, mg per flask	protein, mg · g^{-1} d.wt.	d.wt., mg per flask	protein, mg per flask	protein, mg · g^{-1} d.wt.	d.wt., mg per flask	protein, mg per flask	protein, mg · g^{-1} d.wt.	d.wt., mg per flask	protein, mg per flask	protein, mg · g^{-1} d.wt.
Control	220	4.12±0.3	1.27	309	16.33±1	3.19	196	25.21±1	3.57	142			
	218*										31.88±2	5.75	180
Glutathione	133	3.63±0.1	1.25	344	14.11±1	3.12	221	26.16±1	4.18	160			
10^{-5} M	160*										37.28±1	7.31	196
Cysteine	112	3.65±0.1	1.2	329	13.13±1	2.68	204	23.25±1	3.43	148			
10^{-5} M	156*										30.1 ±1	5.94	197
Ascorbic acid	98	3.38±0.1	1.1	307	11.2 ±0.9	1.59	142	18.8 ±1	2.75	146			
10^{-5} M	158*										25.3 ±0.9	4.94	195
Pyrocatechol	150	4.23±0.1	1.0	236	11.0 ±0.1	2.34	212	23.0 ±0.9	3.31	144			
10^{-5} M	152*										28.6 ±0.9	5.73	200

* = potential after 24 h, d.wt. = dry weight.

production of dry mass. When cysteine and reduced glutathione were used, most favourable proved the highest concentration that is 5×10^{-6} M; with ascorbic acid, however, the lowest concentration was found to be best, that is 10^{-8} M. The protein harvest also increased under the action of reducing agents, but their optimal concentrations did not correspond to those which enhanced most dry mass production. Stimulation of dry mass production including protein was most distinct after 6 days of culture; after 12 days this effect ceased, and appeared once more after 18 days, although less pronounced and only for total dry mass and not for protein (Table 1).

INFLUENCE OF REDUCING AGENTS IN REFERENCE TO THE REDOX POTENTIAL UNDER THE PHOTOPERIOD L:D=17:7

In this experiment none of the substances applied in the concentration which previously produced stimulation increased dry mass production after five days of culture. When pyrocatechol was used the dry weight increment was so low that it could not be determined. A slight stimulation was noticeable as late as after 20 days under the influence of cysteine and glutathione. The latter enhanced somewhat protein production after 15 days. It was found similarly as before that towards the end of the culture the protein content in dry mass decreased in all the combinations of the experiment (Table 2).

The redox potential in the control flasks without algae remained at a constant level to the end of the experiment. In the control flasks containing algae it fell with time and after 20 days was lower than the initial value by about 50 mV. The potentials depressed by addition of a reducing agent at the beginning of the experiment rose with time considerably in the flasks free of algae and in those with algae the potential also increased slightly in the combinations with cysteine and glutathione, it increased greatly in the combinations with ascorbic acid and pyrocatechol (Table 3).

INFLUENCE OF SIMILAR REDOX POTENTIAL DEPRESSION BY MEANS OF VARIOUS REDUCING AGENTS UNDER PHOTOPERIOD L:D=17:7

The result as regards biomass production was similar as in the previous experiment, with the difference that this time pyrocatechol, used it is true in a lower concentration, did not inhibit biomass production as was the case in the preceding experiment (Table 4).

In this case the redox potential of the medium also remained during culture at a constant level in the control flasks without algae and mark-

Table 3

Changes in redox potential of medium with time in experiment with reducing agents under photoperiod L: D=17: 7. Means from 5 replications

Combinations	Potential, mV					
	initial	after 24 h	after 5 days	after 10 days	after 15 days	after 20 days
Medium without reducing agents	212	198	206	214	214	209
The same with algae	209		162	177	110	141
Cysteine 5×10^{-6} M in medium without algae	129	169	182	209	239	242
The same with algae	133		169	155	92	137
Cysteine 10^{-5} M	118	156	186	220	207	230
The same with algae	121		150	145	102	150
Glutathione 10^{-5} M	124	165	180	204	238	239
The same with algae	126		160	170	108	136
Glutathione 10^{-4} M	112	167	180	150	242	238
The same with algae	116		143	198	114	136
Pyrocatechol 10^{-4} M	90	108	77	110	143	168
The same with algae	92		82	113	74	150
Ascorbic acid 10^{-5} M	90	158	168	195	192	195
The same with algae	88		110	120	102	197

edly fell in those containing them. In the combinations with reducing agent without algae the potential rose considerably with lapse of time. In the algae-containing flasks the potential rose at the beginning of culture, then it stabilised or eventually fell (Table 5).

Table 5

Changes in medium redox potential with time, when initial potential is adjusted by application of various reducing agents in 10^{-5} M concentrations. Photoperiod L: D= 17: 7. Means from 5 replications

Combinations	Potential of medium at successive measurements					
	initial	after 24 h	after 5 days	after 10 days	after 15 days	after 20 days
Control medium	220	214	223	212	219	230
„ with algae		209	155	136	101	107
Medium+glutathione	133	160	184	240	239	242
„ + „ with algae		167	137	130	92	139
„ + cystein	112	156	149	240	260	245
„ + „ with algae		154	139	124	90	110
„ +ascorbic acid	98	158	181	244	253	239
„ + „ with algae		153	135	133	117	108
„ +pyrocatechol	150	152	186	214	204	210
„ + „ with algae		156	134	130	103	109

INFLUENCE OF OXIDIZING COMPOUNDS UNDER CONTINUOUS ILLUMINATION

In the presence of $KMnO_4$, particularly at a 10^{-6} M concentration, a slight stimulation of dry mass production was noted after 6 days of culture. Dichromate gave a similar effect in a 10^{-7} M concentration. Hydrogen peroxide did not stimulate dry mass increment and in a 10^{-3} M concentration destroyed the culture. The influence of the above named substances on protein content was not noticeable. This content in the dry mass was in general much lower after 12 than after 6 days of culture (Table 6).

Table 6

Influence of oxidizing agents on dry mass production and protein content under 24-h illumination

Combinations	After 6 days			After 12 days		
	dry wt., mg per flask	protein, mg per flask	protein, mg · g^{-1} d.wt.	dry wt., mg per flask	protein, mg per flask	protein, mg · g^{-1} d.wt.
Control	9.9 ±0.25	3.13	317	18.3±0.3	2.89	158
$KMnO_4$ 10^{-8} M	12.26±0.25	3.93	319	16.6±0.1	2.82	170
„ 10^{-7} M	12.8 ±0.24	3.73	299	19.0±0.5	2.93	154
„ 10^{-6} M	13.1 ±0.12	3.24	246	19.1±0.1	2.99	157
„ 5×10^{-6} M	12.73±0.16	3.73	293	17.6±0.2	2.91	165
$K_2Cr_2O_7$ 10^{-8} M	10.0 ±0.14	3.40	340	17.9±0.3	2.70	151
„ 10^{-7} M	13.06±0.17	3.88	285	16.6±0.1	2.71	163
„ 10^{-6} M	11.6 ±0.1	3.12	269	16.6±0.2	2.71	163
„ 10^{-5} M	10.5 ±0.13	3.84	364	19.4±0.1	2.81	144
Control	7.33±0.18	2.20	300	15.4±0.2	3.35	218
H_2O_2 10^{-6} M	6.86±0.05	1.78	260	15.7±0.2	3.75	246
„ 10^{-5} M	7.40±0.15	1.95	264	17.2±0.8	3.67	213
„ 10^{-4} M	3.20±0.15	0.80	240	12.3±0.1	3.02	244
„ 10^{-3} M	no growth			no growth		

INFLUENCE OF OXIDIZING AGENTS UNDER THE PHOTOPERIOD L:D=17:7

In this case all the three oxidizing agents produced a strong stimulation of dry mass production including protein. As previously, the protein content in the dry mass diminished at the end of the culture (Table 7).

INFLUENCE OF OXIDIZING AGENTS UNDER THE PHOTOPERIOD L:D=17:7 IN REFERENCE TO THE REDOX POTENTIAL OF MEDIUM

Like in the preceding experiment $KMnO_4$ in concentrations of 10^{-8} and 10^{-7} M markedly accelerated dry mass production including protein. When $K_2Cr_2O_7$ was applied stimulation appeared distinctly at a 10^{-7}

Table 7

Influence of oxidizing agents on dry mass production and protein content under photoperiod L: D=17:7. Means from 5 replications

Combinations	After 5 days			After 10 days			After 20 days		
	d.wt., mg per flask	protein, mg per flask	protein, mg · g^{-1}d.wt.	d.wt., mg per flask	protein, mg per flask	protein, mg · g^{-1}d.wt.	d.wt., mg per flask	protein, mg per flask	protein, mg · g^{-1}d.wt.
Control	6.00±0.12	1.64	274	9.20±1.37	2.67	291	21.86±0.17	4.67	214
H_2O_2 10^{-6} M	6.51±0.08	2.04	313	17.72±0.32	3.81	215	27.00±1.13	4.81	178
„ 10^{-5} M	9.23±0.23	2.49	371	17.46±0.88	3.32	190	26.66±2.13	4.20	158
„ 10^{-4} M	7.52±0.09	2.15	287	13.32±0.29	3.70	278	26.80±2.03	4.36	163
$KMnO_4$ 10^{-8} M	7.00±0.07	2.04	292	16.24±0.86	4.03	248	29.86±1.41	4.76	159
„ 10^{-7} M	8.04±0.12	2.15	269	19.46±1.62	3.81	196	33.60±1.07	4.63	138
„ 10^{-6} M	6.52±0.37	2.08	333	12.66±0.94	3.33	263	35.40±1.14	4.71	133
„ 10^{-5} M	5.54±0.07	2.52	455	12.80±0.78	2.61	205	30.92±0.92	5.21	169
$K_2Cr_2O_7$ 10^{-8} M	9.20±0.13	2.16	235	18.00±1.12	3.61	201	23.86±1.17	4.08	171
„ 10^{-7} M	9.60±0.36	2.31	241	20.26±0.88	4.11	202	23.32±0.96	4.46	191
„ 10^{-6} M	10.02±0.14	2.15	215	19.06±1.02	3.60	189	28.66±0.14	4.35	152
„ 10^{-5} M	8.00±0.08	2.16	270	17.86±0.25	4.03	226	26.12±1.05	4.67	179

d.wt. = dry weight

Table 8

Influence of oxidizing agents and medium redox potential on dry mass and protein production under photoperiod L:D=17:7 Means from 5 replications

Combinations	Initial potential, mV	After 5 days		After 10 days		After 15 days	
		d.wt., mg per flask	protein, mg per flask	d.wt., mg per flask	protein, mg per flask	d.wt., mg per flask	protein, mg per flask
Control	204	3.10±0.71	0.71	7.30±0.98	1.61	16.20±1.12	3.40
H_2O_2 10^{-6} M	252	3.30±0.04	0.82	9.31±1.13	1.85	6.00±0.72	0.96
,, 10^{-5} M	259	2.32±0.08	0.52	6.00±0.91	1.10	6.70±0.82	1.38
,, 10^{-4} M	273	1.10±0.02	0.26	3.20±0.45	0.64	4.80±0.64	1.17
Control	208	1.50±0.06	0.33	2.80±0.34	0.60	8.80±1.14	1.31
$KMnO_4$ 10^{-8} M	272	4.20±0.14	1.10	4.40±0.27	0.86	12.10±1.52	1.89
,, 10^{-7} M	237	5.22±0.9	1.31	12.40±0.83	2.19	9.20±0.54	1.10
,, 10^{-6} M	248	3.10±0.72	0.66	7.00±0.48	1.15	6.40±0.34	0.88
,, 5×10^{-6} M	310	1.20±0.08	0.16	4.80±0.04	0.77	4.20±0.17	0.39
Control	204	7.20±0.93	1.71	9.20±0.86	1.59		
$K_2Cr_2O_7$ 10^{-8} M	234	5.40±0.46	1.15	7.22±0.62	1.04		
,, 10^{-7} M	234	10.21±0.72	2.08	22.00±0.98	3.18	experiment not continued	
,, 10^{-6} M	235	7.24±0.10	1.26	14.83±1.77	2.40		
,, 10^{-5} M	245	3.62±0.57	0.68	6.61±0.62	0.83		

d.wt. = dry weight

and 10^{-6} concentration, and H_2O_2 gave only a slight stimulation at a 10^{-6} M concentration. It may, thus, be said that the stimulating effect appeared when the redox potential was increased by 30-50 mV; a greater rise of potential decreased already the biomass production (Table 8).

The redox potential of the medium remained constant for 15 days that is to the end of the culture in the control flasks without algae, it diminished somewhat in the control ones containing algae. In the combinations with $KMnO_4$ the potential fell slightly in the flasks free of algae with time, and in those with algae the depression was more pronounced. In the combination with the use of $K_2Cr_2O_7$, in the absence of algae, the potential fell with time to the level recorded in the medium free of oxidizing agents. The depression of potential was more pronounced in the flasks with algae. The medium potential in the combinations with H_2O_2 and without algae remained more or less at the same level, and in the flasks containing algae it fell considerably (Table 9).

Table 9

Changes in medium redox potential with lapse of time in experiment with oxidizing agents under photoperiod L: D = 17: 7. Means from 5 replications

Combinations	Potential, mV							
	after 1 day		after 5 days		after 10 days		after 15 days	
	no algae	with algae	no algae	with algae	no algae	with algae	no algae	with algae
H_2O_2								
0	204	195	205	204	204	204	204	189
10^{-6} M	252	260	237	215	239	203	246	185
10^{-5} M	259	266	245	232	244	227	253	170
10^{-4} M	273	272	266	247	265	247	265	189
$KMnO_4$								
0	208	208	208	210	206	208	207	192
10^{-8} M	227	225	213	207	208	198	207	171
10^{-7} M	237	236	210	211	207	194	206	175
10^{-6} M	248	250	210	215	212	203	203	198
5×10^{-5} M	310	305	212	215	206	200	205	196
$K_2Cr_2O_7$								
0	204	206	190	195	206	175	208	110
10^{-8} M	234	234	194	188	207	170	205	115
10^{-7} M	234	233	195	185	208	169	205	123
10^{-6} M	235	233	195	174	208	174	205	123
10^{-5} M	245	237	190	195	205	186	202	121

DISCUSSION

Confrontation of the results indicates that under continuous light the reducing agents distinctly stimulate biomass production, whereas under the photoperiod L:D=17:7 stimulation is most pronounced when oxidizing compounds are applied. This contrast cannot, however, be considered as categorical since cysteine and reduced glutathione have a tendency in some cases, under diurnal changes of light and darkness, to enhance dry mass production, and potassium permanganate under 24-h illumination also has a slightly stimulating effect. The fact should be noted that the compounds applied by us may have affected the organism of the alga not only because of their reducing or oxidizing properties, but also on account of their specific participation in metabolism. Especially cysteine may have been incorporated into the proteins, and potassium permanganate may have been utilised as a source of manganum in the photosynthesis process. At any rate the influence of the agent applied by us should not be considered one-sidedly, solely as a reducing or oxidizing action on the environment.

Analysis of changes of the redox potential occurring with lapse of time in the medium under L:D=17:7 indicates that the growing population of algae causes reduction in the environment. Measurements of the redox potential, however, performed with a smooth platinum electrode are completely reliable only when the pH of the environment remains constant. In the present case they should be regarded with reserve since the pH of the medium was not stabilised. The application of a sufficiently strong buffer did not seem possible because of chemical and, above all, biological complications. Actually the medium became alkaline in the course of growth of the algal population, and this fact undermines the conclusion concerning reduction in the medium by the algal organism.

It is possible that, in the course of the light phase within 24 h, photosynthetic oxygen release caused a rise of the redox potential of the medium, whereas during the dark phase only the reducing action of the respiratory metabolism of the alga was in operation, similarly as is the case in cultures of heterotrophic bacteria. Such an argumentation would explain the results which indicate that, under constant illumination, the reducing substances in suitable concentration stimulate as a rule an increase in biomass, and when the light conditions changed within 24 h this effect disappeared, whereas oxidizing agents evoked a distinct stimulation not under continuous light, but just when darkness occurred periodically. The difficulty in interpretation of the influence of depression or eventually increase of the redox potential of the medium without referring it to definite redox substances, consists in the individual action of these agents and in the differences in their optimal concentration.

The noted decrease in protein content in the course of the culture when converted to dry weight may be explained by an earlier inhibition of cell division, while the population dry weight still continues to increase. Earlier inhibition of cell proliferation as compared with the dry mass increment of the population of *Scenedesmus quadricauda* was reported by Kyć (1970) who determined dry weight and counted the cells. The fact should, however, be stressed that the acceleration in dry mass production due (under appropriate conditions) to the redox compounds applied was also connected with an increase in protein production.

It seems that for a more precise knowledge of the phenomena observed by us, further investigations should be undertaken which would take into account simultaneously changes in pH. This would make possible the expression of the redox potential in terms of the rH value without the necessity of buffering the medium. Moreover, it might be rewarding to study the changes in pH and rH immediately after the periods of darkness and light in the course of 24 h.

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Wpływ związków redukujących i utleniających oraz potencjału redox pożywki na biomasę Scenedesmus quadricauda (Turp.) Bréb.

Streszczenie

Badano wpływ kilku stężeń niektórych związków redukujących i utleniających na przyrost suchej masy i białka zielenicy *Scenedesmus quadricauda* (Turp.) Bréb. Sterylne hodowle prowadzono w fototermostacie na pożywce Uspenskiego (1927) uzupełnionej mikroelementami. Reduktory (cysteina, zredukowany glutation, kwas askorbinowy i pirokatechina) wywoływały wyraźną stymulację przyrostu biomasy przy oświetleniu ciągłym kultur, natomiast utleniacze ($KMnO_4$, $K_2Cr_2O_7$ i H_2O_2) powodowały podobny efekt przy dobowych zmianach światła i ciemności (L:D=17:7). Efekty te były zależne od stężenia poszczególnych związków oraz od wywoływanej przez te związki wysokości potencjału redox pożywki. Ponadto ujawniły się różnice w działaniu poszczególnych związków niezależnie od ich właściwości utleniająco-redukujących. W miarę upływu czasu hodowli obserwowano obniżanie potencjału redox pożywek pod wpływem glonów; jednakże pomiary takie wykonano tylko przy fotoperiodzie L:D=17:7. Zawartość białka w suchej masie malała z upływem czasu hodowli.