Increase in the biomass of some green algae species in nitrate and ammonium mediums depending on auto-, mixo- or heterotrophic conditions

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Abstract

The increase in total dry mass and protein in cultures of *Chlorella pyrenoidosa*, *Scenedesmus quadricauda* and *Ankistrodesmus acicularis* was studied. Under autotrophic conditions, increases in dry mass were, as a rule, larger in the nitrate medium than in the ammonium one, under mixotrophic conditions the situation was reversed and in the case of heterotrophy, the individual species reacted differently. The dependence of the protein content increase on the nitrate or ammonium form of the medium was not clear. Changes in time of the pH and rH of the mediums were followed and the interdependence of these changes with the production of biomass is discussed.

Key words: algae, nitrates, ammonium salts, redox potential

INTRODUCTION

In an earlier paper (Gumiński et al. 1983) on the effect of reducing or oxidizing agents on the increase of biomass in the same species of green algae as studied in this paper, we presented, among others, differences in their growth in nitrate and ammonium mediums. These were experiments conducted under constant illumination and exclusively on mineral mediums; under those conditions, all of the studied species grew better in the nitrate than in the ammonium medium.

The utilization of NO_3^- and NH_4^+ ions by algae has been studied by relatively long ago by a large number of physiologists, and a discussion of their results can be found in the compendious works by Syrett (1962) and Morris (1974). However, comparative studies on the effectivity of these ions under auto-, mixo- and heterotrophic conditions have not been carried

out and the problem of redox potential changes during culture has been overlooked. These topics have become the subject of the study done presently by determining dry mass and protein.

MATERIAL AND METHODS

The experiments were done on pure cultures of *Chlorella pyrenoidosa*, *Scenedesmus quadricauda* and *Ankistrodesmus acicularis*; material from these cultures was also used in previous studies (Gumiński et al. 1983).

In the experiments, Uspenski's nitrate medium (1927) and its ammonium variety worked out by us (Table 1) were used.

Table 1
Nutrient composition in $\text{g} \cdot \text{dm}^{-3}$

Compound	Nitrate medium	Ammonium medium
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	0.207	—
KNO_3	0.025	—
KH_2PO_4	0.025	0.025
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.050	0.050
K_2CO_3	0.035	0.069
CaCO_3	—	0.087
$\text{Fe}_2(\text{SO}_4)_3$	0.002	0.002
$(\text{NH}_4)_2\text{SO}_4$	—	0.132

Both mediums also contained microelements in the following amounts (mg per dm^3): H_3BO_3 — 0.6, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ — 0.4, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ — 0.05, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ — 0.05, $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$ — 0.02. In the appropriate variant, 0.1% glucose was added to the mediums. The basic cultures grew on nitrate mediums, without glucose.

The composition of the ammonium medium was chosen after testing 8 preliminary variants which depended on substitution of the cations accompanying the anion NO_3^- and on buffering the medium. The chosen variant was shown to be relatively the most favourable in autotrophic cultures.

The algae were grown in the experimental cultures for about 3 weeks. Every few days part of the cultures was liguidated in order to perform the analyses. The cultures were kept in a photothermostat room at a temperature of about 24°C . In the variants with illumination, lighting was given constantly at an intensity of about 7000 lx (approx. $10 \text{ W} \cdot \text{m}^{-2}$). This is for autotrophic (without glucose) and mixotrophic (with glucose) cultures. Fluorescence lamps (tubes) were used. Heterotrophic cultures were grown with the addition of glucose, in the dark.

Inoculations were done by introducing into 25 cm³ of medium in a 100 cm³ Erlenmayer flask, 1 cm³ of a suspension of algae from a two week-old basic culture. Five replicates were used for each analysis during the culture.

The dry mass of the algae and their protein content were determined. Protein was determined using Lowry's methods as described by Mejbaum-Katzenellenbogen and Mochnacka (1968). More details on this subject can be found in an earlier paper (Gumiński et al. 1984).

The pH and redox potential of the mediums were determined electrometrically. Accepted as an indicator of the redox potential was the rH value calculated from the formula: $rH = \frac{Eh}{29} + 2$ pH, where Eh is the sum of the potentials of a smooth platinum electrode in respect to a calomel electrode and the standard potential of a calomel electrode in respect to a hydrogen one, expressed in milivolts with measurements done at room temperature. The initial pH of the medium was about 7, rH about 28.

When dry mass was determined, average deviations were calculated for 5 replicates.

RESULTS

Chlorella pyrenoidosa

In the culture grown in the light without glucose (autotrophy), dry mass increased much more in the nitrate medium than in the ammonium one, however, when glucose was added (mixotrophy), the situation was reversed; with that growth was much better in the medium with glucose. In the dark, where glucose was always added (heterotrophy), dry mass increased however, more in the medium with nitrate than in the one with ammonium. In the light, in mediums with glucose and either NH₄⁺ or NO₃⁻, the dry mass increased continuously to the end of culture, that is, until the 19th day. Without glucose, it increased until the end only in the presence of NO₃⁻ whereas in the case of NH₄⁺, only until the 16th day. In the dark, both with NO₃⁻ and NH₄⁺, increase in dry mass was found only until the 12th day, after which its amount lessened (Fig. 1).

The protein content rose, as a rule, only until the 16th day of culture. Under conditions of autotrophy it increased more rapidly in the nitrate medium than in the ammonium one, however, at the end, it was higher in the latter. In the case of mixotrophy, the reverse was true. Under heterotrophic conditions, the protein yield was initially higher in the ammonium medium; in the later stages of culture, in the nitrate medium (Fig. 2).

The pH was maintained on a generally higher level in nitrate mediums than in ammonium ones and was lower in heterotrophic conditions than

Fig. 1. Yield of dry mass in *Chlorella pyrenoidosa* cultures. Vertical bars denote the standard error

Fig. 2. Yield of protein in *Chlorella pyrenoidosa* cultures

in auto- and mixotrophic ones. Decided acidification of the medium took place only under heterotrophic conditions in the presence of the ammonium form of nitrogen on the 8th day of the experiment. Generally speaking, the pH of the ammonium medium rose with time from slightly acidic to slightly alkaline (Table 2).

Table 2

Changes of pH and rH values *Chlorella pyrenoidosa* cultures

Type of medium		Type of measurement	Days of cultivation				
			4	8	12	16	19
Auto-trophic	NO ₃ ⁻	pH	8.15	9.6	9.3	9.2	8.42
		rH	28.48	29.53	29.82	30.3	30.52
	NH ₄ ⁺	pH	6.83	7.2	6.9	6.9	7.25
		rH	27.75	28.69	27.24	28.18	29.90
Mixo-trophic	NO ₃ ⁻	pH	8.3	9.2	9.0	8.2	8.3
		rH	30.32	31.12	29.90	30.43	30.63
	NH ₄ ⁺	pH	6.63	6.8	6.7	7.4	7.77
		rH	29.83	28.88	28.73	29.45	29.94
Hetero-trophic	NO ₃ ⁻	pH	6.7	7.5	8.2	8.2	8.45
		rH	29.53	29.25	30.70	30.64	31.19
	NH ₄ ⁺	pH	6.33	4.8	5.5	6.9	7.65
		rH	28.11	24.68	27.01	30.16	30.36

The rH values was as a rule higher in nitrate mediums than in ammonium ones. In the case of heterotrophy, in the latter mediums, this value fell on the 8th day of culture by about 4 units; on this same day, as was already mentioned, decided acidification in these same mediums was also found. Aside from this, the rH value either rose with time or remained on the same level in both the nitrate and ammonium mediums (Table 2).

Scenedesmus quadricauda

The effect of the studied experimental factors on the increase of dry mass in *Scenedesmus* was similar as in *Chlorella*. However, under heterotrophic conditions, the increases in biomass in both nitrate and ammonium mediums were equivalent (Fig. 3).

Increases in protein were slightly higher in the nitrate medium than in the ammonium one in all three variants, that is, under auto-, mixo- and heterotrophic conditions (Fig. 4). The highest amount of protein was usually found on the 16th day of culture.

Fig. 3. Yield of dry mass in *Scenedesmus quadricauda* cultures. Vertical bars denote the standard error

The pH in the appropriate experimental variants was usually higher in the nitrate mediums than in the ammonium ones. Changes in the redox potential did not show a definite tendency. As in *Chlorella* cultures, a drop in the rH potential in the ammonium medium under heterotrophic conditions was found; however, in *Scenedesmus* ones, it took place more clearly after 4 rather than 8 days of culture. In addition, this phenomenon also took place after 16 days culture in the case of autotrophy (Table 3).

Fig. 4. Yield of protein in *Scenedesmus quadricauda* cultures

Table 3

Changes of pH and rH values in cultures of *Scenedesmus quadricauda*

Type of medium		Type of measurement	Days of cultivation				
			4	8	12	16	19
Auto-trophic	NO ₃ ⁻	pH	7.66	9.4	9.4	9.5	9.06
		rH	29.08	30.12	30.02	30.56	30.48
	NH ₄ ⁺	pH	7.2	7.5	7.4	7.3	7.65
		rH	28.49	29.42	28.16	26.50	29.44
Mixo-trophic	NO ₃ ⁻	pH	8.83	9.5	8.3	9.0	8.5
		rH	31.41	30.01	30.50	30.43	31.16
	NH ₄ ⁺	pH	6.9	8.2	8.6	9.1	7.1
		rH	29.11	30.12	29.45	31.40	30.23
Hetero-trophic	NO ₃ ⁻	pH	6.7	7.3	7.7	8.3	8.28
		rH	29.53	28.17	29.70	30.64	30.24
	NH ₄ ⁺	pH	7.0	7.2	7.3	7.3	7.6
		rH	26.24	28.49	29.93	32.61	30.83

Ankistrodesmus acicularis

The increase in dry mass under conditions of autotrophy was higher in the nitrate medium than in the ammonium one (as in the previous two species). In the case of mixotrophy, the increase in dry mass dominated initially in the ammonium medium. However, after 12 days, the production of dry mass in the ammonium medium stopped and was equal in both the ammonium and nitrate variants. As a result, at the end of culture, the largest dry mass was found under autotrophic conditions in the nitrate medium. In the case of heterotrophy, the highest increase in dry mass was shown by the ammonium variant (Fig. 5). The increase in protein

Fig. 5. Yield of dry mass in *Ankistrodesmus acicularis* cultures. Vertical bars denote the standard error

in the autotrophic variant was higher in the nitrate than in the ammonium medium. In the case of mixotrophy and heterotrophy these differences disappeared. The maximum amounts of protein in the individual variants fell on different days culture (Fig. 6).

Fig. 6. Yield of protein in *Ankistrodesmus acicularis* cultures

The pH value in the appropriate variants (auto-, mixo- and hetero-trophic) was higher in the nitrate mediums than ammonium ones.

The rH value was maintained on a higher level in the nitrate variants than in the ammonium ones, similarly as was found with the two previous species. However, in *Ankistrodesmus* a fall in the pH and rH values in the initial stages of culture under heterotrophic conditions did not take place as had been found in *Chlorella* and *Scenedesmus* cultures (Table 4).

Table 4

Changes of pH and rH values in cultures of *Ankistrodesmus acicularis*

Type of medium		Type of measurement	Days of cultivation				
			4	8	12	16	19
Auto-trophic	NO ₃ ⁻	pH rH	7.6 28.49	9.4 29.98	9.5 29.88	9.4 30.02	9.06 30.43
	NH ₄ ⁺	pH rH	7.0 28.09	7.3 27.66	7.0 27.82	7.2 29.04	7.65 29.67
Mixo-trophic	NO ₃ ⁻	pH rH	6.5 30.14	7.0 28.84	7.5 28.96	8.2 30.40	8.5 30.68
	NH ₄ ⁺	pH rH	6.8 28.71	6.7 28.68	7.1 29.51	7.1 30.23	7.1 27.88
Hetero-trophic	NO ₃ ⁻	pH rH	6.6 29.60	7.1 29.14	7.6 30.19	8.3 30.64	8.3 29.90
	NH ₄ ⁺	pH rH	6.8 28.71	7.3 28.85	6.9 29.33	7.1 30.30	7.6 30.43

DISCUSSION

It was already known from previous studies (Gumiński et al. 1983) that all of the three studied species when feeding only autotrophically, grow better on mediums containing the nitrate form of nitrogen rather than the ammonium form. Presently, we have found that under the conditions of mixotrophy and heterotrophy (in the presence of exogenous glucose) the increase in dry mass can be much higher in the ammonium medium than in the nitrate one. On the basis of literature (Syrett 1962, Morris 1974) this can be explained in the following manner. First of all, exogenous glucose can prevent poisoning by excess of ammonia, secondly, an organism uses much less energy to assimilate ammonia than nitrate. It would seem that in our case, the eventual consequences of physiological acidity of ammonium sulfate were successfully prevented by buffering the medium. However, under conditions of autotrophy, and in the case of *Chlorella*, also of heterotrophy (in the presence of exogenous glucose) the nitrate

variant dominated in the production of dry mass over the ammonium variant. The superiority of the nitrate over the ammonium form not only under conditions of autotrophy but also of heterotrophy in *Chlorella* is cause for thought and leads us to treat this organism as clearly nitrate-philic or ammoniumphobic.

Mixotrophy gave better conditions than auto- or heterotrophy, and therefore the quickest increase in dry mass under mixotrophic conditions was what was expected. Also the dominance of the ammonium variant over the nitrate one in mixotrophy is not surprising.

Increase in protein under autotrophic conditions lasted for a shorter period than the increase in total dry weight, from which we can conclude that the multiplication of cells stopped sooner than the production of non-protein biomass. This would be in agreement with the results of Kyć (1970) who studied both cell multiplication and increase in dry mass of *Scenedesmus quadricauda* in autotrophic cultures. In the case of heterotrophy we did not observe such a phenomenon; in the case of mixotrophy the situation was unclear. It seems that two factors at the same time play an important role here: light and exogenous glucose.

The drop in dry mass from the 12th or 16th day of culture under heterotrophic conditions which was observed in all three species, and in *Ankistrodesmus acicularis* also under mixotrophic conditions, indicates the prevalence of catabolism over anabolism in the later phases of culture. This phenomenon also took place in relation to protein although with a certain shift in time. The drop in biomass at the end of culture could have been caused by either exhaustion of nutrients or by the accumulation of harmful metabolic products.

The lowering of the pH of the ammonium medium by the plants growing on it is a well known and understood fact. In order to prevent this negative phenomenon, we buffered the medium which in practice did not allow it to be permanently acidified. But the finding of a lower redox potential in the ammonium mediums than in the nitrate ones during the culture of the algae is something new and indicates the biological reduction of the environment when the ammonium ion is utilized. This phenomenon was clearly visible in *Chlorella* and *Scenedesmus* cultures, less clearly in *Ankistrodesmus* ones. This organism, however, showed the lowest increase in biomass and may be this is the reason this phenomenon was not so distinct as in the case of the other two species. The relatively parallel lowering of the pH and rH of the ammonium medium allows the assumption to be made that in both cases it is connected with the secretion of hydrogen cations in exchange for ammonium cation uptake. It is known that the acidification of the environment favours its reduction or, eventually, hinders its oxidation.

It remains questionable whether the rH value is the appropriate measure of the redox potential of the medium in which algae are cultivated. However, since Eh measurements in solutions of variable pH cannot be accepted as an objective estimation of the redox potential of that solution, and pH changes of the medium during cultivation of algae are inevitable, in spite of everything, we accepted rH as a better indicator of the medium's redox potential.

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Przyrost biomasy wybranych gatunków zielenic w pożywce azotanowej lub amonowej w zależności od warunków auto-, mikso- czy heterotroficznych

Streszczenie

Badano przyrost całkowitej suchej masy i białka roślin w kulturach *Chlorella pyrenoidosa*, *Scenedesmus quadricauda* i *Ankistrodesmus acicularis*. Sucha masa przyrastała najsilniej w warunkach mikсотroficznych, słabiej w przypadku autotrofii i o wiele słabiej w wariancie heterotroficznym. Nie stwierdzono jednoznacznych różnic w przyroście białek. W przypadku autotrofii ilości suchej masy były znacznie większe w pożywce azotanowej niż w amonowej,

w warunkach miksotrofii było odwrotnie a w warunkach heterotroficznych poszczególne gatunki reagowały niejednakowo. Przyrosty białka nie wykazywały określonych różnic w zależności od azotanowej czy amonowej formy pożywki. Przyrost białka w warunkach auto- i miksotroficznych trwał na ogół krócej niż przyrost całkowitej suchej masy. Odczyn kwasowo-zasadowy utrzymywał się w pożywce amonowej na niższym poziomie niż w odpowiednich wariantach doświadczalnych w pożywce azotanowej. Podobnie potencjał redox pożywki amonowej był w odpowiednich wariantach doświadczalnych niższy niż azotanowej.