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THE INFLUENCE OF THE REDOX POTENTIAL OF THE MEDIUM ON THE GROWTH OF SOME CHLOROPHYCEAE

1. INTRODUCTION

The effect of the environmental redox potential on cultures of microorganisms has been widely studied, but information is very scarce as far as algae are concerned. The oxidative-reducing properties of the environment in which algae grow were assumed to be an ecological and physiological factor analogous to the acid-base equilibrium of that environment. In consequence, some experiments were performed to evaluate the influence of some oxidizing and reducing substances on algal growth. Using these substances, the initial redox potential of the nutrient medium at a given level was determined.

2. MATERIAL AND METHODS

Three species of Chlorophyceae were used: Scenedesmus quadricauda, Chlorella pyrenoidosa and Ankistrodesmus acicularis. All of them came from the Collection of Autotrophic Algae Cultures of the Czechoslovak Academy of Sciences in Prague. Each species was cultivated under sterile conditions in Uspenski's medium [6], supplied with certain microelements according to Czopek [1]. The composition of this medium was: $Ca(NO_3)_2 \cdot 4H_2O$ — 0.207; KNO_3 — 0.025; KH_2PO_4 — 0.025; $MgSO_4 \cdot 7H_2O$ — 0.05; K_2CO_3 — 0.035; $Fe_2(SO_4)_3 \cdot H_2O$ — 0.002 grams per litre of distilled water and 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; $H_2MoO_4 \cdot 4H_20$ — 0.02 milligrams per litre of macroelement solution. The pH of this medium was 6.9, the rH about 29.

The algae were cultivated in a photothermostat at a temperature of about 24°C and a light intensity of ca. 7000 lx. In individual experiments, the cultures were illuminated by "Flora" — fluorescent tubes — either continuously or for 17 h per day.

The algae cultures containing an oxidizing or reducing agent were placed (25 ml) in a 100 ml Erlenmayer flask, and incubated for several days. Every few days some of the cultures were killed and analysed, and the dry weight of these organisms determined. As necessary, the protein, iron, phosphorus and chlorophyll contained in them were also estimated.

Cysteine, glutathione (reduced form), pyrocatechol, pyrogallol and ascorbic acid were used as reducing agents. Hydrogen peroxide, potassium permanganate, potassium dichromate and potassium hexacyanoferrate (II) were used as oxidizing agents. Using different concentrations of these substances, the initial redox potential of the medium at a desired lowered or raised level was obtained. When sampling the algae for analysis, changes in the pH and Eh of the media were recorded. In this way, it was possible to compare the changes in pH and redox potential with the increase in dry weight and the content of the investigated organic or mineral substances in the algae.

pH and Eh were measured using a glass or platinum electrode in conjunction with a calomel electrode. The redox potential was calculated from the formula:

$$rH = \frac{Eh}{29} + 2pH$$

where Eh is expressed in millivolts.

The protein content in the dry weight of algae was estimated by Lowry's method [5], iron was estimated colorimetrically using dipyridyl [4], phosphorus — also colorimetrically using Fiske-Subbarow's method [5]. Chlorophyll was determined in the fresh biomass of algae by measuring the extinction in a 90% solution in acetone separately for chlorophyll "a" and "b" [3]; a Zeiss VSU-2P spectrophotometer was used.

3. RESULTS

3.1. Cultures in liquid media

The dry weight of algae increased rapidly during the first week, then the growth rate gradually decreased until a constant value was reached after about three weeks cultivation. Protein increase ceased much earlier than that of the total dry weight.

The P/Fe ration in the dry weight increased, while the chlorophyll content decreased with time. The dry weight increase was greater in the medium containing nitrate than in the one containing ammonia. The former medium became more alkaline, the latter more acid. Addition of calcium carbonate inhibited the acidification of the ammonia medium and was accompanied by an increase in dry weight production.

It was found that the increase in dry weight in cultures growing under continuous illumination was accompanied by an increase in the redox potential of the medium. In experiments conducted under conditions of alternating light and darkness, it was found that after the period of illumination (day) the dry weight, as well as the pH and rH of the solution increased while the protein content in the dry weight decreased. After the period of darkness (night), reverse effects were observed.

The effect of the various oxidizing and reducing agents on the redox potential of the medium was fairly short-lived: the difference in potentials with respect to the control media disappeared after a few days. This equalisation took place not only in the media containing algae, but also, though much more slowly, in those media without algae. Nevertheless, these substances significantly affected the growth of the algae and also the biological and biochemical properties investigated.

In general, it may be stated that this effect is more a property of the given compound than of its oxidizing or reducing abilities. At suitably low concentrations of cysteine and ascorbic acid, the biomass production of algae increased; however, other reducing agents at concentrations reducing the rH by an identical amount had the reverse effect. The very slight raising of the rH by the various oxidizing agents either increased the algal growth rate, or had no effect at all. Potassium permanganate was the most effective oxidizing agent. Higher concentrations of either oxidizing or reducing agents inhibited growth or even killed the algae, oxidizing agents being the more toxic. The addition of reducing agents to the medium tended to increase the chlorophyll concentration in fresh algae whereas oxidizing agents usually had the reverse effect.

3.2. Cultures in solid media

Experiments performed in media solidified with agar showed that, in the case of illuminated cultures, these species of algae grew not only on the surface of the agar, but also in the deeper layer. In media without sucrose, growth of algae in the deeper layer was observed only in the control cultures and in the presence of pyrocatechol. On adding sucrose, the algae also grew in the presence of oxidizing agents. No

growth in the deeper layer of the medium took place in darkness. Neither in liquid nor in solid media were any distinct differences noted in the reactions of the three species of algae to the various physiological factors.

4. DISCUSSION

This study has shown that the strains of Scenedesmus quadricauda, Chlorella pyrenicosa and Ankistrodesmus acicularis are adapted to life in an environment with a high redox potential (rH=ca. 30). By giving off oxygen during photosynthesis, this potential is maintained at a high level. This apparently does not affect the algae, as they do not require intervals of darkness during which the redox potential of the medium decreases. These algae grow much better in media containing nitrates than in ammonium salts. The less intensive growth in ammonium salts can be explained not only by the acidification of the environment, but also by the harmful action of excess ammonia on the algal cells. The addition of calcium carbonate did prevent the acidification of the medium, but growth was even so, less intensive than in media with added nitrates. Bearing in mind that ammonia inhibits photosynthetic phosphorylisation [2], it is very probable that this happened during our experiments with Chlorophyceae. Also, as Warburg and Negelein [7] showed, nitrates are metabolic oxidizing agents. Furtheremore, in darkness and heterotrophic conditions, these algae grew only on the surface of the solid agar medium and should be considered as requiring strongly oxidizing conditions. These organisms in conditions favourable to photosynthesis are able to produce a high "reducing power" which is needed for efficient biomass production.

However, the observation that deep-layer growth taking place in a solid medium and appearing only in the presence of light is inhibited by oxidizing agents, and that this inhibition can be reversed by adding sucrose, indicates that when the removal of photosynthetic oxygen is rendered difficult, the tolerance of algae towards the oxidizing properties of the environment is exceeded.

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WPŁYW POTENCJAŁU OKSYDACYJNO-REDUKCYJNEGO POŻYWKI NA WYBRANE GATUNKI ZIELENIC

Streszczenie

Na pożywce Uspenskiego, uzupełnionej 5 niezbędnymi mikroelementami, hodowano aseptycznie w fototermostacie Scenedesmus quadricauda, Chlorella pyrenoidosa i Ankristodesmus acicularis. Do pożywki dodawano związki redukujące lub utleniające, obniżając lub podwyższając jej potencjał redox. Ponadto stosowano porównawczo formę azotanową i amonową azotu. W niektórych doświadczeniach zestalano pożywkę agarem.

W pożywkach płynnych sucha masa glonów przyrastała w ciągu kilkunastu dni, przyrost białka trwał znacznie krócej. Stosunek P/Fe w glonach wzrastał z czasem, a zawartość chlorofilu malała. Przy stałym oświetleniu potencjał redox pożywki wzrastał wraz z przyrostem suchej masy. Przy dobowych zmianach światło — ciemność po dniu stwierdzano wzrost rH pożywki i suchej masy glonów, po nocy było odwrotnie.

Wpływ poszczególnych związków redukujących lub utleniających na glony był indywidualny i nie udało się znaleźć ogólnej prawidłowości co do wpływu wysokości potencjału redox pożywki.

Przyrost biomasy glonów na pożywce azotanowej był większy niż na amonowej, nawet przy buforowaniu pożywki.

Na pożywkach zestalonych glony rosły wgłębnie jedynie w świetle.

Wszystkie trzy gatunki zielenic reagowały podobnie na zmiany poszczególnych czynników.

REFERENCES

1. Czopek M., *Metody hodowli Lemnaceae,* Wiadomości Botaniczne, 7(2), 1963.
2. Hofstra J., T. Koch-Bosma, *Organic phosphate in ammonium and nitrate fed tomato plants,* Acta Nederlandica, 19, 1970.
3. Jeffrey S. W., G. F. Humphrey, *New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton,* Biochem. Physiol. Pflanzen., 167, 1975.
4. Marczenko Z., *Kolorymetryczne oznaczanie pierwiastków,* Warszawa 1968.
5. Mejbaum-Katzenellenbogen W., I. Mochnacka, *Kurs praktyczny z biochemii,* Warszawa 1968.
6. Uspenski E. E., *Eisen als Faktor für die Verbreitung niederer Wasserpflanzen,* Pflanzenforschung, 9, 1927, Jena.
7. Warburg O., E. Negelein, *Über die Reduktion der Salpetersäure in grünen Zellen,* Biochem. Z., 110, 1920.