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FURTHER EXPERIMENTS WITH ALGAL CULTURES FOR DETERMINING SOME PROPERTIES OF WATER OF LAKE BALATON

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In a previous paper two problems were investigated by culturing a pure algal strain and by measuring its photosynthetic power (FELFÖLDY 1959): (a) could the filtered water of Lake Balaton be used as nutrient medium for mass culturing of unicelluar algae and (b) which properties of natural lake water are the factors limiting phytoplankton productivity.

These investigations were carried out with the algal strain, 7K *Chlorella vulgaris* BEIJER., which is one of the most special strains in the collection of algae of the Biological Research Institute, Tihany. The strains of this collection isolated from Lake Balaton, from pond Belső tó at Tihany and from calcareous, more or less alkaline soils of the neighbourhood of our Lake are for the most part basophilic, photosynthesizing more intensively in presence of bicarbonate ions than in solutions containing free carbon dioxide (FELFÖLDY 1960). The 7k *Chlorella* strain is, however, slightly acidophilous (pH optimum at about 6,8). This strain, if it is grown in preparatory culture made of KNOP solution modified by PRINGSHEIM (1946, 35) aerated with 3 per cent carbon dioxide in air (pH 6,5—6,8), is able to utilize only free carbon dioxide and its photosynthesis decreases parallel with the diminution of CO_2 content.

In the course of other previous investigations the importance of physiological properties of algal strains and the significant role of age, past history and training in their development were studied (FELFÖLDY 1960a).

The results of experiments performed in Balaton water (FELFÖLDY 1959) are presented in *Table 1.*

As it is shown by these experiments the rate of photosynthesis in lake water is not or only a little increased by the addition either of nitrate (N° 2) or by phosphate of equal pH (N° 5,9). Dosage of KH_2PO_4 has definite stimulating effect, which, however, might be attributed to the decrease of pH to 6,8. Photosynthetic activity of *Chlorella vulgaris* in lake water increases parallel with the rising hydrogen ion concentration (expts. N° 3,4,7,8,10). An increase of 68 per cent was observed also after addition of hydrochloric acid which otherwise has no effect on photosynthesis (expt. N° 8).

Knowing that in many plants, e. g. the plankton alga *Chlorella pyrenoidosa* (EMERSON and GREEN 1938), other algae (OUELLET and BENSON 1952, LORENZEN 1958, KESSLER 1953, 1953a, KESSLER and KRAMER 1960 etc.) and the higher aquatic *Myriophyllum spicatum* (STEEMANN NIELSEN 1960) the rate of photosynthesis rapidly becomes independent of pH over a wide range of pH at an optimal concentration of the carbon

Table 1. Photosynthetic rate (P_k = mg $O_2/10^9$ cells/one hour) of strain 7K Chlorella vulgaris suspended in variously treated water of Lake Balaton. The nutrient salts were added to the suspension as crystals (FELFÖLDY 1959, 215—216)

1. Táblázat : 7K Chlorella vulgaris fotoszintézise (P_k) különféleképpen kezelt Balaton-vízben

Number of experiment	Treatment	P_k	rel. P_k pure Balaton water = 100	pH
1	Pure lake water	4,53	100,0	7,8
2	+1g KNO_3/litre	4,65	102,6	7,8
3	+0,313g KH_2PO_4/l	7,91	174,8	6,8
4	+0,313g KH_2PO_4+1g KNO_3/l	7,42	163,7	6,8
5	+0,200g K_2HPO_4/l	3,68	81,2	8,0
6	Pure lake water	3,84	100,0	8,0
7	+0,313g KH_2PO_4/l	6,70	174,5	6,8
8	+5 ml 0,05 N HCl/l	6,47	168,5	6,9
9	+0,200g K_2HPO_4/l	5,08	132,2	8,0
10	+0,100g K_2HPO_4 + 0,156g KH_2PO_4/l	6,58	171,1	7,4

source, and knowing, that in the case of Balaton water changes in hydrogen ion concentration cannot be separated from changes occurring in the carbonate-bicarbonate-free carbon dioxide ratios, the following inferences might be drawn from these experiments. The quantities of N and P present in water of Lake Balaton are sufficient for the photosynthetic activity of phytoplankton and these experimental results emphasize the importance of inorganic carbon cycle in the productivity of Lake Balaton and of other lakes similarly alkaline and rich in calcium.

Several other experiments performed with other the strains of the axenic (MCLAUGHLIN 1960) culture collection of the Biological Institute necessitate the revision of these results and conclusions.

Materials and methods

The 953. *Coelastrum microporum* NÄG. strain of the living algal collection of the Biological Institute, Tihany was chosen for these new experiments. The nature of this strain was known, for it had been examined thoroughly in previous experiments. This strain might be placed among strains belonging to the third photosynthetic type, which are able to utilize both free carbon dioxide and hydrocarbonate ions, and which after certain lag-period photosynthesize more vigorously in solutions containing hydrocarbonates and surplus of carbonates than in the presence of free carbon dioxide (FELFÖLDY 1960, 198). It is suggested that hydrocarbonate assimilation is the result of some training. The lag period occurring before the start of hydrocarbonate assimilation may be interpreted as a period that is needed by the cells to develop or activate a system for absorption of hydrocarbonate ions. No lag period was observed, naturally, in suspensions cultured previously in nutrient media containing no free carbon dioxide (pH 8,5) (FELFÖLDY 1960a, 183).

The stock cultures are maintained on agar slants containing glucose, peptone, yeast hydrolysate and the inorganic salts of KNOP—PRINGSHEIM solution (PRINGSHEIM 1946, 35). For preparatory cultures algae were scarped from agar slants and were suspensed in one litre of liquid KNOP—PRINGHEIM medium in two litre ERLENMEYER flasks. The initial pH was adjusted before autoclaving to 7 with some drops of N NaOH solution. The cultures were aerated with 3% carbon dioxide in air filtered through cotton plugs. During aeration the pH decreased to about 6,8. The culture vessels were illuminated by 40 W fluorescent tubes (Tungsram F2 "warm white") ca. 7000 lux. Temperature in the flasks was 22—25° C.

For photosynthetic measurements aliquots were taken from the three month old preparatory cultures. The cells were separated from the nutrient solution by centrifuging and were washed twice with the experimental medium.

10 ml algal suspension was pipetted into the main compartment of WARBURG vessels and the release of oxygen was measured by the usual manometric method (25 ± 0,1° C; 40 W fluorescent tube, Tungsram F_2 "warm-white", c. 5000 lux; shaking velocity of about 110 cycles per minute with a travel of 4,5 cm). The results are given in mg O_2/1 mg dry matter of algae units. Determination of dry matter was carried out by filtering the cells through Macherey—Nagel Nr. 640 d 5,5 cm ⌀ filter paper and drying them at 105° C to constant weight. The experimental medium was either pure filtered off shore water of Lake Balaton (filtered through the above mentioned Macherey—Nagel paper) or the same water enriched with one of the following nutrient salts as potassium nitrate, potassium dihydrophosphate, dipotassium phosphate and hydrochloric acid.

The chemical composition of the water of Balaton does not exclude the presence either of free carbon dioxide or of carbonate. Its pH fluctuates between 8,42—8,44 (in extraordinary cases 8,7). The chemically detectable CO_3^{--} content varies between 0—19 mg/l in unfiltered Balaton water. A considerable part of this carbonate consists of the fine calcium carbonate precipitate resulting from the photosynthetic activity of phytoplankton ("biogene marl"). In consequence of the preceding filtration the greater part of carbonates was missing from our experimental solutions (the pH of this filtered water was always lower than the pH of off shore water).

On the other hand, as regards determination of the small amounts of free carbon dioxide present in water of Lake Balaton at these higher pH

Table 2. Chemical analysis of the off shore water of Lake Balaton at Tihany (MÜLLER 1928)

2. Táblázat : Balatonból származó nyíltvizi minták kémiai analízisének eredménye

	Al^{+++}	Fe^{++}	Ca^{++}	Mg^{++}	K^{+}	Na^{+}	Cl^{-}	SO_4^{--}	HCO_3^{-}	CO_3^{--}	H_2SiO_3
Surface water ...	1,25	0,04	28,8	40,5	5,16	22,8	9,4	46,4	260,15	15,85	13,1
Depth of 5 m ...	1,25	0,04	29,0	40,6	5,16	22,6	9,2	45,3	255,05	18,95	13,5

values useful results were obtained only by computations from the pH and bicarbonate alkalinity. According to our computations (FELFÖLDY 1959) on basis of symbols of KLUT—OLSZEWSKI (1945, 59) and analytical data of B. ENTZ (1953, 33) the free carbon dioxide content of off shore water is about 0,2—0,4 mg/l.

To demonstrate the chemical composition of the off shore water the analytical data of MÜLLER (1928) are collected in *Table 2.*

Concentration of nitrogen and phosphorus compounds are low as it is seen below (MAUCHA ap., G. ENTZ and SEBESTYÉN 1946, 247):

	mg/l
Albuminoid ammonia	0,497
Ammonium ion (H_4N^+)	0,149
Nitrite (NO_2^-)	0,021
Nitrate (NO_3^-)	0,267
Phosphate (PO_4^{---})	0,031
Total nitrogen	1,028
Inorganic phosphorus	0,010

The chemistry of water of Lake Balaton has been investigated by several authors (SZABÓ 1930, CSEGEZY 1938, WOYNÁROVICH 1941, SEBESTYÉN *et al.* 1951, B. ENTZ 1953, 1959), nevertheless, nothing new has been given as regards the composition of off shore water except for the description of smaller changes and local differences.

Results and discussions

In *Fig. 1* the photosynthetic curves of *Coelastrum microporum* suspensions in pure lake water are presented. For photosynthetic measurements the cells were taken from two preparatory cultures, from which the one was aerated with 3% carbon dioxide in air (1) while the other was not aerated (2). In the first case a lag-period of 180 minutes was observed, whereas there was no lag-period in the case of cells originating from non-aerated preparatory

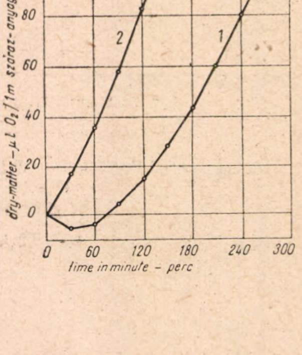

Figure 1. Rate of photosynthesis in *Coelastrum microporum* NÄG cells suspended in pure lake water and originating from two different preparatory cultures: 1. KNOP—PRINGSHEIM medium aerated with 3% carbon dioxide in air (pH = 6,7); 2. Knop-Pringsheim solution without aeration (pH = 8,8).

1. Ábra : Két különböző előkészítő tenyészetből származó *Coelastrum microporum* NÄG sejtek fotoszintézise tiszta Balatonvízben. 1. Elővelés 3% széndioxidot tartalmazó levegővel szellőztetett KNOP—PRINGSHEIM tápoldatban (pH = 6,7); 2. elővelés szellőztetés nélkül (pH = 8,8).

Рис. 1. Фотосинтез клеток *Coelastrum microporum* NÄG. из двух различных предварительных культур в чистой воде из Балатона. 1) Проращивание в питательном растворе Кнопа—Прингсхейма, проветренном содержащим 3% угольной кислоты воздухом (pH = 6,7); 2) Проращивание без проветривания (pH = 8,8) cultures. Here photosynthesis began just at the start of illumination. Should we attempt to illustrate numerically the photosynthetic power of cells in experiment N° 1, only data referring to the photosynthetic process following lag period may be used for calculations. For this reason experiments were performed with cells originating from non-aerated preparatory culture (pH = 8,8). The results are given in *Table 3*.

The results summarized in this table are entirely different from those seen in *Table 1*. The increase of hydrogen ion concentration either by acid phosphate or by hydrochloric acid definitely inhibits photosynthesis similar to the results obtained in previous experiments with *Coelastrum microporum* (FELFÖLDY 1960, 196; 1960a, 179).

Table 3. Photosynhesis (μl O_2/1 mg dry matter of algae/1 hour) in Coelastrum microporum suspended in variously treated water of Lake Balaton. The salts were added in crystal form to 50 ml portions of suspension.
3. Táblázat. 953. Coelastrum microporum Näg. fotoszintézise különféleképpen kezelt Balatonvízben

Number of experiment	Treatment	μl O_2/1 mg dry matter/ 1 hour	Relative photosynthesis. Pure Balaton water = 100	pH
11	Pure lake water	52,0	100,0	8,0
12	+0,313g KH_2PO_4/litre	35,2	67,7	6,9
13	+5ml 0,05 N HCl/litre	40,0	76,9	7,0
14	+0,200g K_2HPO_4/litre	58,4	112,2	8,1
15	+1g KNO_3/litre	50,8	97,8	8,0

According to present experiment the intensity of photosynthesis is the function of pH. Recent investigations (FELFÖLDY 1962) confirm the results of previous authors namely that pH is not a deciding factor within a fairly large pH range in the photosynthesis of algae assimilating free carbon dioxide (EMERSON and GREEN 1938, OUELLET and BENSON 1952, KESSLER 1953, LORENZEN 1958, KESSLER and KRAMER 1960, STEEMANN NIELSEN 1960), whereas in the case of algal strains utilizing hydrocarbonates a definite pH optimum was observed (ÖSTERLIND 1949, KRATZ and MYERS 1955). The position of this optimum is dependent on the past history of the culture (FELFÖLDY 1962).

Experimental data given in *Table 3* confirm the previous establishment (FELFÖLDY 1959) that the quantities of N and P present in water of Lake Balaton are sufficient at a given moment for the photosynthesis of phytoplankton, or at least photosynthesis is not stimulated by the addition of these elements. The other establishment made in the paper cited previously, namely that the advantageous effect of increased hydrogen ion concentration may be attributed to a parallel change in the carbonic acid system, can be upheld only in the case of strains requiring free carbon dioxide for their photosynthesis similar to *Chlorella vulgaris*. The algae living in Lake Balaton have adapted themsemves in the course of phylogeny to the utilization of hydrocarbonates (e. g. see the studies made on eight different *Scenedesmus* strains, FELFÖLDY 1960c) and the amount of hydrocarbonates always present in the water of

Balaton, showing only a small fluctuation, always meets the carbon requirement of these strains.

It should be emphasized on basis of present paper, that the qualification of natural waters by biological tests, especially by physiological experiments with algae (literature data see FELFÖLDY 1959) is not an easy task. In photosynthetic measurement the reserves of algal cells may constitute a source of errors, because these reserve substances render normal photosynthesis possible also for longer period even in pure $KHCO_3$ solution (about for 60 hours, cf. FELFÖLDY 1960b).

In growth experiments of similar purposes the specific physiological characters of test organism should be taken into consideration. This difficulty might be eliminated either by working with more algal strains (MAC PHEE 1961) or by investigating in detail the physiology of the strain used.

Summary

Studying the effect of chemical properties of water of Lake Balaton on photosynthesis in unicellular algae different results were obtained due to the physiological characters of test organisms. Experiments made with strain 7K *Chlorella vulgaris* BEJER. suggest the insufficiency of inorganic carbon sources because this strain requires free carbon dioxide for its photosynthesis. Photosynthetic activity of this strain is stimulated by decrease of pH in Balaton-water, because by this decrease free CO_2 is released.

If, however, algal strains utilizing easily also the hydrocarbonates dissolved in water are used (eg. *Coelastrum microporum* NÄG.) the increase of hydrogen ion concentration has no stimulating but, moreover, a retarding effect on photosynthesis. Considering that the algae living in the water of Lake Balaton are mainly basophilic, utilizing hydrocarbonates, that conclusion of the previous article reported in the introduction of this paper that the amount of inorganic carbon is insufficient in Lake-water, has to be revised.

As the present investigations show, the water of Lake Balaton contains sufficient P and N reserves at a given moment for the photosynthesis of algae. Nor would it be correct to speak of insufficient carbon supply in the case of basophilic algal strains either.

It should be pointed out on basis of these results that the characterization of natural water bodies by biological tests, especially by physiological experiments with algae is not simple, and the reliability of results greatly depends on the suitable physiological characters of the test organism.

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ÚJABB KÍSÉRLETEK A BALATON-VÍZ TULAJDONSÁGAINAK VIZSGÁLATÁRA ALGA TISZTATENYÉSZETEKKEL

Felföldy Lajos

Összefoglalás

Egy régebbi dolgozatban (FELFÖLDY 1959) megaállapítást nyert az *1. táblázat*ban közölt kísérleti eredmények alapján, hogy a Balaton-vízhez kevert nitrát és azonos pH értékű foszfát oldat nem, vagy alig növeli a teszt-növényként használt 7K *Chlorella vulgaris* BEIJER. asszimilációs tevékenységét. Savanyú foszfát, sőt a fotoszintézisre egyébként hatástalan sósav oldat viszont stimulálólag hat, azaz a hidrogén ion koncentráció növelésével párhuzamosan nő a fotoszintézis. Balaton-vízről lévén szó, a hidrogén ion koncentráció változása elválaszthatatlan a benne levő karbonát-, hidrokarbonát-szabad széndioxid-rendszer arányainak megváltozásától. Irodalmi adatokból tudjuk, hogy az első sorban szabad széndioxidot igénylő növények fotoszintétikus tevékenységére a hidrogén ion koncentráció alig van hatással, elég tág pH-határok között. Az *1. táblázat*-ban közölt eredmények alapján feltételeztük, hogy a Balaton-víz szervetlen szénforrásainak aránya nem kedvező a fitoplankton algák fotoszintézise számára.

Az egysejtű algák szervetlen szénforrásait illető évekig tartó kísérletek arra figyelmeztettek, hogy a 7K *Chlorella* törzs ilyen szempontból kivételes tulajdonságokkal rendelkezik alga tisztatenyészet gyűjteményünk többi tagjaival összehasonlítva, amennyiben azok bazofiliájával és élénk hidrokarbonát hasznosításával szemben ez a törzs gyengén acidofilnek bizonyult mely fotoszintéziséhez szabad széndioxidot igényel, különösen ha 3% széndioxidot tartalmazó levegővel szellőztetett tápoldatban neveljük elő: pH6,5—6,8.

A Balaton-víz tulajdonságait kutató kísérleteket megismételtük egy alaposan ismert bazofil törzzsel (953. *Coelastrum microporum* NÄG). Ha ezt a törzset 3% széndioxidot tartalmazó levegővel szellőztetett KNOP-PRINGSHEIM oldatban (pH = 6,5—6,8) neveljük elő, majd tiszta Balaton-vízben szuszpendáljuk, a hidrokarbonát-asszimilációra jellemző lappangási periódus után indul meg a fotoszintézis (*1. ábra* 1. görbe). Ha a sejteket szellőztetés (széndioxid utánpótlás) nélküli oldatban neveljük (pH = 8,8), tiszta Balaton-vízben szuszpendálva a fotoszintézis a megvilágítás kezdetekor, lappangási idő nélkül indul jelezve, hogy az alga élettani tulajdonságai már az előkészítő tenyészetben átálltak a hidrokarbonát ionok hasznosítására (*1. ábra* 2. görbe). Ilyen hidrokarbonáthoz szoktatott anyaggal végeztük el a *3. táblázat*ban részletezett kísérleteket 10—10 ml szuszpenzióval, melyeket a táblázatban feltüntetett anyagokkal kevertünk. (WARBURG monometrikus módszere, 25 ± 0,1 C°, 40 W-os fénycső Tungsram F2 „warmwhite"; c. 5000 lux.)

A kísérletek eredménye szerint a Balaton-vízben pillanatnyilag elégséges N és P van a planktonalgák fotoszintéziséhez, sőt a tihanyi alga gyűjteményben nagy számmal található bazofil törzsek számára a Balaton-víz tetemes hidrokarbonát tartalma a szénellátást is megfelelően biztosítja. A pH érték fontossága melyet a hidrokarbonátot hasznosító algák esetében többen hangsúlyoztak, ezekből a kísérletekből is kitűnik.

Eredményeink figyelmeztetnek a teszt-növények élettani ismeretének fontosságára a természetes vizek értékelését célzó biológiai módszerek kidolgozásánál.

НОВЫЕ ОПЫТЫ ДЛЯ ВЫЯСНЕНИЯ СВОЙСТВ ВОДЫ ОЗЕРА БАЛАТОН ПРИ ПОМОЩИ ЧИСТЫХ КУЛЬТУР ВОДОРОСЛЕЙ

Л. Фельфёльди

Резюме

На основе экспериментальных данных, приведенных в таблице 1, было установлено в предыдущей статье (фельфёльди 1959), что примешанный к воде из Балатона нитрат и фосфатный раствор тождественного значения pH не или только в небольшой мере увеличивают ассимиляционную деятельность примененного в качестве пробного растения 7k *Chlorella vulgaris* BEYER. Кислый фосфат, но и раствор соляной кислоты, вообще не влия-

ющий на фотосинтез, имеют стимулирующий эффект, т. е. параллельно увеличению концентрации водородных ионов увеличивается и фотосинтез. Так как речь идет о воде из Балатона, изменение концентрации водородных ионов неотделимо от изменения соотношений содержащейся в ней системы карбонат-гидрокарбонат-свободная угольная кислота. Из литературы известно, что концентрация водородных ионов едва воздействует на фотосинтетическую деятельность растений, требующих в первую очередь свободной угольной кислоты. Это наблюдается в довольно широких пределах pH. На основе приведенных в таблице 1 данных можно предполагать, что соотношение неорганичных угольных истоков воды Балатона не благоприятствует фотосинтезу фитопланктонных водорослей.

Многолетнее экспериментальное исследование неорганических угольных источников одноклеточных водорослей свидетельствуют о том, что по сравнению с другими членами нашей коллекции альгиновых культур штамм 7К Chlorella в этом отношении располагает исключительными свойствами, поскольку — в отличие от их базофилии и живого потребления гидрокарбоната, этот штамм оказался асидофильным, т. е. его фотосинтез требует свободной угольной кислоты, особенно если проращивается в питательном растворе, провентилированном содержащим 3% угольной кислоты воздухом: pH 6,5—6,8.

Опыты были повторены с хорошо известным базофильным штаммом (953. Coelastrum microporum). Если этот штамм разращается в растворе Кнопа—Прингсхейма, провентилированном содержащим 3% угольной кислоты (pH = 6,5—6,8), потом взвешивается в чистой воде из Балатона, то фотосинтез начинается после характерного дря ассимиляции гидрокарбоната латентного периода (кривая 1, рис. 1). Если клетки разрашиваются в растворе без вентиляции (без добавления израсходованной угольной кислоты) (pH = 8,8), затем взвешивается в чистой воде из Балатона, то в начале освещения фотосинтез начинается без латентного периода, указывая на то, что биологические свойства водосорлей переключились на израсходование ионов гидрокарбоната при предварительном выращивании (кривая 2, рис. 1). Такие вещества, приуроченные к гидрокарбонату, были применены в опытах, подробно изложенных в таблице 3, с 10 мл суспенсией каждый. К каждому веществу были прибавлены указанные в таблице вещества. (Манометрический метод Варбурга, 25 ± 0,1 С, 40 ваттовая неоновая трубка Тунгсрам F2 "Warmwhite"; c. 5000 lux.)

По результатам опытов, в настоящее время имеется достаточное количество и P в воде Балатона для фотосинтез водорослей, причем высокое гидрокарбонатное содержание воды Балатона обеспечивает необходимый уголь для базофильных штаммов, имеющихся в большом количестве в коллекции водорослей в Тихане. Важность значения pH, часто подчеркнутая авторами в связи с водорослями, потребляющими гидрокарбонат, подтверждается этими опытами.

Опыты авторов обращают внимание на важность знания биологических свойств пробных растений при разработке биологических методов для оценки природных вод.