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CULTIVATION OF THE GREEN ALGAL STRAIN 5618. *SCENEDESMUS OBTUSIUSCULUS* IN ARTIFICIAL SEA WATER

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The salinity is one of the most important factors in the life and distribution of some marine and brackish organisms or populations (e.g. PILLAI 1955, BRAARUD 1951, 1961, 1962, CARPELAN 1964). The green algae especially the Chlorococcales play no important role in the phytoplankton of the sea (IYENGAR 1951), though there are many useful data at disposal on their appearance in coastal waters of some marine areas (BUTCHER 1952, HALME and MÖLDER 1958, SKOLKA 1960, UHERKOVICH 1962 and others). Especially the genus *Scenedesmus* is lacking in typical sea water (salinity at about 35‰), but it rather frequently occurs in labile or stable oligo-beta-mesohalyn waters of ca. 10—18‰ salinities (UHERKOVICH 1962). The salt tolerance of any fresh water algal species may be of importance not only from ecological viewpoint but also because of the mass culturing of algae. The marine unicellular algae are not suitable for this purpose (LOOSANOFF 1951, EDDY 1956, GIBOR 1956). It was thought therefore worth investigating the behaviour of some species of our algal collection in artificial marine water.

The experiments were performed also with the aim of obtaining further data on our most intensively investigated 5618. *Scenedesmus obtusiusculus* strain (FELFÖLDY 1962, 1965, FELFÖLDY, SZABÓ and TÓTH 1962, 1964, UHERKOVICH & al. 1962).

Material and method

The studies were carried out with 5618. *Scenedesmus obtusiusculus* strain belonging to the collection of living algae of the Biological Research Institute of the Hungarian Academy of Sciences.

The algal material kept on agar slants in a northern window was scraped from the agar surface under aseptic conditions and transferred into 1 litre Erlenmeyer flasks containing liquid medium of the following composition:

NH_4NO_3	500 mg
KH_2PO_4	250 mg
$MgSO_4 \cdot 7\ H_2O$	75 mg
$FeSO_4 \cdot 7\ H_2O$	25 mg
disodium ethylenediamin-tetra-acetate (EDTA)	6 mg
HCl	0,2 ml
Arnon's solution of trace elements	0,2 ml

in one litre of Tihany tap water.

The pH of this solution was adjusted to about 7 by the addition of n-HCl or n-NH_4OH. (The total salt concentration of Tihany tap-water according to the analysis of Mr. ERNŐ SZABÓ is: 308 mg/litre without hydrocarbonate ions).

Culturing vessels were put on a transparent plate placed horizontally above 40 W Tungsram "warm white" fluorescent tubes (c. 9000 Lux). Compressed air containing 1.5 per cent carbon dioxide was bubbled through the cultures.

For the purpose of the experiments artificial sea water was prepared from laboratory reagents. Several formulae have been in use out of which the following due to LYMAN and FLEMING (1940) includes all the major constituents and yields a water of Cl = 19.00 ‰ and of 34.33 salinity (HARVEY 1955).

NaCl	23,477 g	$NaHCO_3$	0,192 g
$MgCl_2$	4,981	KBr	0,096
Na_2SO_4	3,917	H_3BO_3	0,026
$CaCl_2$	1,102	$SrCl_2$	0,024
KCl	0,664	NaF	0,003

in 1000 ml of deionized water (prepared with the combination of ion exchange resins "Varion K" and "Varion AK"). The pH of this artificial sea water after aeration was 8.15.

In the course of the experiment this artificial sea water was enriched pro litre with 500 mg NH_4NO_3, 250 mg KH_2PO_4, 25 mg $FeSO_4 \cdot 7H_2O$, 0.6 mg EDTA and 0.2 ml ARNON's solution (ARNON 1938). Salinity increased after the addition of these salts with 0.8 g/litre ($S‰ = 35.13$).

The dilutions of sea water was prepared with Tihany tap-water. The composition and other properties of nutrient solutions used in these studies are presented in *Table 1.*

Table 1 — 1. Táblázat

Composition and properties of experimental nutrient solutions
A kísérleti tápoldatok összetétele és tulajdonságai

	Sea water (sw) — Tengervíz	sw / 2	sw / 4	Control — Kontroll
Artificial sea water — Mesterséges tengervíz	1000 ml	500 ml	250 ml	0 ml
Tap water — Csapvíz	0 ml	500 ml	750 ml	1000 ml
NH_4NO_3	500 mg	500 mg	500 mg	500 mg
KH_2PO_4	250 mg	250 mg	250 mg	250 mg
$MgSO_4 \cdot 7\ H_2O$	0 mg	37 mg	55 mg	75 mg
$FeSO_4 \cdot 7\ H_2O$	25 mg	25 mg	25 mg	25 mg
Arnon's solution — Arnon-féle oldat	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Salinity ‰ — Szalinitás	35.18	18.18	9.66	0.31
pH	8.15	8.2	7.6	7.0

The growth rate of algae was not measurable with optical methods, because of the great differences in colour produced by different treatments. Thus it was determined by measuring the dry matter content of suspension filtered through tared filter paper and dried to constant weigth at 105° C.

The number of cells present in 1 ml suspension was determined with BÜRKER haemocytometer at the end of the experimental procedures.

From the properties of the cell mass produced the total nitrogen content was determined with KJELDAHL method. Cells separated and washed with distilled water by centrifuging were dried at 105° C, ground to fine powder, and digested in concentrated sulphuric acid by the addition of a little amount of hydrogen peroxide. After alkalinization the samples were distilled in a PARNASS—WAGNER microdestillation apparatus. The distilled ammonia absorbed in 4% boric acid solution was titrated with 0.1N HCl solution. Protein content = 6.25N.

For the cultures had different colouring it was considered important to determine the composition of pigment content in samples. This was performed with the spectrophotometric measurements of the acetonous extract usual in hydrobiology. Aliquots of suspensions were filtrated through filter papers of fine quality (MACHEREY—NAGEL N° 640 d) and the filter paper together with the filtrated cells was put above boiling water, pulpified by grinding in a porcellain mortar with a mixture of little amount of magnesium oxide and glass powder, and was left to stand for one night in 90% acetone. Next day it was filtrated crystal-clear through asbestos filter, washed with some drops of acetone and filled up to a volume of 50 ml. The transmittancy of this pigment extract was measured with BECKMAN DU spectrophotometer against 90% acetone. As neither c-chlorophyll nor astacin type carotenoids were present in the extract, the amount of the various types of chlorophylls was computed by the formulae of FELFÖLDY et al. (1962):

$$C_a = 0{,}1554\ A_{665} - 0{,}0221\ A_{645}$$

$$C_b = 0{,}2273\ A_{645} - 0{,}0559\ A_{665}$$

$$C_{nac} = 0{,}0493\ A_{480} - (0{,}0094\ C_a + 0{,}0670\ C_b)\ ,$$

where C_a and C_b are concentrations of a and b chlorophylls expressed in mg/litre, C_{nac} = the quantity of non astacin type carotenoids expressed in mSPU/litre units (SPU = specified pigment unit). A $m\mu$ = absorbancy at wavelengths of 480.645 and 665 $m\mu$ (RICHARDS and THOMPSON 1952).

For photosynthetic measurements the cells grown in sea water of different concentrations were separated from nutrient solution by centrifuging. A suspension of 1 g/litre density was prepared of the separated cells with fresh nutrient solution. 3 ml of this suspension was pipetted into flat-bottomed WARBURG vessels of 26—28 ml capacity. The measurements were performed at an about 9000 Lux illumination and 25 ± 1° C temperature with the WARBURG technique. The results were computed from the averages of measurements performed at 20 minute intervals and are expressed in units of μl O_2/ mg dry matter/hour. In pure sea water photosynthesis was not measurable in the original cells, not even in cells grown in control nutrient solution and freshly suspended in sea water.

Morphological examinations were carried out in living material and in those conserved with 2% formaldehyde (ZEISS NfPK microscope and phase-contrast adapter).

Results

Physiological and chemical observations

Experiences obtained in different dilutions of sea water are summarized in *Table 2* and *Figure 1.*

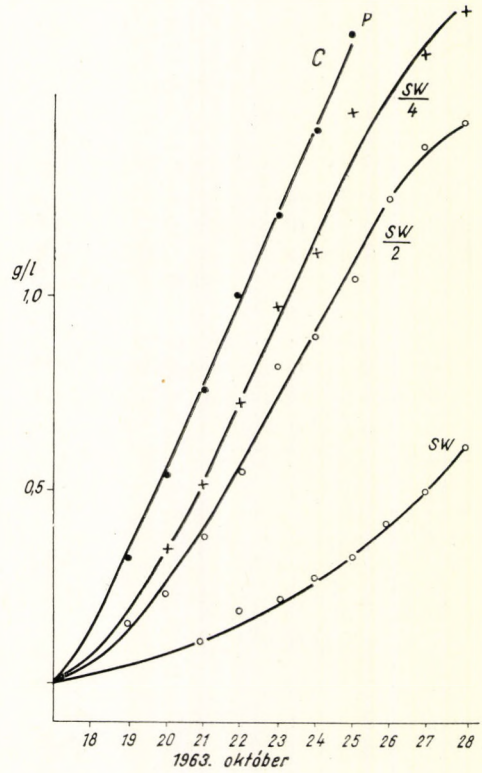


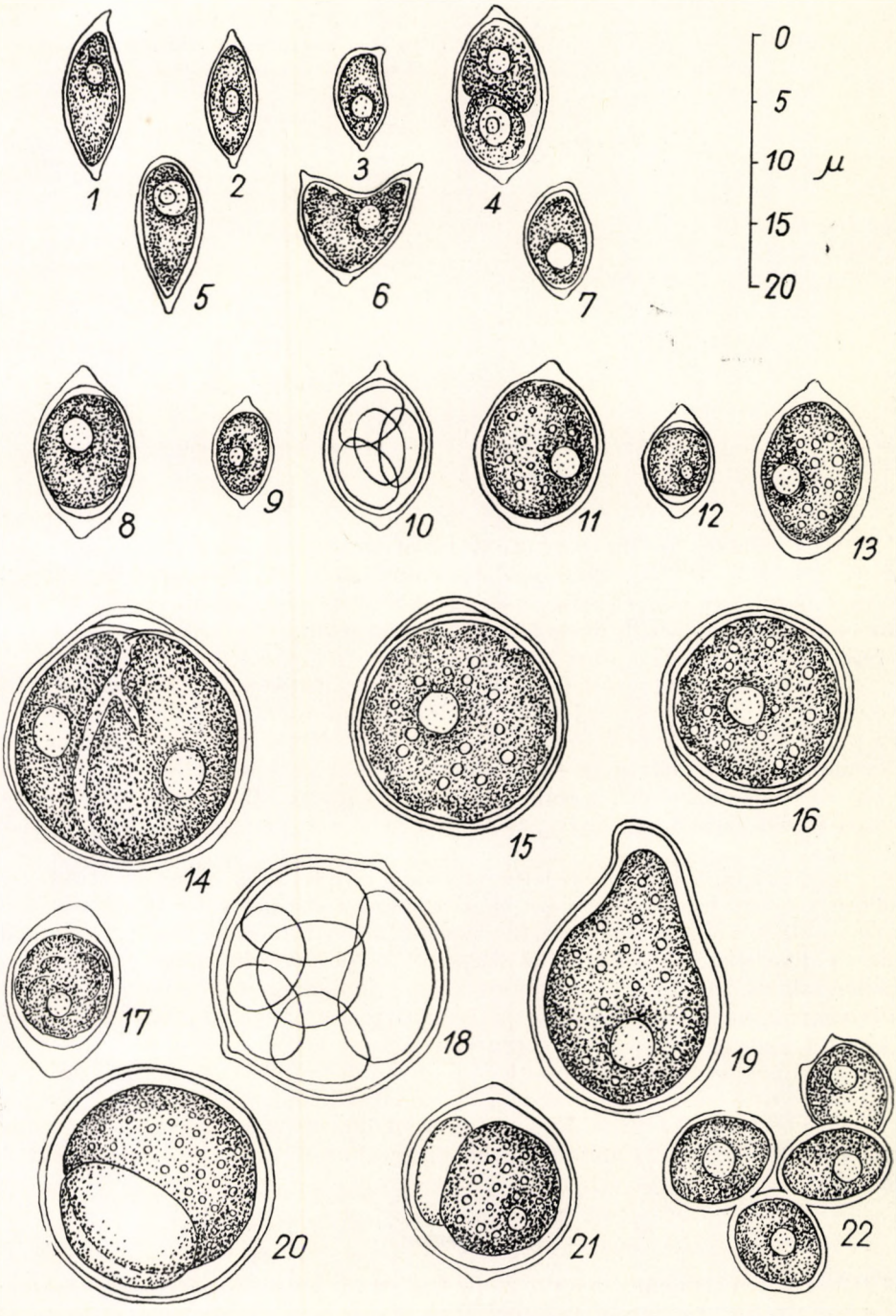
Figure 1. Growth curves of *Scenedesmus obtusiusculus* in nutrient media with different grades of salinity

1. ábra. Scenedesmus obtusiusculus növekedési görbéi különböző só-koncentrációjú tápoldatokban

Morphological observations

The general appearance — *facies culturalis* — of control cultures shows the normal microscopic picture of cell populations grown in other balanced nutrient solutions (UHERKOVICH et al. 1962). The dimension of the single cells varies between $8.5-14\times4-8\ \mu$, but their majority is of $12\times5.5\ \mu$ size. The cells are spindle shaped, monodesmoid condition is predominant and only few four-celled coenobia are present *(Plate 2, N° 1—7).*

Plate 2 — 2. Tábla



Explanation see text — Magyarázat a szövegben

Table 2 —

Properties of 5618 Scenedesmus obtusiusculus
5618 Scenedesmus obtusiusculus tulajdon-

	S%	Dry matter after 10 days — Szárazanyag 10 nap után, g/litre	%	Cell number — Sejtszám, 10^6/ml	%	Crude protein % in dry matter — Nyers fehérje szárazanyag %-ban
Sea water (sw) — Tengervíz	35.2	0.61	25.8	2.5	5.0	29.0 24.0
$\frac{sw}{2}$	18.2	1.46	61.6	19.2	38.3	30.9 34.8
$\frac{sw}{4}$	9.7	1.86	78.5	40.6	81.1	47.2 43.9
Control — Kontroll	0.3	2.37	100.0	50.1	100.0	48.2 49.7

Cells growing in the 3 : 1 mixture of freshwater—sea water are more spherical = 8.5—13.5×6—8.5 μ, but more often broadly spindle shaped monodesmoid forms of 13—7 μ size, or seldom forming irregular chains with four or eight cells, which do not resemble the regular coenobial arrangement of *Scenedesmus* species *(Plate 2, N° 8—10)*.

In the 1 : 1 mixture of fresh-water and sea water (sw/2) the size of cells varies between 6.5—14.5×4.5—11 μ, but the diameter of the majority is 14×8 μ. They are broadly spindle-shaped or broadly ellipsoidal. In the latter ones papilla characteristic of the genus is present very often only at one end of the cells, while the other end seems to be rounded. Besides these common monodesmoid form irregularly granulated cells are also observable *(Plate 2, N° 11—13)*.

In artificial sea water (sw) the cells are of 14—21 μ diameter, they are globose, or very broad ellipsoidal monodesmoid cells and very often only one or two obtuse papillae indicate the descent from the original spindle-shaped cells. Seldom these broad spindle shaped cells observable in the cultures are smaller (about 10×14 μl), and more seldom similar or even smaller cells are united into four-celled coenobia of irregular shape or cell aggregates. In case of greater globose cells a very often transparent vesicle (filled with water or salt water) is also observable besides the green granulated cell body inside the cell wall, which seems to be double (daugher cell enclosed into the mother cell?) *(Plate 2, N° 14—22)*. Even in case of more rounded cells at least one papilla at the cell end reminds of the original spindle shape (14, 15, 21 in *Plate 2)*.

Discussion

As the experimental results show the strain 5618. *Scenedesmus obtusiusculus* has a rather moderate salt tolerance and this establishment corresponds to the observations of field algology, namely that the distribution of *Scene-*

2. Táblázat

in artificial sea water of different dilutions
ságai különböző hígítású tengervízben

Chlorophyll % in dry matter — Klorofill % szárazanyagban, a	b	Total carotenoids % of dry matter — Össz karotin szárazanyag %-ában	Colour of the suspension — Szuszpenzió színe	Photosynthesis μl O_2/1 mg dry matter/hour — Fotoszintézis μl O_2/1 mg szárazanyag/h
1.2	0.4	0.42	wine coloured — borszínű	not measurable — nem mérhető
2.0	0.7	0.54	greenish-yellow — zöldessárga	27.5
3.7	1.3	0.61	dark green — sötétzöld	29.1
3.8	1.3	0.56	dark green — sötétzöld	31.8

desmus genus in marine biotopes is limited to salinities below 16—18‰. The results of the experiments of WETHERELL (1961, 1963) with *S. brasiliensis*, *S. obliquus* and *S. quadricauda* are of the same order of magnitude. UHERKOVICH identified eleven *Scenedesmus* taxa in the Black Sea plankton collected near Costanca at 10.79—17.99‰ salinities. Our growth curves in *Figure 1* show that the growth of *Scenedesmus obtusiusculus* is very good even in sea water diluted to 1 : 1, having 18.2 salinity. In sea water diluted to its half photosynthetic activity is also fairly satisfactory, 86.5 per cent of the control, whereas in pure sea water no photosynthesis was measurable either in cells growing in pure sea water itself or in those grown in the control nutrient solution and freshly suspended in artificial sea water. The decrease in the rate of photosynthesis is parallel with the decrease in growth.

As it is evidenced by morphological observations the shape of cells becomes gradually broader and the size of cells increases simultaneously with increasing concentration. This tendency is well visible in *Figure 2* in which cell number (scale on the left) and the dry matter content in an unit volume of suspension (scale on the right) is compared. In pure sea water and in its dilution to 1 : 1 the values of cell number are surpassed by those of dry matter due to the increased volume of the single cells. A similar increasing effect of salt concentration on the dimension of cells was recorded also by SOEDER (1960). Disturbancies in cell division and formation of clumps by aggregated cells were observed by SOEDER (1960) and WETHERELL (1963). This was observed by the latter also in 10% sea water in the case of *Scenedesmus obliquus*.

The colour of the cultures deserves also attention. The experiences of MCLACHLAN (1961) suggest that changes in pigment content are not indicative of salinity. A variety of reactions was produced in the case of different species. In Scenedesmus obtusiusculus maximum growth and photosynthesis is parallel with maximum a-chlorophyll content.

As a final conclusion it may be said that 5618 *Scenedesmus obtusiusculus* is not an immoderately salt resistant strain. It does not grow sufficiently in sea water even if it is diluted to 1 : 1. Nevertheless, it yields a rich and healthy culture when grown in a 1 : 4 mixture of sea water and fresh-water.

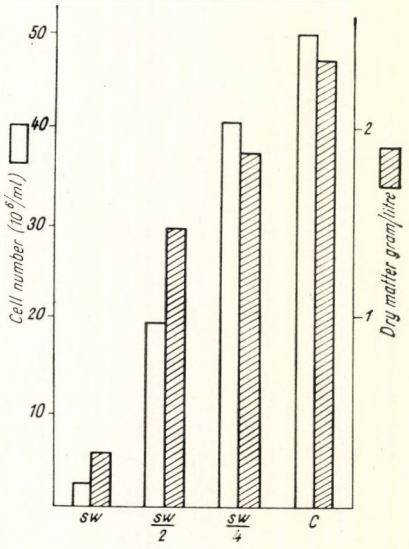


Figure 2. Comparison of cell number and dry matter content in suspensions of *Scenedesmus obtusiusculus* grown in nutrient media with different salinities

2. ábra. Különböző sókoncentrációjú oldatban nevelt *Scenedesmus obtusiusculus* sejtszáma és szárazanyagtartalma közötti összefüggés

Summary

The strain 5618. *Scenedesmus obtusiusculus* CHOD. belonging to the collection of algae of the Biological Research Institute at Tihany was cultured in artificial sea water and in 1/2 and 1/4 dilutions thereof ($S‰ = 35.2, 18.2, 9.7$). The following properties of suspension decreased with increasing salinities: dry matter content, cell number, protein and chlorophyll content of cells, photosynthetic activity. On the other hand a little increase in carotenoid content of dry cell material was demonstrable.

The experimental results well agree with the experiences in field algology, namely that the members of genus *Scenedesmus* are not to be found in marine habitats above 18‰ salinity.

This strain is not suitable for mass cultivation in pure sea water, but it will have some possibilities in brackish waters ($S‰ < 10-12$).

Grateful aknowledgement is due to Mr. ERNŐ SZABÓ for putting the analytical data of Tihany tap-water at our disposal, and to Mrs. BRIGITTA SZABÓ for her technical assistance in the experimental work. Thanks is due to Mr. GYULA HÁMORY for the careful performance of protein analyses.

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AZ 5618. *SCENEDESMUS OBTUSIUSCULUS* ZÖLD ALGATÖRZS TENYÉSZTÉSE MESTERSÉGES TENGERVIZBEN

Összefoglalás

Felföldy Lajos és Uherkovich Gábor

A tihanyi Biológiai Kutatóintézet gyűjteményének 5618. *Scenedesmus obtusiusculus* Chod. törzsét tenyésztettük mesterséges tengervízben és annak 1/2 és 1/4-es hígításaiban ($S°/_{oo}$ = 35,2, 18,2, 9,7). Kontroll tápoldatként ammónium nitráttal gazdagított csapvíz szolgált ($S°/_{oo}$ = 0,3). A szalinitás emelkedésével a szuszpenzió szárazanyag tartalma, a sejtszám, a sejtek fehérje- és klorofilltartalma valamint fotoszintetikus tevékenysége csökken, a karotinoid tartalom kissé emelkedik.

A kísérleti adatok jól egyeznek a hidrobiológia tapasztalataival, hogy 18 $°/_{oo}$ szalinitás felett *Scenedesmus* a tengerben nem található.

Ez a törzs nem alkalmas arra, hogy tömegtenyésztéséhez tengervizet használjunk.

КУЛЬТИВИРОВАНИЕ ШТАММА ЗЕЛЕННЫХ ВОДОРОСЛЕЙ (SCENEDESMUS OBTUSIUSCULUS № 5618 В ИСКУССТВЕННОЙ МОРСКОЙ ВОДЕ

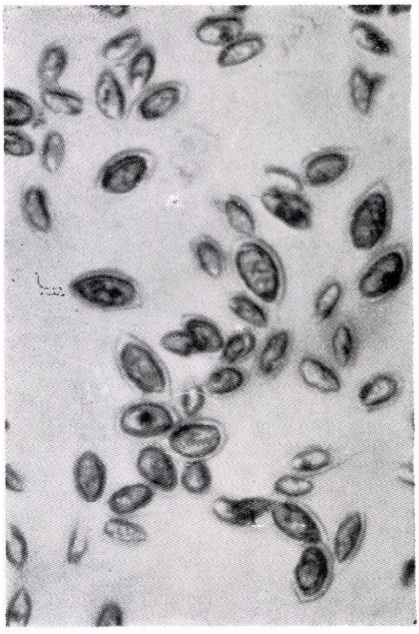
Л. Фелфелди, Г. Ухеркович

Были Культивированы штаммы Scenedesmus obtusiusculus Chod. № 5618 из коллекции Биологического Института в Тихани в разведенной на 1/2 и 1/4 части и концентрированной искусственной морской воде ($S°/_{oo}$ = 35,2 — 18,2 — 9,7). Контрольной питательной средой служила водопроводная вода, содержащаяся нитрат аммония (0 $°/_{oo}$ = = 0,3). С увеличением соленности наблюдалась снижение сухого остатка суспенсии, числа клеток, содержания белка и хлорофилла клеток и также фото-синтетической деятельности, а содержание каротиноидов несколько увеличивалось.

Экспериментальные данные совпадают с даннымы морской алгологии согласно которым выше 18 $°/_{oo}$ соленности Scenedesmus в море не обнаруживается.

Для массового культивирования этого штамма нельзя использовать морской воды.

Plate 1 — 1. Tábla



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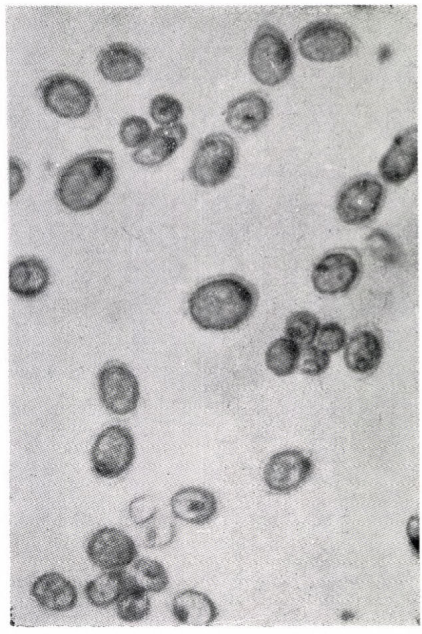

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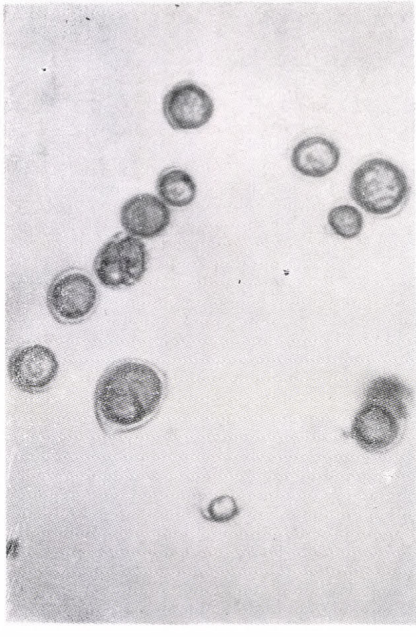

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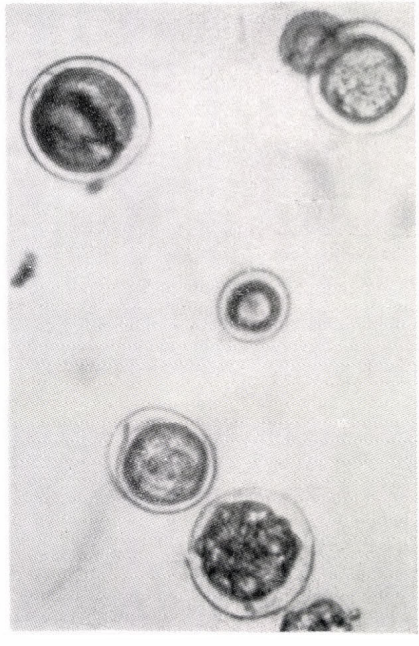

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Plate 1 — 1. Tábla

Different forms of "facies culturalis" of Scenedesmus obtusiusculus suspensions in control nutrient medium (1), in four times- (2), twice- (3) and un-diluted (4) sea water. (1^0 in the scale: 10 μ)

Scenedesmus obtusiusculus tenyészetek "facies culturalis"-a kontrol tápoldatban (1), négyszer (2) és kétszer hígított (3) és hígítatlan tengervízben (4). (Egy skálabeosztás 10 μ),