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A SIMPLE APPARATUS FOR CULTURING UNICELLULAR ALGAE IN LARGE AMOUNTS FOR LABORATORY PURPOSES

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In June of 1959 an attempt was made in the Biological Research Institute of the Hungarian Academy of Sciences to cultivate unicellular algae in large scale. As a first task the various pure algal strains of the Institute were tested for usefulness in mass culturing.

To become familiar with the problems of algal cultivation and to obtain a simple continuous system, glass columns of 7 cm diameter and of 120 cm length were constructed (Cook 1950). In these columns aeration is provided

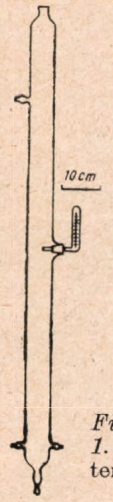

Figure 1. Sketch of glass colum.
1. ábra. Üvegcsőből készült algatenyésztő edény vázlata.

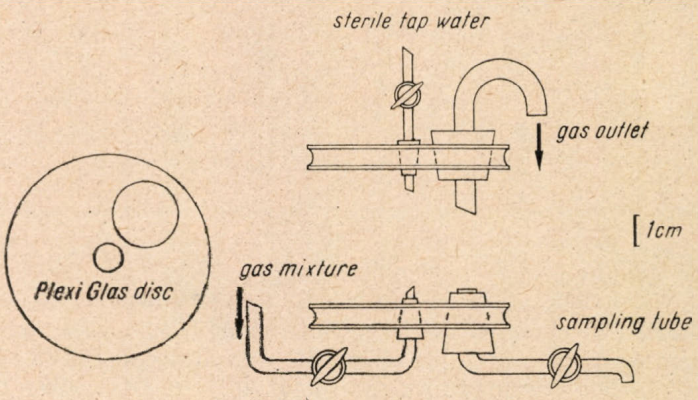

Figure 2. Plexi-Glas closing discs.
2. ábra. A plexi zárólapok szerkezete.

by means of an inlet tube which enters at the conical bottom. Two other tubes are sealed to the column near the bottom, one for connection with another tube and the other for sampling. The tube in the upper part of the column serves for the introduction of fresh culture medium. In *Fig. 1* one of these glass units is presented. The manipulation of these glass units is not simple. It is easily breakable and its cleaning and fitting is difficult. In 1961, therefore, another equipment of similar dimension was constructed of plastic tube. The use of Tygon and polyethylene tubings for similar purposes is known in literature, but these equipments are operating in horizontal position and centrifugal pumps are used to circulate the algal suspensions (Davis *et al.* 1953, 113, Burlew 1953, 243).

Description of plastic tube unit

Culture units of any length may be prepared from commercial polyethylene plastic tube. This tubing has a diameter of 70 mm and a thickness of 0,2 mm. The culture units prepared of these tubings were closed on both ends with „Plexi-Glas" discs of 73 mm diameter. The grooved edge of the disc was fastened with string to the ends of the tubes. Two openings were cut into the top disc. The one in the middle for the introduction of germ free tap water, the other for an U-shaped glass tube serving as gas outlet. In the other

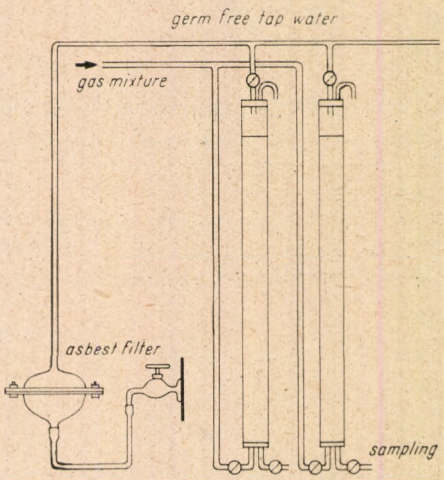

Figure 3. Schematized representation of the plastic tube units.
3. ábra. A műanyag csövekből összeállított tenyésztő egység vázlata.

disc, which constituted the bottom of the culture unit there were also two openings. The one in the middle served as an inlet of aeration gas mixture, the other was made for sampling purpose. Glas tubes of suitable shape were inserted into these openings. Concentrated solution of the nutrient salts was added on the top by removing the exhaust pipe and pouring the solution through its opening.

The construction of these discs is illustrated in *Fig. 2.*

Four plastic tube units of 170 cm length each (effective capacity of 6 litres) were suspended in greenhouse and were installed according to the schema given in *Fig. 3.* The sterilization both of the tube units and of the pipe lines was made with formaldehyde gas. For nutrient medium Tihany tap water was used. It was filtered through a sterile filter of 20 cm diameter (Seitz EK asbestos filter) and enriched with nutrient salts to a final concentration per litre as follows:

1 g KNO_3	0,01 g Fe-citrate
0,3 g KH_2PO_4	0,01 g citric acid
0,1 g Na_2SO_4	1 ml Hoagland A—Z solution.

The concentrated solutions of nutrient salts were sterilized in autoclave before feeding. The aeration mixture (1,5% CO_2 in air) was controlled by pressure reducers and needle valves on a compressed air line and cylinders of compressed carbon dioxide. The quantity of air was measured with a gas-meter, the carbon dioxide current with a rotameter. Oil gas filters were used

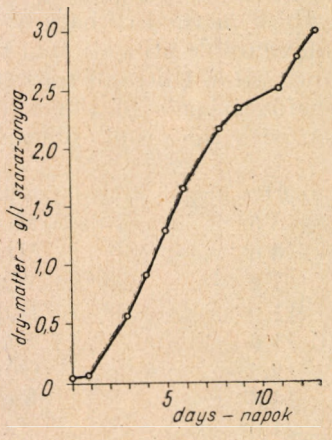

Figure 4. Initial phase of growth curve of algae in plastic tube apparatus.

4. ábra. A műanyag-csőből készült készülékben szaporodó algatenyészet növekedési görbéjének eleje.

and all connections were sterilized to prevent contamination and the so called semi-sterile techniques were employed.

The inoculation of this sterile system was made on 13th July from a pure liquid culture of the 5618 *Scenedesmus obtusiusculus* CHOD. strain. The growth rate in the first phase of cultivation is graphed in *Fig. 4.* In the second phase, when the growth curve reached the region of decreasing slope, at a suspension density of about 3,0 g dry matter/litre (measured by drying at 105° C after filtration through Macherey—Nagel N° 640 d 5,5 cm ∅ filter paper), harvesting periode was started. Three different harvest-methods were tried out. The results are given in *Table 1.*

When working according to the first method (the half of the suspension was harvested at every third day) the maximum cell-density of the suspension

Table 1. Productivity of plastic tube algal culturing system under greenhouse conditions (13th July—30th September. 1961).

Method of harvest	Harvested dry matter (g)/l unit/1 day (average)	Harvested dry matter (g)/m²/day (8 tubes/m²)	Daily nutrient solution requirement (litre)	Harvested dry matter g/day/1 L nutrient solution
Halving on every third day	1,57	12,56	1,0	1,6
Two third parts of suspension taken on every third day	2,39	19,12	1,3	1,8
Five sixth parts of suspension taken on every third day	2,36	18,88	1,6	1,5

(3,2 g dry matter/litre) decreased to a concentration of 1,0—1,5 g dry matter/litre at which level it remained nearly constant for a half month long period.

The second method of harvest, when two thirds of the suspension were drained off and replaced with freshly prepared nutrient solution in every third day, resulted an increase both in dry matter concentration (1,2—1,8) and in productivity (about 30% surplus).

The third method of harvest resulted again a decrease in productivity and an unfavourable utilization of the nutrient solution (only 1,5 g dry matter was obtained after 1 litre of nutrient medium).

The effectiveness of the second method of harvest is fairly good in comparison with the results obtained by different authors:

dry matter g/m²	author
3,5	MITUYA *et al.* 1953, 280
6,6	MEFFERT and STRATMAN 1954, 13
7,2	WASSINK *et al.* 1953, 60
11,0	BURLEW 1953, 252
11,6	MEFFERT 1955, 89
12,8	OORSCHOT 1955, 235
14,8	FELFÖLDY 1961, 157
21,0	MAYER *et al.* 1955, 58
28,0	MORIMURA *et al.* 1955, 173

To facilitate and simplify the separation of cells from the nutrient media the harvested suspension was poured into a plastic container of a capacity of 15 litres where the cells were allowed to settle during the night. Next day the upper half part of the supernatant was siphoned with a pipe shaped tube and the rest was centrifuged in a supercentrifuge (type FS 45 Zuglói Gépgyár Budapest). This settling procedure resulted in a loss of about 4% of total dry matter only.

The values of some chemical constituents of the centrifuged mass are presented in the followings:

Dry matter in percent of wet weight (Water content: 73,6—76,9%)	23,1—27,4
	per cent of dry matter
Kjeldahl-N	6,9—9,3
Protein (N × 6,15)	43,3—57,8
Chlorophyll *a*	4,2—4,6
Chlorophyll *b*	1,0—1,4
α-carotene	0,02—0,03
β-carotene	0,07—0,08
other carotenoids	0,48—0,49

Averages were not computed from the values because the results were obtained from samples originating from different harvests.

It was regarded important to investigate if there were any unfavourable changes occurring in the chemical composition of the algal masses during sedimentation. For this reason an algal suspension of 0,96 g dry matter/litre

concentration was left to stand in laboratory in a covered plastic container for 10 days (10th—20th September).

The results of these analyses are presented in the table below.

	Dry matter g/litre	Kjeldahl-N % of dry matter	Protein N × 6.25	Chlorophyll	
				a	*b*
0 day	0,96	8,6	53,7	4,6	1,3
5 days	0,93	9,3	57,8	4,7	1,3
10 days	0,95	9,3	57,8	4,8	1,3

It is seen from this table that the algal mass loses 2—3% of its original dry-matter content, when it is kept living in darkness and in its own nutrient medium for ten days. This may be due primarily to the loss of its carbohydrate content, which causes a relative increase of both protein and pigment content in the cells (if, altogether, a 2% increase of pigment content may be regarded significant !)

Grateful acknowledgement is made here to Miss GIZELLA SÓLYMOSY for helpful assistance during these experiments.

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LABORATÓRIUMI MÉRETŰ EGYSZERŰ KÉSZÜLÉK FOLYAMATOS ALGATENYÉSZTÉSRE

Felföldy Lajos

Összefoglalás

Az *1. ábrán* látható üveg-tenyésztőcsövek helyett 1961-ben hasonló nagyságrendű alga-tenyésztő egységet konstruáltunk 0,2 mm falvastagságú, 70 mm átmérőjű polietilén tömlőből. A *2. ábrán* látható 10 mm-es plexi-ből készült zárólapok segítségével (melyekre legcélszerűbb erős zsineggel rögzíteni a műanyagcső végeit), bármilyen hosszú tenyésztő-cső előállítható. Az alsó zárólap középső furatába illő vinidur csövön a gázkeverék (1,5% széndioxidot tartalmazó levegő) lép be, az oldalra kivezető csapos cső mintavételre szolgál. A felső zárólap középső furatán a steril vízvezeték vezethető be, míg az oldalsó nyílásba illő pipa alakú csövön a gázfelesleg távozik. A tenyésztő csöveket a *3. ábra* vázlata szerint csoportosítjuk. A rendszert formalin gázzal sterilizáljuk, majd alapos szellőztetés után a műanyag csöveket SEITZ EK azbeszt-szűrőn sterilizált csapvízzel feltöltjük. A külön sterilizált tömény tápsó oldatot a kipufogó cső dugójának kivétele után a felső nagyobb nyíláson át töltjük be. Ugyanitt oltjuk be az alga-inokulumot is. „Aratáskor" a szuszpenzió megfelelő mennyiségét a mintavevő csövön át leengedjük, a csövet steril csap-vízzel feltöltjük és friss tápsó oldatot is adunk. A tápoldat tihanyi csapvíz, melybe annyi tápsót pótolunk, hogy a végső koncentráció az angol szöveg 96. oldalán közölt legyen.

5618 *Scenedesmus obtusiusculus* CHOD. törzzsel 1961. július 13.-án négy 170 cm hosszú műanyagcsövet oltottunk be. A sejt-szaporodás első fázisa a *4. ábrán* látható (szárazanyag meghatározás 105°-on MACHEREY—NAGEL N° 640 d 5,5 cm ∅ szűrőpapíron). Mikor a szaporodás lassul (kb. 3 g szárazanyag literenként) kezdetét vette az aratás. A három féle aratási mód termelékenységét az *1. táblázat*ban állítottuk össze. A legjobb eredménnyel zárult mód (kétharmadrésznyi szuszpenzió három naponként) összehasonlítása külföldi eredményekkel a 98. oldalon látható (MORIMURA és MAYER eredményei jobbak csak nála). A dolgozat végén a termék kémiai összetételére és annak állás közbeni változására vonatkozóan közlünk adatokat.