ANNAL. BIOL. TIHANY | 31 | 177—183 | HUNGARIA 1964

EXPERIMENTS TO SELECT STRAINS FOR ALGAL MASS CULTURE

LAJOS J. M. FELFÖLDY

Received: March 15th 1964

The scientific examination of algal mass culture technology has to be started with selection of algal strains suitable for this purpose. According to the opinion of many famous authors (SOROKIN and MYERS 1953, 330, TAMIYA 1957, 318, STAGNO D'ALCONTRES et al. 1960, 352, etc.) only local algal strains can be used successfully, which have been adapted to the climate and waters of the region or country in question.

In 1960 some strains of the algal collection in the Biological Research Institute at Tihany have been investigated as to their suitability for mass cultivation.

Material and methods

Bacteria-free pure algal strains growing in the collection of algae in the Biological Research Institute at Tihany were used in these selection experiments (FELFÖLDY and KALKÓ 1959, KALKÓ and FELFÖLDY 1959). The choice fell on strains excelling in their good growth rate on agar slants and in our previous experiments described elsewhere (FELFÖLDY 1960).

The experiments were run in 10 litre „Ergon" sphaerical bottles. The suitable mounting of these bottles is illustrated on the *Fig. 1.*

It is clearly impracticable to begin comparing the growth rates of algae by determining experimentally for each the most suitable culture solution. Instead, it is necessary to begin by using the medium which was used for different purposes in our laboratory, and which will also be suitable for mass cultivation in fairly large dimensions. The composition of this medium is the following:

10 litres of germ free tap water (filtered through SEITZ EK asbestos filter)
10 g KNO_3
3 g KH_2PO_4
1 g $Na_2SO_4 \cdot 10H_2O$
0.1 g ferric citrate
0.1 g citric acid
0.25 g disodium ethylenediamine tetraacetate (EDTA)
10 ml „A—Z" solution according to HOAGLAND (HOAGLAND and SNYDER 1933, SCHROPP 1951, 167)
10 ml N-HCl

(See FELFÖLDY 1961, 1962).

In practice two stock solutions were prepared. These were sterilized separately in autoclave and after cooling were poured into the germ free tap water through the inoculation tube *e* in *Fig. 1.*

The stock solutions were:

„NPS-solution": 1000 ml glass destilled or by ion exchange deionized water
200 g KNO_3
60 g KH_2PO_4
20 g $Na_2SO_4 \cdot 10H_2O$
50 ml ad 10 litres of germ free tap water.

Fe-solution: 250 ml „A—Z" solution according to HOAGLAND
250 ml N-HCl
2.5 g ferric citrate
2.5 g citric acid
6.0 g EDTA
20 ml is given to 10 litres of liquid medium.

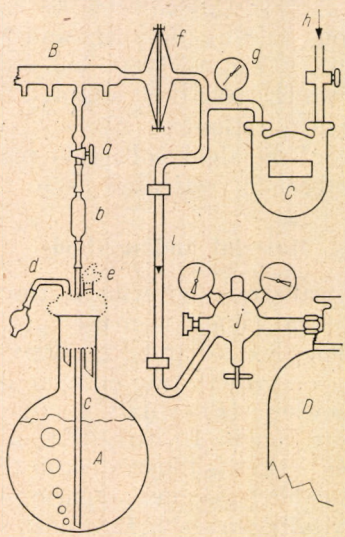

Fig. 1. The mounting and supply of 10 litre culture vessels
A — Sphaerical flask, *B* — copper manyfold for distribution of the gas mixture; *C* — gas meter for measuring the quantity of air; *D* — Carbon-dioxide gas cylinder. *a* — regulator stop-cock; *b* — cotton filter; *c* — bubbling tube (inside ⌀ 6—7 mm); *d* — gas outlet tube (inside ⌀ 7—8 mm) with a filter filled with glass-wool; *e* — inoculation tube (inside ⌀ 14—15 mm) provided with a cotton plug; *f* — asbestos filter for producing germ-free gas-mixture (200 mm ⌀ SEITZ EK filter); *g* — a manometer for measuring the pressure of air-carbon dioxide mixture; *h* — inlet of compressed air; *i* — rotameter for measuring carbon dioxide; *j* — reduction ventile with manometers for the feeding of carbon dioxide

1. ábra. A 10 literes lombik-kultúra szerelése és ellátása
A — gömblombik; *B* — gázkeverék elosztó, *C* — gázóra a levegőmennyiségének mérésére; *D* — széndioxidpalack. *a* — Szabályozó csap; *b* — vattaszűrő; *c* — buborékoló cső (belső ⌀ 6—7 mm); *d* — gázkivezető cső (belső ⌀ 7—8 mm) végén üveggyapottal töltött szűrővel; *e* — oltócső (belső ⌀ 14—15 mm) kisebb vattadugóval; *f* — azbeszt szűrő a gázkeverék csíramentesítésére (200 mm ⌀ SEITZ EK szűrőlap); *g* — a levegő—széndioxid keverék nyomását mérő feszmérő; *h* — a szűrített levegő belépése; *i* — rotamérő a széndioxid mérésére; *j* — nyomáscsökkentő szelep feszmérőkkel a széndioxid adagolására

It is also possible, if it is preferred, to prepare the complete nutrient solution in one vessel and sterilize it in autoclave at 2 atm. for 10 minutes. After cooling the precipitates arising from autoclaving may be brought again into solution by aeration with filtered carbon dioxide gas.

A modification of this medium was used in previous experiments (FELFÖLDY 1959). It contained the same anorganic salts in the same amounts, but instead of tap water, Balaton-water was used to its preparation. Balaton-water is chemically very suitable medium for preparing nutrient solutions, but due to the fine suspended precipitates present it cannot be filtered without difficulty. For this reason it had to be rejected in our present experiments.

The sphaerical bottles filled thus with sterile nutrient solution were inoculated with algae scraped off from agar slants and risen in the same nutrient solution in a northern window.

Because in these experiments mainly the growth rate and yield of different strains were of interest, special attention was paid to use equal quantities of algal material in the inoculation based on previous dry matter determination in suspension.

For the cultures carbon may be provided as a gas flow of 1.5—3 per cent carbon dioxide in air blown through the suspension. In these experiments carbon dioxide was administered from a carbon dioxide cylinder. The gas mixture was freed from germs at first by leading it through a washing flask containing water as washing fluid, later on, however, due to the insufficient sterility obtained in this way, the gas mixture was filtered through asbestos and cotton filters respectively. The quantities of air and carbon dioxide were measured with gas meter and rotameter respectively.

The qualification of strains was made primarily on basis of their growth rate expressed by the dry matter content of suspension. The most simple method for measuring dry matter content is to filter the suspension through filter-paper of known weight (dried at least for 24 hours at 105 °C). The strains unfiltrable even through the finest filter paper (Delta 368) were filtered through porcelain filters (A1, A2) after being washed by centrifuging (the pores of porcelain or glass (G4) filters get easily clogged if the suspension is not washed previously).

Finally there were some strains (e.g. *Dictyosphaerium)* which could not be either centrifuged or filtered. In this case the ash-free dry-matter content of suspension was determined. This was carried out by pipetting a known quantity of suspension into porcelain crucible and drying it either on water-bath or in a ventillation drying-chamber (60 °C). Thereafter the dry residue was kept for at least 24 hours at 105 °C and its weight was measured. Subsequently it was put for one and a half hour into an electric muffle furnace at 600 °C temperature. By this technique it was rendered possible to take into correction the excess of weight caused by the nutrient salts present in the suspension.

Besides the rate of growth a special attention was paid in these experiments to the so called technological properties of the various strains as: separability by filtration or centrifuging, foaming, homogeneous, granulous or lamellated distribution, adhesion to the walls of culture vessel etc.

Concerning the chemical constituents of the various strains investigated, only their protein content was determined in these experiments. Data on the chemical composition of certain strains of ours as lipid-, sterol- (SZABÓ et al.

1961) and pigment content (FELFÖLDY et al. 1962) were published previously. Protein determination was done by the usual KJELDAHL's method using centrifuged and washed dry matter samples dryed to constant weight at 105 °C and grinded flourfine. The wet digestion was performed in concentrated sulphuric acid in the presence of a catalizator mixture containing yellow mercuric oxide and a few drops of hydrogen peroxide. The ammonia was distilled into 4% boric acid solution and titrated with 0.1 N hydrochloric acid. The quantity of crude protein was calculated by multiplying the KJELDAHL nitrogen content by 6.25.

The characteristics of strains investigated are summarized in *Table 1.*

As it is seen from *Table 1* the following strains excelled by good growth: 3153 *Chlorocloster terrestris*, 5618 *Scenedesmus obtusiusculus*, 953 *Coelastrum microporum*, 3556 *Dictyosphaerium pulchellum*, 3615 *Chlorococcum botryoides*, 3602 not determined, 1893 *Ankistrodesmus falcatus*, 512 *Ankistrodesmus* sp., whereas strains with the slowest rate of growth were: 4086, 2250 and 644 *Chlorella* spp., 2500 *Cosmarium* sp., this being not even able to reach a density of 0.5 g dry matter/litre in a month. There are certain strains, which though exhibiting excellent growth rate, cannot be regarded suitable for mass culturing due to their bad filtrability and bad separability by centrifuging as the two *Dyctyosphaerium* strains (641, 3556), further the 516 *Kirchneriella* which is not separable even with supercentrifuge at 30 000 R.p.m. The best growing strain 3153 *Chlorocloster terrestris* had to be also disregarded because the cells climb up to the foam and stain green all parts of the equipment (The foam is colourless in the case of most strains). The strains: 7K *Chlorella*, 85 *Scenedesmus acutus*, 4061 *Scenedesmus quadricauda* and 5640 *Chodatella balatonica* did not excel with good properties in the present experiments, though it was expected on basis of experiences obtained in the course of previous experiments of other technology.

It should be stated the 10 litre sphaerical bottles are not very suitable vessels for culturing algae due to the unadvantageous thick layer of suspension causing the self screening of the cells. The data reported here are therefore of relative value, nevertheless they furnish valuable information for our future experiments.

Summary

Thirty-five strains of unicellular green algae were tested for their rate of growth with non-continuous culturing method in 10 litre sphaerical flasks, under greenhouse conditions. Strains of excellent growth rate and good technological characteristics are: 5618 *Scenedesmus obtusiusculus*, 953 *Coelastrum microporum*, 3615 *Chlorococcum botryoides*, 1893 *Ankistrodesmus falcatus*, 512 *Ankistrodesmus* sp. and No 3602 undetermined strain. The other strains investigated are less suitable partly technologically (3153 *Chlorocloster terrestris*, 3556 *Dictyosphaerium pulchellum)* or their rate of growth is insufficient.

On the basis of these experiments the 5618 *Scenedesmus obtusiusculus* strains was chosen for our experiments on the mass culturing of algae.

The helpful assistance of Mrs. F. KALKÓ ZSUZSA in the experiments is gratefully acknowledged. The author thanks Miss GIZELLA SÓLYMOSY for her skilled technical assistance in the greatest part of the analytical work.

Table 1

Characteristics of thirty-five algal strains investigated for their suitability in mass culturing.

Strain No	Name	Production per month dry matter g/l	Protein content in percentage of dry matter	Notes
71	Ankistrodesmus convolutus det.: G. Tamás	0.98	33.4	Foams up, unfiltrable even through the finest filter paper. Not suitable.
1893	Ankistrodesmus falcatus (Corda) Ralfs. det.: G. Uherkovich	2.18	34.6	
512	Ankistrodesmus sp.	2.01	41.6	Technologically good strain
3147	Chlorella pyrenoidosa Chick.	1.18	49.6	Unfiltrable through filter paper
3515	Chlorella pyrenoidosa Chick. det.: G. Uherkovich	0.60	44.6	
7K	Chlorella vulgaris Beyer.	1.11	41.1	
12K	Chlorella vulgaris Beyer.	1.74	42.4	
645	Chlorella sp.	0.30		Grew scarcely. Unsuitable
951	Chlorella sp. det.: G. Tamás	0.51		Unsuitable due to its low rate of growth
2250	Chlorella sp.	0.41	—	Reduced capacity of growth
3501	Chlorella sp.	1.32	49.1	Passes through the filter paper
4081	Chlorella sp. det.: G. Uherkovich	0.44	52.6	Sticks to the glass wall, its density is unsatisfactory
4086	Chlorella sp.	<0.2	—	Unsuitable
3153	Chlorocloster terrestris Pascher det.: G. Uherkovich	3.41	38.6	Its foam stains the glass surface green
3615	Chlorococcum botryoides Rabenh. det.: G. Uherkovich	2.86	27.8	Heat resistant strain
484	Chlorococcum infusionum (Schrank) Menegh. det.: G. Tamás	0.60	27.9	Sticks up, lumpy, forms a film on the surface of the liquid
1329	Chlorosphaera angulose (Corda) Klebs det.: G. Uherkovich	0.94	18.5	Forms thin lamellae in the suspension
5640	Chodatella balatonica Scherffel	1.24	51.3	
177	Coccomyxa lacustris Chod. det.: G. Tamás	1.06	54.3	
953	Coelastrum microporum Näg. det.: G. Tamás	3.00	40.1	Excellent strain, grows well in tap water and in Balaton water
2500	Cosmarium sp.	0.38	31.6	Forms mucuous gralunes. Unsuitable.

Strain No	Name	Production per mont dry matter g/l	Protein content in percentage of dry matter	Notes
641	Dictyosphaerium pulchellum Wood. det.: G. Uherkovich	1.60	45.2	Mucuous strain, unfiltrable and cannot be centrifuged either
3556	Dictyosphaerium pulchellum Wood.	2.89	37.5	Mucuous strain, unfiltrable and cannot be centrifuged either
2148	Haematococcus pluvialis Flotow.	0.80	36.6	Sticks to the glass surface, granulous, not suitable for mass culturing
516	Kirchneriella sp	1.88	41.5	Cannot be sedimented with supercentrifuge
3145	Oocystis sp.	1.81	31.8	
2507	Scenedesmus acuminatus Chod. det.: G. Uherkovich	0.55	—	Its capacity of growth is small
85	Scenedesmus acutus Meyen. f. alternans Hortob. det.: G. Uherkovich	1.41	35.0	
5618	Scenedesmus obtusiusculus Chod. det.: G. Uherkovich	3.29	44.7	Very excellent strain
4061	Scenedesmus quadricauda (Turp.) det.: G. Uherkovich	0.62	34.6	
532	Stichococcus bacillaris Näg. det.: B. Fott	0.88	43.0	Its foam is green and sticks to the glass surface
3520	Stichococcus bacillaris Näg. f. pallescens Chod. det.: E. Kol	0.51	35.4	
2580		—	—	Suspension light green. Adheres to the bottom. Unsuitable
2639		0.43	31.1	
3602		2.46	41.5	Very good strain technologically

REFERENCES

FELFÖLDY L. (1959): A balatonvíz tulajdonságainak vizsgálata algaélettani kísérletekkel. (Experiments with algal cultures for determining some properties of Balaton lake water.) — *Annal. Biol. Tihany* **26**, 211—222 (In Hungaran with English summary).

FELFÖLDY, L. J. M. (1960): The role of age and training in carbonate assimilation of unicellular algae. — *Acta Biol. Acad. Sci. Hung.* **11**, 175—185.

FELFÖLDY, L. J. M. (1960a): Photosynthetic experiments with unicellular algae of different photosynthetic type. — *Annal. Biol. Tihany* **27**, 193—220.

FELFÖLDY, L. J. M. (1961): Effect of temperature on photosynthesis in three unicellular green algal strains. — *Acta Biol. Acad. Sci. Hung.* **12**, 153—159.

FELFÖLDY, L. J. M. (1962): A simple apparatus for culturing unicellular algae in large amounts for laboratory purposes. — *Annal. Biol. Tihany* **29**, 95—100.

FELFÖLDY, L. J. M. and ZSUZSA F. KALKÓ (1959): Some methodical observations on the use of antibiotics for preparing bacteria-free algal cultures. — *Acta Biol. Acad. Sci. Hung.* **10**, 95—99.
FELFÖLDY L., SZABÓ E. és TÓTH L. (1962): Egysejtű algák pigment tartalmáról (On pigment content in some unicellular algae.) — *Annal. Biol. Tihany* **29**, 101—106. (In Hungarian with English summary.)
HOAGLAND, D. R. and W. C. SNYDER (1933): Nutrition of strawberry plant under controlled conditions: (a) Effects of deficiencies of boron and certain other elements. *Proc. Amer. Soc. Hort. Sci.* **30**, 288—294. *Expt. Sta. Rec.* **72**, 625 (1935).
KALKÓ, ZSUZSA F. and L. J. M. FELFÖLDY (1959): Notes on the method for preparing bacteria-free cultures of green algae by ultra-violet irradiation. — *Annal. Biol. Tihany* **26**, 343—347.
SCHROPP, W. (1951): Die Methodik der Wasserkultur höherer Pflanzen. In: R. HERRMANN (Herausg.) Handb. landw. Versuchs- und Untersuchungsmeth. VIII., *Neumann Verl. Berlin*, 1—313.
SOROKIN, C. and J. MYERS (1953): A high-temperature strain of Chlorella. — *Science* **117**, 330—331.
STAGNO d' ALCONTRES, G., G. LAMONICA, MARIA POLIMENI CONTI (1960): Cultura massiva di alghe. I. Selezione di ceppi, rese e composizione. — *Atti Soc. Peloritana* **6**, 349—382.
SZABÓ E., RUFF F. és FELFÖLDY L. (1961): Egysejtű algák össz-szterin tartalmáról. (On sterol content in some unicellular algae.) — *Annal. Biol. Tihany* **28**, 139—131. (In Hungarian with English summary.)
TAMIYA, H. (1957): Mass culture of algae. — *Ann. Rev. Plant Physiol.* **8**, 309—334.

KISÉRLETEK TÖMEGTENYÉSZTÉSRE ALKALMAS ALGATÖRZSEK KIVÁLASZTÁSÁRA

Felföldy Lajos

Összefoglalás

35 egysejtű algatörzset próbáltunk ki abból a szempontból, hogy növekedési erélyük és más technológiai tulajdonságaik (centrifugálhatóság, szűrhetőség stb.) szerint alkalmasak-e tömegtenyésztési kísérletek céljaira?

A kísérleteket 10 literes gömblombikokban, üvegházban állítottuk be 1960 nyarán az *1. ábrán* látható megoldással. A törzseket egy hónapig tenyésztettük. Az ekkorra elért szárazanyag koncentráció, a termék fehérjetartalma (6,25. Kjeldahl-N) és a törzsek tenyésztés és kitermelés alatti viselkedése voltak az elbírálás alapjai. A kísérlet tihanyi csapvízből készült káliumnitrát-tartalmú tápoldatban történt (177. old.). A vizsgált törzsek felsorolása és a kísérletek eredménye az *1. táblázat*ban látható.

Feltétlenül meg kell jegyeznünk, hogy a 10 literes gömblombik alakjánál fogva nem célszerű algatenyésztő edény, mert a túl nagy rétegvastagság miatt a sejtek önárnyékolása révén már a 0,5 g szárazanyag/liter koncentrációjú szuszpenzióban tetemes fényhiány van. Így kísérleteink eredményének elsősorban relatív értéke van, de a további munka szempontjából sok hasznos tapasztalatot szereztünk. Ez a tenyésztő berendezés folyamatos termelést nem tesz lehetővé, de ha több törzset kell egy időben tenyésztenünk, igen jó szolgálatot tesz.

ВЫБОР ШТАММОВ ВОДОРОСЛЕЙ, ПРИГОДНЫХ ДЛЯ ЭКСПЕРИМЕНТАЛЬНОГО МАССОВОГО КУЛЬТИВИРОВАНИЯ

Л. Фэлфёлди

Было изучено 35 штаммов одноклеточных водорослей, чтобы выяснить по скорости выращивания и другим техническим свойствам (их центрифугируемость, фильтрируемость и т. д.) пригодность их для массового культивирования.

Опыты были поставлены летом 1960 года в 10 литровых шарообразных колбах в оранжерее; общий вид установки изображен на рисунке № 1. Шаммы культивировались в течение месяца. Концентрация сухого вещества, содержание белка продукта (6,2о

Кйельдал-Н) и поведение штаммов во время культивирования и выращивания служили критериями для их выбора. Эксперименты проводились на питательной среде, содержащей калий и нитрат и приготовленной на тиханьской водопроводной воде. Пересчет исследуемых штаммов и экспериментальные результаты видны на таблице № 1.

Мы должны отметить, что 10-литровая шаробразная колба по своей форме непригодна для культивирования водорослей, потому что обладает слишком толстым размером слоя и поэтому уже при концентрации 0,5 г сухое вещество/литр. наступает значительный недостаток света вследствие самозатемнения клеток. Таким образом наши экспериментальные данные имеют только относительное значение, но все же для дальнейшей работы мы приобрели некоторый опыт. При помощи этой установки продолжительное вырашивание невозможно, но успешно применяется для одновременного культивирования нескольких штаммов.