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TERESA BEDNARZ, MIECZYSŁAW NOWAK

Selekcja glonów dla potrzeb kultur masowych

The selection of algae for mass culture purposes

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A b s t r a c t — From various natural environments of Poland 950 algae strains were isolated. They belonged to the genera *Chlorella, Scenedesmus, Ankistrodesmus, Tetraëdron, Coelastrum, Protococcus, Stichococcus,* and *Chlamydomonas,* and were subsequently subjected to a three-stage selection.

The selection aimed at choosing productive algae strains with a high content of crude protein in the biomass. 56 productive algae strains were obtained, belonging to the genera *Chlorella, Scenedesmus,* and *Ankistrodesmus.* The content of crude protein in the biomass of the selected algae strains varied from 43 to 65 per cent.

In the forties of the present century the possibility was considered of a mass culture of algae as a possible means of production of protein, carbohydrates, and fat on an economic scale (B u r l e w ed. 1953). This concerns especially the genera *Chlorella* and *Scenedesmus,* which, developing under optimum conditions, can give a production of up to 100 tons of dry matter from one hectare annually (T a m i y a 1966). On account of the rich and valuable chemical composition of the cells of some algae, the investigations carried out tended towards the isolation from natural conditions and selection, i. e. the choice of the most productive varieties of algae (B u r l e w ed. 1953, K o k 1952, S o r o k i n 1953, 1959, L e w i n ed. 1962, V l a d i m i r o v a, S e m e n e n k o 1962). As can be seen from publications and other sources of information, studies on the selection of algae have hitherto been carried out on a rather limited scale. Investigations of this type were started in Poland in 1961. Productive alga strains isolated from natural Polish localities began to be sought for J a n k o w s k i (1964).

The present work is a continuation of previous investigations. It was

carried out with the view of choosing productive alga strains with a high content of crude protein in the biomass.

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Methods of investigations

A selection was made of 950 monocultures of alga strains of the genera *Chlorella, Scenedesmus, Tetraëdron, Ankistrodesmus, Chlorococcum, Protococcus, Coelastrum, Chlamydomonas,* and *Stichococcus,* isolated from nature from various biological habitats according to the method of inoculation on agar mineral in Petri dishes (Jankowski 1964) during the period from October 1964 to August 1966. The samples were taken from the terrain of southern Poland, the great majority of them procceding from warmed drainage, eutrophic or eutrophicated waters and natural hot springs (Table I, fig. 1).

For comparative purposes, cultures of algae were used proceeding from the Laboratory of Mass Culture of Algae at Třebonia, ČSSR (*Scenedesmus quadricauda*$_1$), from the Fodder Institute in Moscow, USSR (*Chlorella* Co 10/25/62), and from the collection of the Institute of Zootechnics at Zator, PPR (*Chlorella* 366, *Scenedesmus* 315).

All strains were kept on a mineral medium L_nm+z, solidified with agar (Jankowski 1964) in a refrigerated counter, at a temperature of 8—10°C and illumination of 200 lux.

The choice of strains was determined by three stages of selection: The first stage — extensive culture (preliminary selection). The cultures were grown in conical Erlenmayer's flasks of 500 ml capacity, containing 250 ml of alga suspension. During 24 hours the cultures were shaken on shakers of the WS — 2 type. The cultivation was carried out during 10 days at an illumination of 2000 lux/16 hr/day and temperature of 22—24°C on an L_nm+z medium (Jankowski 1964).

During cultivation the increase in the density of cultures was determined every other day by means of a Zeiss nephelometer of the III/b/4 type. The initial density was fixed at 0.4 E extinction, which corresponded to about 0.1 g of dry matter (dry weight) per litre.

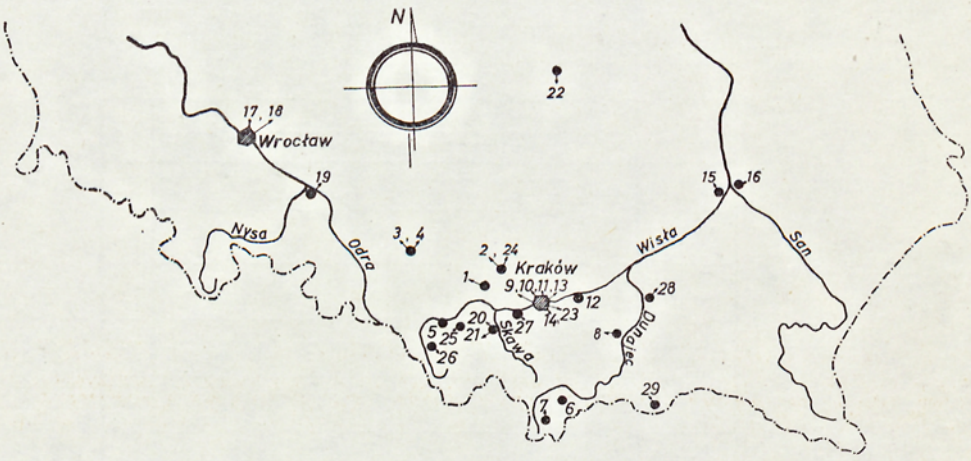
The initial density, the time of illumination, the culture vessels, and the volume of cultures were maintained in all stages of selection.

An example of the curve of dependence of extinction on the content of dry matter for the genus *Chlorella* and *Scenedesmus* is presented in fig. 2A. In spite of the considerable morphological differences of these algae, the calibration curves run almost identically. Hence the authors

Tabela I. Lista szczepów glonów, wyizolowanych z poszczególnych stanowisk

Table I. List of algae strains isolated from particular sampling points

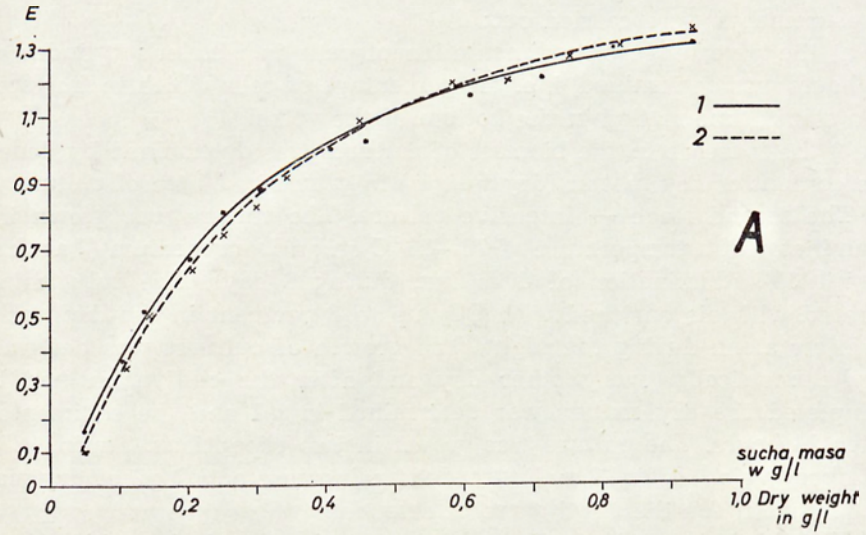
Number stanowiska / Number of sampling point — Rodzaj / Genus	Chlorella	Scenedesmus	Ankistrodesmus	Tetraédron	Chlorococcum	Radiococcus	Gloeocystis	Coelastrum	Protococcus	Stichococcus	Hormidium	Ulothrix	Oedogonium	Tribonema	Chlamydomonas	Non determinans	Ogółem Total
1	17	23						4								4	48
2	19	12	1					1								2	35
3	12	18	6													3	39
4	10	22	4														36
5	16	10														2	28
6	11	12						1								8	32
7	4	6	1													6	17
8	9	7								1						1	18
9	15	37	6	1												2	61
10	5	5	1													2	13
11	18	25	3		1			1								2	50
12	24	13	1					2		3						1	44
13	19	12	2					3								4	40
14	3	9	1							2						3	18
15	8	9	1							1						3	22
16	15	20	2													4	41
17	25	17	2				1								1	3	49
18	20	38	7													8	73
19	10	31	2					2	1						1	1	48
20	36	22								3						5	66
21	25	19	6	1	1			2								4	58
22	3	1							1							2	7
23	5	1														2	8
24	4	2														3	9
25	2	2		1													5
26		2	1														3
27	5	2	4														11
28	1	2								1							4
29	5	1															6
inne - other	2	17	11	1		1		6		6	1	2	1	3		10	61
Ogółem Total	348	397	62	4	2	1	1	22	2	17	1	2	1	3	2	85	950



Ryc. 1. Mapka stanowisk. 1) Staw parkowy w Chorzowie, 2) Potok Rawa przed Sosnowcem, 3) Wody chłodnicze elektrowni w Zabrzu, 4) Osadnik wód przemysłowych w Makuszowej, pow. Zabrze, 3) Rzeka Wisła przed zbiornikiem zaporowym w Goczałkowicach, pow. Pszczyna, 6) Potok Białka w Białce Tatrzańskiej, pow. Nowy Sącz, 7) Zakopane, ciepłe źródło na Antałówce, 8) Rzeka Dunajec przed zbiornikiem zaporowym w Rożnowie, pow. Nowy Sącz, 9) Staw parkowy w Balicach, pow. Kraków, 10) Bajorko w Balicach, pow. Kraków, 11) Rzeka Rudawa przed Mydlnikami, pow. Kraków, 12) Rzeka Wisła w Niepołomicach, 13) Bajorko w Bonarce, pow. Kraków, 14) Rzeka Wilga, poniżej ujścia ścieków Krakowskich Zakładów Sodowych, 15) Rzeka Wisła przed Sandomierzem, 16) Rzeka San przed ujściem, 17) Ścieki miejskie Wrocławia, 18) Rzeka Odra poniżej ujścia Nysy Kłodzkiej, 19) Rzeka Nysa Kłodzka przed ujściem, 20) Stawy karpiowe w Zatorze, pow. Oświęcim, 21) Rzeka Skawa w Zatorze, pow. Oświęcim, 22) „Niebieskie Źródła" — rezerwat k. Tomaszowa Mazowieckiego, 23) Aleksandrowice, pow. Kraków, ścieki z obory, 24) Rzeka Brynica k. Sosnowca, 25) Rzeka Białka w Czechowicach, pow. Bielsko, 26) Rzeka Wisła poniżej Skoczowa, pow. Cieszyn, 27) Rzeka Skawinka poniżej ujścia wód chłodniczych elektrowni w Skawinie, pow. Kraków, 28) Rzeka Dunajec w Tarnowie, 29) Źródła i hodowla masowa glonów w Tyliczu k. Krynicy, pow. Nowy Sącz

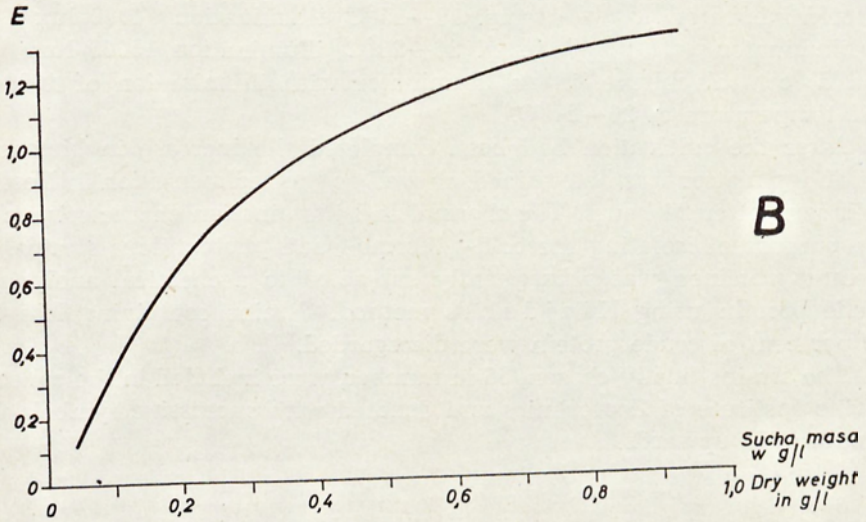
Fig. 1. The map of sampling places: 1) The park pond at Chorzów, 2) Stream Rawa above Sosnowiec, 3) The cooling waters of the Power Station at Zabrze, 4) The settler of industrial sewages at Makuszowa, distr. Zabrze, 5) River Vistula above the Goczałkowice dam-reservoir, distr. Pszczyna, 6) Stream Białka at Białka Tatrzańska, distr. Nowy Targ, 7) Zakopane, the hot springs on Antałówka, 8) River Dunajec, above the Rożnów dam-reservoir, distr. Nowy Sącz, 9) The park pond at Balice, distr. Kraków, 10) A slough at Balice, distr. Kraków, 11) River Rudawa, above Mydlniki, distr. Kraków, 12) River Vistula, at Niepołomice, 13) A slough at Bonarka, distr. Kraków, 14) River Wilga below entrance point of wastes of Kraków Soda Works, 15) River Vistula, above Sandomierz, 16) River San, above its mouth, 17) The municipal sewages of Wrocław, 18) River Odra, below the mouth of the River Nysa Kłodzka, 19) River Nysa Kłodzka, above its mouth, 20) The carp ponds at Zator, distr. Oświęcim, 21) River Skawa at Zator, distr. Oświęcim, 22) „Niebieskie Źródła" the reservation near Tomaszów Mazowiecki, 23) Aleksandrowice, distr. Kraków, the farm sewages, 24) River Brynica near Sosnowiec, 25) River Białka, at Czechowice, distr. Bielsko, 26) River Vistula below Skoczów, distr. Cieszyn, 27) River

Skawinka below the entrance point of cooling waters of the Power Station at Skawina, distr. Kraków, 28) River Dunajec at Tarnów, 29) The springs and the mass culture of algae at Tylicz near Krynica, distr. Nowy Sącz



Ryc. 2A. Krzywe zależności ekstyncji od suchej masy glonów, dla rodzajów *Chlorella* i *Scenedesmus;* 1. *Chlorella,* 2. *Scenedesmus.*

Fig. 2A. The curves of dependences of extinction from dry weight of algae for genera *Chlorella* and *Scenedesmus*; 1. *Chlorella*, 2. *Scenedesmus*



Ryc. 2B. Krzywa wzorcowa przyrostów gęstości kultur

Fig. 2B. Standard curve of the increase in density of cultures

determined not to plot calibration curves for the particular genera of algae, referring the increase in density of all cultures to the standard curve (fig. 2B).

After completing the cultivation of the first stage, the following measurements were carried out:

a) the percentage of sedimentation of cells in Nessler's cylinder after 24 hours was determined, i. e. the percentage of cells which in free setting at a stated time settled in the bottom of the vessel,

b) the increase in the biomass of cultures was determined by denoting the content of dry matter (dry weight) in grams in 1 litre of culture (g/l).

The second stage — intensive culture. The culture was grown in an illuminator of a temperature of 26—28°C on an L_5 m medium (J a n k o w s k i 1964), illumination of 4000 lux, during 6 days. The cultures were aerated with air enriched with CO_2 up to 3 per cent in the daytime and with air alone during the night. The growth of cultures was determined nephelometrically every other day and after the cultivation had been completed the dry matter was determined in g/l and the sedimentation after 24 hours. The culture was grown in two repetitions. Strains, 250 in number, which attained and exceeded an increase of 1.2 g/l of dry matter were chosen for the third stage of selection.

The third stage of selection. To characterize the strains with regard to the thermal and illumination requirements the culture was grown in three variants, the previous parameters, such as the medium, aeration, and duration of the cultivation being maintained:

a) thermoilluminostat — temp. 36—38°C, illumination 4000 lux
b) cold incubator — temp. 13—15°C, illumination 4000 lux
c) illuminator — temp. 26—28°C, illumination 15000 lux

and d) control culture, in an illuminator with illumination of 4000 lux and temperature of 26—27°C.

After the cultivation had been completed the increase in biomass in g/l of dry matter was determined, as well as the sedimentation in Nessler's cylinders, after 24 hours. The thermal and illumination characteristics had the purpose of isolating the cold-, thermo-, and photophilous strains. The biomass obtained was subsequently subjected to a chemical analysis for crude protein using K i e j d a h l's method. Strains containing less than 40 per cent of crude protein were disregarded.

The strains finally chosen, 56 in number, were included in the collection of the Institute of Zootechnics and denoted with successive symbols from number 654 upwards.

Results

Table II and fig. 3 show the results of 10-days' extensive culture of 950 alga strains. 77 strains, chiefly of the genus *Chlorella* and *Scenedesmus*, representing 8.1 per cent of all investigated strains, did not attain the minimum increase in biomass amounting to 0.1 g/l of dry matter.

Tabela II. Częstotliwość występowania szczepów glonów w różnych przedziałach przyrostu biomasy, po 10 dniach hodowli ekstensywnej, wyrażonej suchą masą w g/l

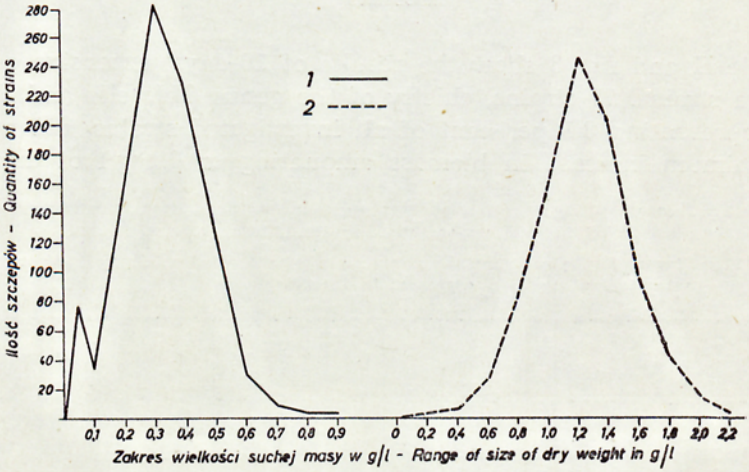
Table II. Frequency of algae strains in various intervals of biomass growth, after 10 days extensive cultivation, expressed in dry weight g/l

Rodzaj / Genus — Sucha masa g/l / Dry weight g/l	0,1	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	>0,8	Ogółem Total
Chlorella	37	10	61	130	80	25	2	2		1	348
Scenedesmus	31	9	36	95	116	83	20	5	1	1	397
Ankistrodesmus	1	4	26	25	5	1					62
Tetraedron			1		2		1				4
Coelastrum	1	2	6	5	5	2		1			22
Gloeocystis		1									1
Protococcus					1	1					2
Radiococcus							1				1
Chlorococcum	1			1							2
Tribonema		1	2								3
Ulothrix		1						1			2
Stichococcus	3	4	6	1	2		1				17
Hormidium			1								1
Chlamydomonas	1			1							2
Oedogonium							1				1
Chlorophyceae non det.	2	6	18	20	19	15	5				85
Ogółem - Total	77	38	157	278	230	127	31	9	1	2	950

These strains were disregarded. The remaining 873 strains passed to the second stage of selection. 54 per cent attained an increase equalling 0.3—0.4 g/l of dry matter. Some cultures of algae necrotized immediately after inoculation or after 3 to 5 days of cultivation. This phenomenon was preceded by a sudden loss of the green colouring of the culture and a fall in extinction, noted during the nephelometric measurement.

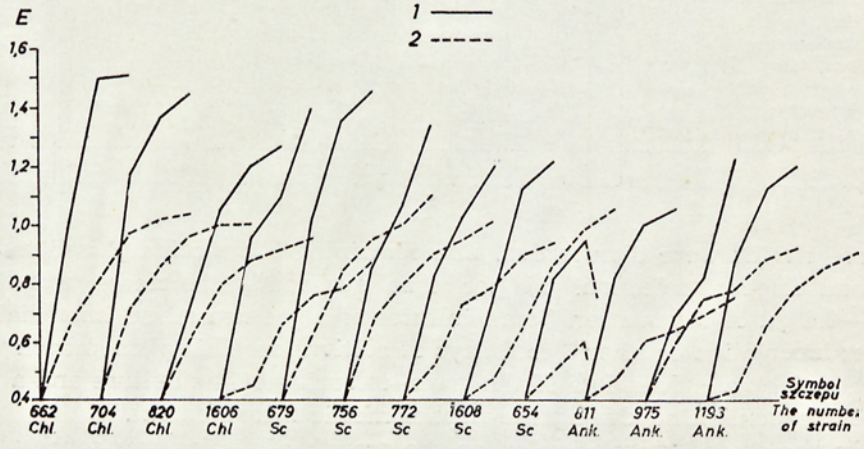
In the second stage of selection the growth of strains was much more rapid. Already after two or at the latest after four days the strains attained such an increase in biomass as was obtained in the end in cultures grown extensively (fig. 4). Strains necrotizing during the first stage of selection began to grow quite well under conditions of intensive cultivation. However, after two to four days a phenomenon identical to that previously observed was seen. The authors did not succeed in explaining the causes bringing about the necrosis of these cultures.

The increase in the biomass of cultures varied within a fairly wide range — from 0.1 to values exceeding 2 g/l of dry matter (Table III). 80.3 per cent of strains attained an increase in biomass ranging from



Ryc. 3. Częstotliwość występowania szczepów glonów w różnych przedziałach suchej masy w g/l; 1. hodowla ekstensywna, 2. hodowla intensywna

Fig. 3. The frequency of occurrence of algae strains in different intervals of biomass growths, expressed in dry weight g/l; 1. extensive culture, 2. intensive culture



Ryc. 4. Nefelometryczne krzywe wzrostu glonów; 1. hodowla intensywna, 2. hodowla ekstensywna

Fig. 4. Nephelometric curves of growth of algae; 1. intensive culture, 2. extensive culture

0.8 to 1.0, 1.0 to 1.2, and 1.2 to 1.4 g/l of dry matter. The value of 1.4 g/l of dry matter was exceeded by 17.9 per cent of strains. The others did not attain 0.8 g/l of biomass growth (Table III, fig. 3). Two hundred and fifty strains, representing 28.8 per cent of strains cultivated under intensive conditions, attained and exceeded 1.2 g/l of biomass growth expressed in dry matter. These strains were assigned for the third stage of selection.

Tabela III. Częstotliwość występowania szczepów glonów w różnych przedziałach przyrostu biomasy po 6 dniach hodowli intensywnej, wyrażonej suchą masą w g/l

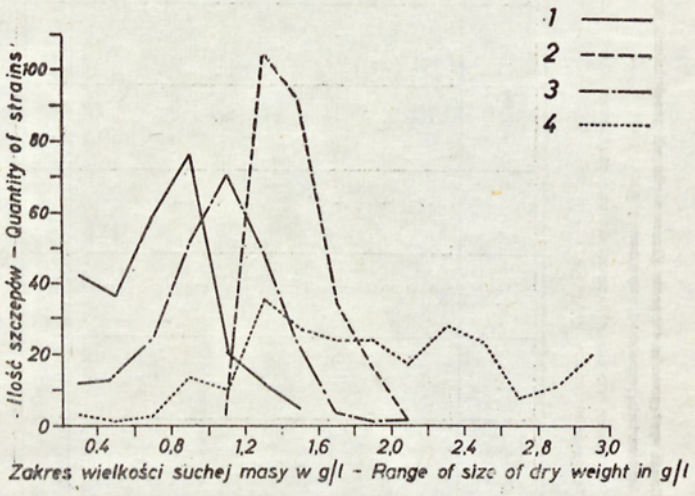
Table III. Frequency of algae strains in various intervals of biomass growth, after 6 days intensive cultivation, expressed in dry weight g/l

Rodzaj / Genus — Sucha masa g/l / Dry weight g/l	0,1-0,4	0,4-0,6	0,6-0,8	0,8-1,0	1,0-1,2	1,2-1,4	1,4-1,6	1,6-1,8	1,8-2,0	2,0	Ogółem Total
Chlorella	2	8	30	50	91	62	38	16	7	1	307
Scenedesmus		6	20	54	103	109	48	21	7		368
Ankistrodesmus	1	3	7	15	17	13	4	2			62
Tetraëdron			1	1	1	1					4
Coelastrum	3	1	7	1	7	1	1				21
Gloeocystis					1						1
Radiococcus					1						1
Chlorococcum					1						1
Protococcus						2					2
Stichococcus		3	5	4	2		1				15
Hormidium		1									1
Ulotrix			1	1							2
Oedogonium				1							1
Chlamydomonas			1								1
Tribonema	2	1									3
Chlorophyceae n.det.	1	4	7	27	20	14	7	2	1		83
Ogółem Total	9	27	79	156	244	202	99	41	15	1	873

The greatest increase in biomass among them, exceeding 1.8 g/l of dry matter, was attained by 15 strains, 8 of which were of the genus *Chlorella* and 7 of the genus *Scenedesmus*.

In the third stage of selection the dependence of biomass growth on temperature and illumination was investigated. It was found that the majority of strains increase well in volume at all temperatures applied. The frequency of occurrence of alga strains within various ranges of dry matter was different for each temperature, varying within fairly wide limits (Table IV, fig. 5). It was only at a temperature of 27°C that all algae attained a growth ranging from 1 to 2.2 g/l of dry matter, which would indicate that this is the optimum temperature. The investigated alga strains can therefore be assigned to the group of mesophilous strains.

No typically cold-loving strain was found. Neither was any decrease in biomass growth noted at 15000 lux; on the contrary, all strains attained the greatest increase in biomass at this illumination. In contradistinction to the results obtained by J a n k o w s k i (1964), at a temperature of 37°C, a smaller growth of algae was observed as a rule than at a temperature of 27°C. Only one strain, *Scenedesmus acutus* No 1608, isolated as



Ryc. 5. Częstotliwość występowania szczepów glonów w różnych przedziałach suchej masy w g/l; 1. hodowla w 14°C przy 4000 lux oświetlenia, 2. hodowla w 27°C przy 4000 lux oświetlenia, 3. hodowla w 37°C przy 4000 lux oświetlenia, 4. hodowla w 27°C przy 15 000 lux oświetlenia

Fig. 5. The frequency of algae strains in different intervals of biomass growth expressed in dry weight g/l; 1. culture in 14°C by 4000 lux lighting, 2. culture in 27°C by 4000 lux lighting, 3. culture in 37°C by 4000 lux lighting, 4. culture in 27°C by 15 000 lux lighting

Tabela IV. Częstotliwość występowania szczepów glonów w różnych przedziałach przyrostu biomasy, w czterech wariantach hodowli, wyrażonej suchą masą w g/l

Table IV. The frequency of algae strains in various intervals of biomass growth, in four variants of cultivation, expressed in dry weight (g/l)

Sucha masa g/l Dry weight g/l

Rodzaj Genus	Oświetlenie i temperatura Lighting and temperature	0,1- -0,4	0,4- -0,6	0,6- -0,8	0,8- -1,0	1,0- -1,2	1,2- -1,4	1,4- -1,6	1,6- -1,8	1,8- -2,0	2,0- -2,2	2,2- -2,4	2,4- -2,6	2,6- -2,8	2,8- -3,0	0,3 i powyżej 0,3 and over
Chlorella	4000, 14°C	33	21	25	16	6	5	2								
	4000, 27°C					1	40	43	17	6	1					
	4000, 37°C	8	10	13	28	23	18	7	1							
	15000, 27°C		1	3	9	5	20	16	10	9	7	7	10	1	3	7
Scenedesmus	4000, 14°C	8	11	26	55	13	6	3								
	4000, 27°C					2	57	38	16	9						
	4000, 37°C	2	2	8	19	43	29	14	3	1	1					
	15000, 27°C	2			4	5	12	8	10	14	9	18	13	6	8	13
Ankistrodesmus	4000, 14°C	1	1	3	5	1										
	4000, 27°C						4	5	2							
	4000, 37°C	1	1	4	1	1	1	2								
	15000, 27°C	1					3	2	2	1		1		1		
Stichococcus	4000, 14°C			1												
	4000, 27°C							1								
	4000, 37°C					1										
	15000, 27°C							1								
Chlorococcum	4000, 14°C															
	4000, 27°C		1				1									
	4000, 37°C				1											
	15000, 27°C												1			
Chlamydomonas	4000, 14°C															
	4000, 27°C			1												
	4000, 37°C							1								
	15000, 27°C	1			1											
Chlorophyceae n.det.	4000, 14°C															
	4000, 27°C		2	3		1	2	3		1						
	4000, 37°C				2	3	1									
	15000, 27°C						1		2		1	2				
Ogółem - Total	4000, 14°C	42	36	59	76	21	11	5								
	4000, 27°C					3	104	91	35	16	1					
	4000, 37°C	12	13	25	51	71	49	23	4	1	1					
	15000, 27°C	3	1	3	14	10	36	27	24	24	17	28	24	28	11	120

a pollution of mass cultivation of *Chlorella* at Tylicz in 1966, showed a distinctly greater biomass growth at a temperature of 37° (fig. 6C). Thus, the final choice of alga strains was made on the basis of the increase in biomass of algae, obtained from a culture grown at a temperature of 27°C. The exceeding by the strains of 1.3 g/l of dry matter was regarded as the criterion of this choice.

For another culture grown with the view of obtaining the biomass essential to chemical analyses and a control of growth, 63 alga strains were chosen, including 26 of the genus *Chlorella*, 34 of *Scenedesmus*, and 3 of *Ankistrodesmus*. The culture was grown at an illumination of 7500 lux and a temperature of 27°C. The content of protein in the biomass of the investigated algae varied from 35 to 64 per cent; only 7 strains, 6 of the genus *Chlorella* and 1 of *Scenedesmus*, did not exceed 40 per cent of the crude protein content in the biomass. These strains, as low protein, were disregarded.

The lowest content of protein was noted in *Chlorella* — 35 per cent — and the highest in *Scenedesmus* — 64.41 per cent. *Ankistrodesmus* also showed a high content of protein — 56—58.52 per cent. Taken as a whole, *Chlorella* contained less protein than *Scenedesmus* and *Ankistrodesmus* (Table VI).

Tabela V. Sedymentacja szczepów glonów hodowanych w różnych warunkach, po 24 godz., wyrażona w procentach

Table V. Sedimentation of algae strains after 24 h, cultivated in various conditions, expressed in percentage

Rodzaj, gatunek i symbol szczepu / Genus, species and number of strain — Temperatura i oświetlenie / Temperature and lighting	14°C 4000 lux	27°C 4000 lux	37°C 4000 lux	27°C 15000 lux
662 Chlorella sp.	50	60	60	60
669 - -	90	100	100	100
704 - -	60	60	60	50
714 - -	40	50	50	40
817 - -	40	40	40	40
911 - -	60	60	70	60
948 - -	60	70	80	80
1066 - -	70	70	80	80
1324 - -	80	80	90	90
1606 - -	30	30	30	40
756 Scenedesmus acutus Meyen	90	90	90	90
772 - quadricauda (Turp.) Bréb.	40	50	50	40
792 - acutus Meyen	70	80	80	80
824 - spinosus Chod.	40	50	50	40
1018 - brevispina (G.M.Smith) Chod.	80	90	90	90
1057 - quadricauda (Turp.) Bréb.	100	100	100	100
1082 - obtusiusculus Chod.	90	90	90	90
1403 - dispar Bréb.	90	90	90	90
1408 - -	100	100	100	100
1420 - arcuatus Lemm.	90	90	90	90
1608 - acutus Meyen	80	80	90	90
811 Ankistrodesmus braunii Brunth.	80	80	80	90
975 - minutissimus Korschik.	60	60	70	70

Tabela VI Zawartość procentowa białka ogólnego u wyselekcjonowanych szczepów glonów

Table VI. Crude proteins in biomass of selected algae strains, expressed in percentage

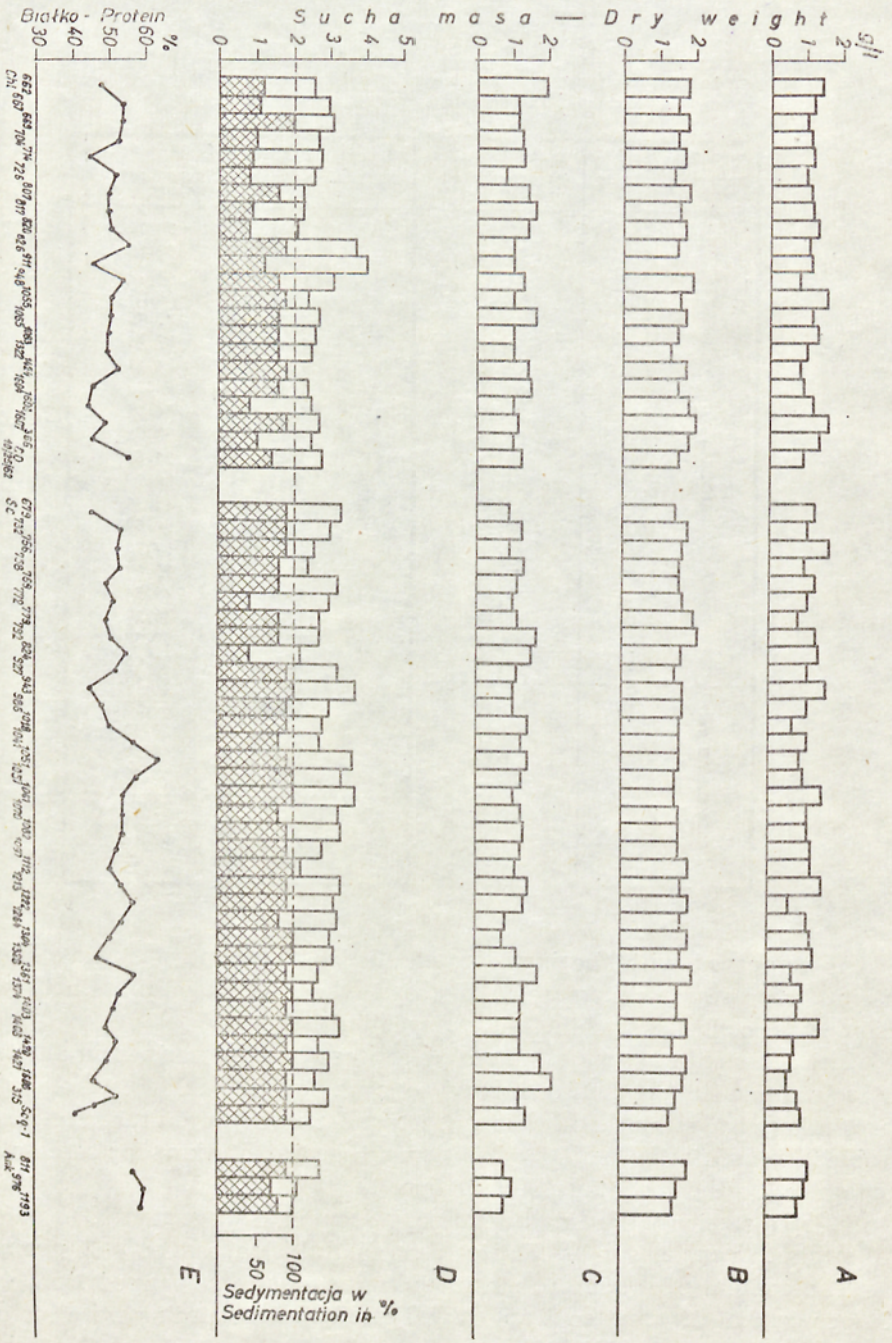
Symbol szczepu Number of strain		Procent białka Percentage of protein	Symbol szczepu Number of strain		Procent białka Percentage of protein
CHLORELLA sp.	662	46,56	SCENEDESMUS sp.	824	54,72
	667	53,04		927	52,15
	669	53,22		943	45,15
	704	51,69		986	48,06
	714	43,53		1018	49,76
	726	50,72		1041	57,45
	806	48,90		1051	64,41
	817	49,28		1057	56,78
	820	50,37		1061	54,35
	826	55,09		1070	53,69
	911	45,28		1082	54,44
	948	54,01		1097	52,30
	1055	49,62		1112	49,94
	1066	50,37		1213	52,69
	1083	49,34		1222	57,34
	1322	48,58		1264	53,27
	1422	51,69		1304	49,54
	1604	45,12		1328	46,36
	1606	43,62		1361	57,38
	1607	48,75		1374	53,06
				1403	51,17
SCENEDESMUS sp.	679	45,39		1408	48,58
	732	52,71		1420	52,38
	756	51,75		1421	49,32
	758	52,72		1608	45,06
	769	48,56			
	772	51,44	ANKISTRODESMUS sp.	811	56,41
	779	49,44		975	58,52
	792	50,23		1193	68,35

As compared with the investigated strains, the control strains showed a similar or smaller increase in biomass. The content of protein in their biomass also varied within the average limits (fig. 6).

The sedimentation expressed in percentage and determined in all stages of selection after 24 hours, proved to be a fairly stable feature for the particular alga strains and was not dependent on the culture conditions. Table V presents the results of settlement (sedimentation) for some algae, obtained for the last stage of selection.

56 alga strains were finally chosen, belonging to 3 genera: *Chlorella* — 20 strains, *Scenedesmus* — 33 strains, and *Ankistrodesmus* — 3 strains.

The content of crude protein in the selected strains ranged from 43 to 64 per cent of dry matter (Table VII, fig. 6E). These strains were included in the collection of productive alga strains of the Institute of Zootechnics at Zator.



Ryc. 6. Sucha masa glonów, uzyskana po 6 dniach hodowli w: A. 14°C, 4000 lux, B. 27°C, 4000 lux, C. 37°C, 4000 lux, D. 27°C, 15 000 lux, na diagramie zaznaczono procent sedymentacji komórek po 24 godz., E. zawartość białka ogólnego w procentach. Szczepy kontrolne zaznaczono na diagramach silniejszym obrysem

Discussion

Comparative investigations on the yield of algae of the genera *Chlorella* and *Scenedesmus* from various collections were carried out in Holland (K o k 1952). It was found that in the majority of algae the greatest increase in biomass was obtained in thermoilluminators at a temperature of 30°C. The results of J a n k o w s k i's (1964) and of the authors' investigations also showed that the maximum increase in biomass of algae was obtained at a temperature of 25—30°C.

S o r o k i n (1953, 1959) obtained by way of selection a thermophilous variety of alga *Chlorella* whose optimum development occurred at a temperature of about 40°C. As a result of investigations carried out by J a n k o w s k i (1964) and by the present authors, several thermophilous alga strains were distinguished, but their temperature optimum was slightly lower, amounting to about 37°C.

Unlike J a n k o w s k i (1964), the authors found no typically cold-loving alga strain. Neither did they observe any decrease in the biomass growth at 15 000 lux; on the contrary, all strains showed the greatest biomass growth at this illumination.

The percentage content of crude protein in alga strains selected by the authors was slightly higher than in those selected in 1964. In contradistinction to the results obtained by J a n k o w s k i (1964), algae of the genus *Chlorella* showed a lower mean content of crude protein than algae of the genus *Scenedesmus*. Also the increase in biomass of the selected algae was higher than that reported in J a n k o w s k i's (1964) work for algae obtained by means of selection in the years 1961—1964. This, however, could be due to the aeration of alga cultures during the night, while those grown by J a n k o w s k i were not aerated at that time. The fact that alga strains of the genus *Chlorella* and *Scenedesmus* included in the selective works being the subject of the present study, for comparative purposes, showed a greater biomass growth than those reported by J a n k o w s k i (1964), seems to corroborate this opinion.

The selective material of the two parts comprised about 1600 alga strains belonging to various genera of the class *Chlorophyceae*. In spite of the initially considerable systematic differentiation, in the final effect algae belonging to only three genera: *Chlorella*, *Scenedesmus*, and *Ankistrodesmus* were regarded as useful for mass culture purposes. This points to the particular predisposition of these groups of algae to the above-mentioned purposes. Authors engaged in a similar subject matter also came to the same conclusion.

←

Fig. 6. Dry weight of algae, after 6 days cultivation in: A. 14°C, 4000 lux, B. 27°C, 4000 lux, C. 37°C, 4000 lux, D. 27°C, 15 000 lux; in the diagram is marked the sedimentation of cells after 24 h, E. contents of crude protein expressed in percentage. The control strains are marked in the diagrams by a stronger outline

Tabela VII. Lista wyselekcjonowanych szczepów glonów, z uwzględnieniem daty i miejsca izolacji

Table VII. List of selected algae strains, with regard to date and place of izolation

Rodzaj i gatunek Genus and species	Symbol szczepu Number of strains	Numer stanowiska Number of sampling point	Data wyizolowania The date of isolation
1	2	3	4
Chlorella sp,	662	3	15.V.1964
- -	667	23	15.V.1964
- -	669	23	15.V.1964
- -	704	1	16.X.1964
- -	714	24	16.X.1964
- -	726	2	16.X.1964
- -	807	27	21.X.1964
- -	817	15	21.X.1964
- -	820	15	21.X.1964
- -	826	16	26.X.1964
- -	911	4	11.XII.1964
- -	948	11	30.XII.1964
- -	1055	21	15.XII.1964
- -	1066	22	13.XII.1964
- -	1083	3	27.II.1965
- -	1322	13	20.V.1965
- -	1424	21	9.VI.1965
- -	1604	29	lato summer 1966
- -	1606	29	lato summer 1966
- -	1607	29	lato summer 1966
Scenedesmus acutus Meyen	732	3	17.X.1964
- -	756	5	17.X.1964
- -	769	9	21.X.1964
- -	792	12	21.X.1965
- -	927	5	12.XI.1964
- -	1041	19	4.XII.1964
- -	1051	21	15.XII.1964
- -	1061	21	15.XII.1964
- -	1070	15	4.XII.1964
- -	1112	1	18.II.1965
- -	1113	19	17.III.1965
- -	1222	1	28.V.1965
- -	1304	12	20.V.1965
- -	1328	13	20.V.1965
- -	1361	17	8.VI.1965
- -	1421	21	9.VI.1965
- - -	1608	29	lato summer 1966
- armatus Chod.	758	5	17.X.1964
- arcuatus Lemm.	1420	21	9.VI.1965
- brevispina (G.M.Smith) Chod.	1018	18	4.XII.1964
- -	1374	18	8.VI.1965
- dispar Bréb.	1403	19	8.VI.1965
- -	1408	19	8.VI.1965
- ecornis (Ralfs) Chod.	779	9	21.X.1964
- obtusiusculus Chod.	1082	3	27.II.1965
- -	1264	6	28.V.1965
- quadricauda (Turp.) Bréb.	679	13	15.V.1964
- -	772	9	21.X.1964
- -	943	7	30.XI.1964
- -	1057	21	15.XII.1964
- -	1097	6	20.II.1965
- spinosus Chod.	824	20	28.X.1964
- wisconsinensis Chod.	986	11	30.XII.1964
Ankistrodesmus braunii Brunth.	811	27	21.X.1965
- minutissimus Korschik.	1193	17	17.III.1965
- -	975	10	30.XII.1964

STRESZCZENIE

W okresie od października 1964 do sierpnia 1966 r. wyizolowano z różnych siedlisk naturalnych (ryc. 1, tabela I) 950 szczepów glonów, należących do rodzajów *Chlorella, Scenedesmus, Ankistrodesmus, Tetraëdron, Coelastrum, Protococcus, Stichococcus* i *Chlamydomonas*. Wyizolowane i przechowywane na agarze mineralnym (Jankowski 1964) monokultury glonów poddano trójetapowej selekcji, mającej na celu wybór produktywnych szczepów glonów o dużej zawartości białka ogólnego w biomasie.

W pierwszym etapie selekcji przebiegającej w warunkach ekstensywnych wybrano 873 szczepy glonów (tabela II), za kryterium przyjęto podwojenie masy wyjściowej szczepów po zakończeniu hodowli. Podczas hodowli dokonywano kontroli przyrostów przy pomocy nefelometru (ryc. 4) i przyrosty biomasy kultur odnoszono do krzywej zależności ekstynkcji od suchej masy glonów (ryc. 2a).

W drugim etapie selekcji hodowano glony w warunkach intensywnych, kontrolując przyrosty biomasy kultur nefelometrycznie (ryc. 4). Za kryterium wyboru przyjęto 13-krotne zwiększenie biomasy kultur na końcu hodowli (tabela III, ryc. 3).

Do trzeciego etapu selekcji, mającej na celu ustalenie wymogów cieplnych i świetlnych, zakwalifikowano 26,8% szczepów hodowanych w warunkach intensywnych. Okazało się, że większość szczepów przyrasta dobrze we wszystkich stosowanych temperaturach i oświetleniu oraz że biomasa końcowa kultur waha się w dość szerokich granicach (tabela IV, ryc. 5). Najmniejsze wahania w przyrostach biomasy szczepów występowały przy temperaturze 27°C, co wskazuje na mezofilność badanych szczepów.

Do analiz na zawartość białka wybrano 63 szczepy najlepiej przyrastające w warunkach hodowli trzeciego etapu selekcji. Zawartość białka ogólnego w biomasie szczepów wahała się w granicach 35—64%, przy czym tylko 7 szczepów miało mniej niż 40% białka. Szczepy te, jako niskobiałkowe, odrzucono (tabela VI). Tabela VII podaje listę wyselekcjonowanych szczepów glonów, scharakteryzowanych pod względem zawartości białka ogólnego w biomasie jak i wymogów termicznych (ryc. 6).

Podczas wszystkich etapów selekcji kontrolowano sedymentację komórek u poszczególnych szczepów glonów, wyrażoną w procentach po 24 godzinach. Okazało się, że jest to cecha dość stała i nie zależna od warunków hodowli (tabela V).

Włączone do hodowli selekcyjnej cztery szczepy kontrolne, pochodzące z Instytutu Paszowego w Moskwie, ZSRR — szczep *Chlorella*, symbol Chl. CO 10/25/62, Laboratorium Masowej Hodowli Glonów w Třebonii, ČSRS — szczep *Scenedesmus*, symbol Sc q_1 i kolekcji Instytutu Zootechniki w Zatorze, PRL — dwa szczepy: *Chlorella*, symbol Chl. 366 i *Scenedesmus*, symbol Sc. 315, wykazały zbliżone lub mniejsze przyrosty biomasy, co pozwala na pozytywną ocenę szczepów uzyskanych drogą selekcji w 1968 r.

Wyselekcjonowane szczepy glonów włączono do kolekcji Instytutu Zootechniki w Zatorze.

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Adres autorów — Authors' address

mgr Teresa Bednarz

mgr Mieczysław Nowak

Zootechniczny Zakład Doświadczalny, Zator, pow. Oświęcim