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Free amino acids in cultures of various algae species

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Abstract — In cultures of unialgal species and of algae with bacterium the concentration and composition of free amino acids dissolved in the medium were investigated. The accumulation of these compounds in the phase of the logarithmic growth of cell number, a decreased content of amino acids in the stationary phase, and a repeated accumulation in the death phase were observed. The amount of amino acids released to the medium in different growth stages were characteristic of the given alga species. The addition of bacterium *Escherichia coli* changed the proportion of amino acids in the media of *Dictyosphaerium pulchellum* and *Diatoma elongatum*, but the concentration of the compounds was markedly increased in the diatom culture only.

Key words: culture of algae, amino acids, extracellular compounds.

1. Introduction

The origin of organic matter dissolved in water has not been completely explained yet, especially with regard to autochtonic substances. Results of laboratory experiments suggest that among other factors the development of algae affects the formation of organic matter (Nalewajko, Lean 1972). According to some authors (Taust 1968, Herbst 1976) the excess of organic matter dissolved in the environment inhibits the growth of algae, this being probably the reason for the ageing of algae cultures.

Of the constituents of organic matter, free amino acids are released by photosynthetizing algae as a result of photorespiration; the compounds can also originate from the autolysis of dead algal cells.

Meffert and Overback (1979) showed that the concentration of extracellular proteins was controlled by the growth of algae. Pimenova et al. (1970) observed that the bacteria isolated from a *Chlorella* culture did not release great amounts of proteolytic enzymes before reaching the sporulation phase of this species. In an earlier work, Matusiak et al. (1965) found that *Chlorella pyrenoidosa* and *Ch. vulgaris* produced less antibacterial substances in the older than in the younger stage.

The aim of the present work was to investigate the effect of different algae species on the content of amino acids in the medium. The subject of the experiment were various species of blue-green algae, diatoms and green algae whose "algal bloom" is most frequently observed. The cultures of algae and a 24-hour test of mixed cultures of some algal species with bacterium *Escherichia coli* were conducted.

2. Material and method

Two- and three-week cultures of algae were carried out. The two-week culture included the following species:

— blue-green algae *Phormidium favosum* (Bory) Gom., *Aphanizomenon flos aquae* (L.) Ralfs.,

— diatoms *Asterionella formosa* Hass., *Nitzschia palea* (Kütz.) W. Sm.,

— and a green alga *Volvox aureus* Ehr.;

while in the three-week culture the following species were grown:

— a diatom *Diatoma elongatum* (Lyngb.) Ag., and

— green algae *Dictyosphaerium pulchellum* Wood and *Volvox aureus* Ehr.

All algae used for inoculations originated from the same physiological phase, i.e., from the advanced growth of cell number (log phase). The cultures were grown in 500 ccm flasks on ASM-1 medium (Gorham et al. 1964) modified by Bombówna et al. (1975), in a thermoluminostate at 20—22°C and 2000 lux.

The biomass of specimens or cells of the different species was determined by weighing the wet mass collected on membrane filters, the cell numbers being simultaneously counted in an inverted microscope. The filters were dried at 60°C, weighed once more, and the dry weight of algae was thereby obtained.

In addition, in a 24-hour test the three-week old cultures of *Dictyosphaerium pulchellum* and *Diatoma elongatum* were inoculated with the bacterium *Escherichia coli*. The cultures were transferred to three

300 ccm flasks and inoculated with the bacterium at a ratio of 5.8×10^8 per 100 ccm of the culture.

The following procedure was used in determining the content of free amino acids: a 200 ccm sample of algae cultures was filtered through Whatman GF/C glass fibre papers with pore diameter of 1.2μ or, in case of the alga-bacterium test, through coli-titer 5 filters. All samples were taken in two replications.

The obtained filtrates of about 200 ccm were shaken for 2 hours with 30 g of Amberlit IRC resin in a H^+ form (Devaux et al. 1970). After the filtrate was decanted, the resin was washed with a small amount of redistilled water and again shaken for 2 hours with 50 ccm of 2 N ammonia. Then, ammonia was evaporated at 45—50°C. This was repeated twice, several cubic centimetres of redistilled water being added to the evaporated residue. The obtained sample was suitably diluted and used for the quantitative determination of a-amino nitrogen by Moore and Stein's method (1954) and for the qualitative analysis of amino acid composition using thin-layer chromatography (Opieńska-Blauth et al. 1967).

For determining the qualitative composition of free amino acids the sample was dissolved in 1 ccm of redistilled water. Then it was put in 0.005, 0.015 and 0.020 ccm on cellulose covered plates. Standard amino acids (0.05 M) in the amount of 0.001 to 0.003 ccm were also placed on the plates. MN cellulose was used as absorbent, a mixture of butanol, acetate acid and redistilled water in the 4 : 1 : 1 V/V/V ratio being used as the mobile phase.

Each chromatogram was developed twice and then, after drying, it was detected with a mixture of ninhydrine in methanol with an admixture of collidine in the ratio of 300 mg : 95 ccm : 5 ccm.

3. Results

At different stages of the logarithmic growth of cell number (exponential phase) considerable amounts of free amino acids accumulated in the media of nearly all cultures.

In the green algae *Dictyosphaerium pulchellum* (fig. 1A Di) and *Volvox aureus* (fig. 1A Vo) and in blue-green algae *Phormidium favosum* and *Aphanizomenon flos aquae* (fig. 1B, Aph, Ph) the logarithmic growth of cell number began simultaneously with the parallel release of great amounts of free amino acids. The simultaneity of the increasing concentration of these substances in the culture and of the growth of cell number was particularly pronounced in experiments with *Volvox aureus*. With double density of the inoculum the adaptation (the lag phase) of this alga to new worse conditions of the environment lasted twice as long as in the culture with 50% density of the inoculum (fig. 1A Vo), no cumulation of free amino acids being observed in the former culture.

However, with diatoms *Diatoma elongatum* and *Asterionella formosa*, a slight cumulation of free amino acids in the suspension was noted at the beginning of the logarithmic growth of cell number (fig. 1C As Dia). An increased concentration of the discussed compounds was not noted before the advanced growth of these algae began. *Nitzschia palea* was an exception among the investigated algae because during the entire period of intense growth of cell number (12 days) the content of free amino acids in the culture of this alga did not increase (fig. 1C Ni).

Between the 12th and 14th day of culture in the steady state a distinct decrease in the concentration of free amino acids was found with nearly all algae (Table I). This did not concern the algae which were still at an earlier physiological phase, i.e., by the end of the logarithmic growth of cell number. These were *Asterionella formosa* and *Nitschia palea* (fig. 1C As Ni) and the blue-green algae *Aphanizomenon flos aquae* (fig. 1B Aph). The blue-green alga *Phormidium favosum* was the only exception, since on the 14th day of the culture it was still in the final growth phase, while the concentration of free amino acids in the culture was already slightly diminished (fig. 1B Ph).

It was found that in the third week of culture of the green algae *Dictyosphaerium pulchellum* (fig. 1A Di) and *Volvox aureus* (fig. 1A Vo), and a diatom *Diatoma elongatum* (fig. 1C Dia) considerable accumulation of free amino acids in the culture was again observed. With green algae it was already observed on the 17th day of growth of culture, still in the steady state, while in the diatom culture it took place later, on the 21st day, at initial stage of the death phase. Moreover, it was observed that unlike the green algae, the diatoms cumulated the largest amounts of free amino acids per unit of dry weight of algae in this phase and not in the

Table I. Free amino acids released by algae in mg isolecine · mg^{-1} dry weight of algae

Onset of experiment	24.VII.1973		20.VIII.1973		5.X.1973				
Test species / Controls	1.VIII	7.VIII	27.VIII	1.IX	9.X	12.X	17.X	22.X	26.X
Blue-green algae:									
Phormidium favosum (Bory) Gom.	0.309	0.057							
Aphanizomenon flos aquae (L.) Ralfs			0.277	0.362					
Diatoms:									
Nitzschia palea (Kütz.) Smith	0.016	0.014							
Asterionella formosa Hassal			0.133	0.216					
Diatoma elongatum Agardh					0.159	0.256	0.031	0.084	0.324
Green algae:									
Volvox aureus Ehr.			0.330	0.170	0.311	0.540	0.025	0.192	0.186
Dictyosphaerium pulchellum Wood					0.313	0.305	0.04	0.123	0.142

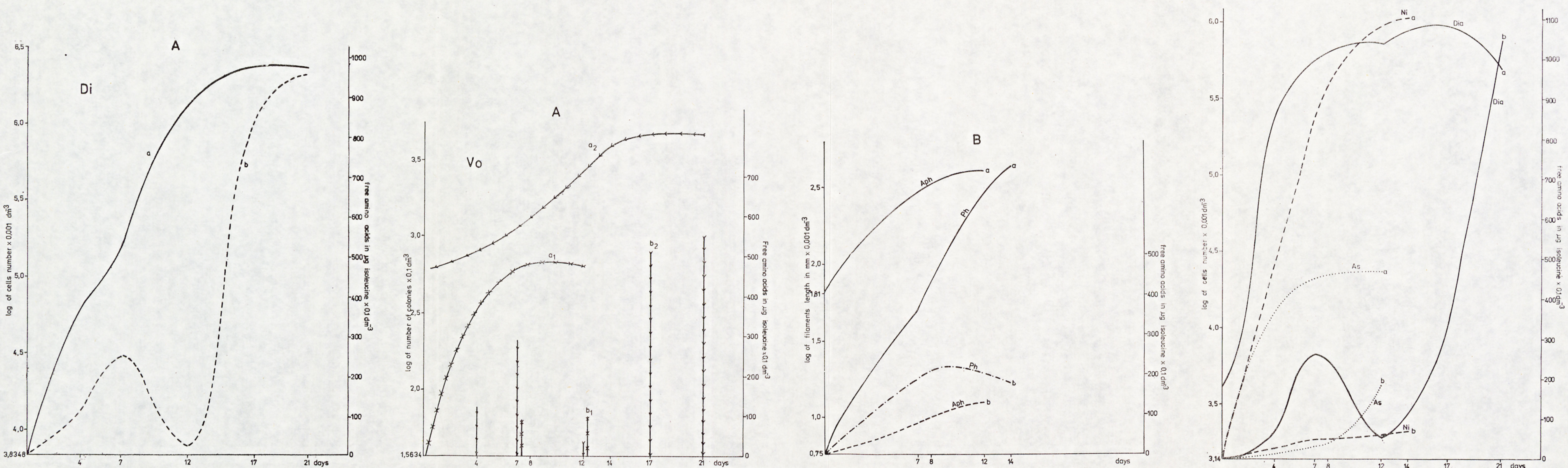

Fig. 1. Growth (a) and free amino acids content (b) in cultures of algae. A — green algae: Di — *Dictyosphaerium pulchellum*, Vo — *Volvox aureus* — single inoculum (1), double inoculum (2); B — blue-green algae: Aph — *Aphanizomenon flos aquae*, Ph — *Phormidium favosum*; C — diatoms: As — *Asterionella formosa*, Dia — *Diatoma elongatum*, Ni — *Nitzschia palea*

phase of the growth of cell number (Table I). On the 21st day of culture, the number of *Diatoma elongatum* cells was ten times larger than that of *Dictyosphaerium pulchellum* (fig. 1A Di C Dia).

Three-week cultures of *Dictyosphaerium pulchellum* and *Diatoma elongatum* were inoculated with the bacterium *Escherichia coli,* the following changes being noted after 24 hours: both in the test of *Dictyosphaerium pulchellum* + bacterium, and in the filtrate with the bacterium, the content of free amino acids was insignificantly different from the control test (fig. 2 Di Dia), while in the test with the diatom inoculated with *Escherichia coli* the content of amino acids was 2.5 × higher and in the algal filtrate (*Diatoma elongatum*) inoculated with the bacterium — 3.2 × higher than in the control not inoculated sample (fig. 2 Di Dia).

It should be stressed that in the control samples the content of free

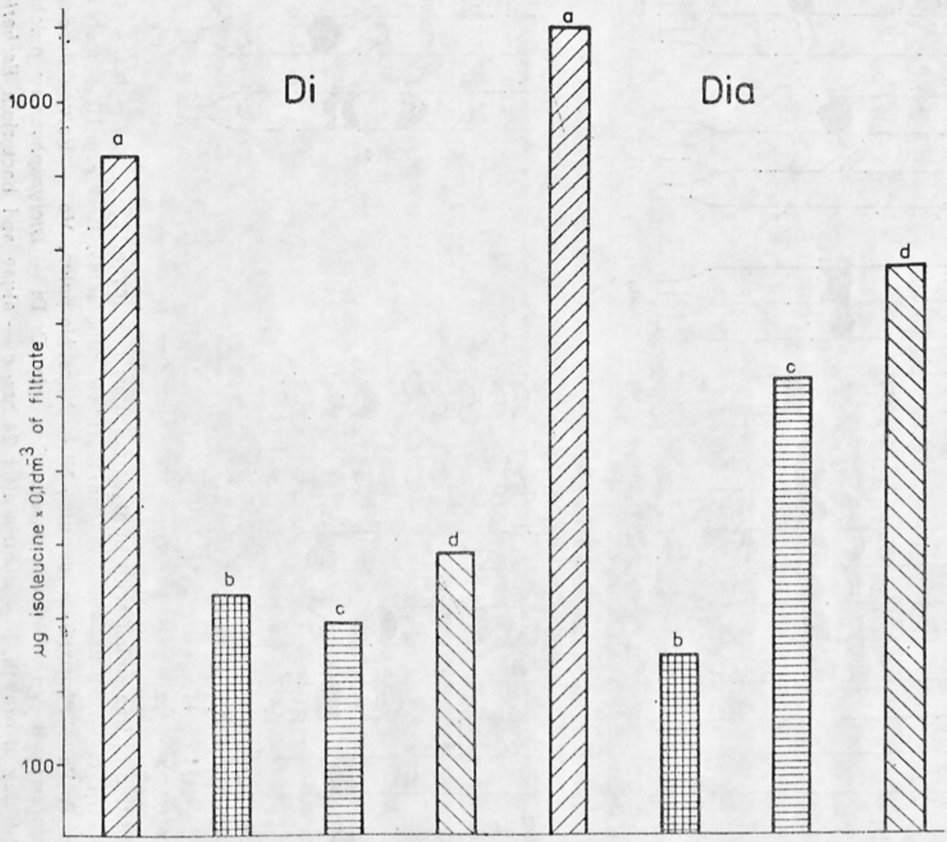

Fig. 2. Free amino acids content in algae — bacterium test: Di — *Dictyosphaerium pulchellum;* Dia — *Diatoma elongatum:* a — algae in declining phase (in 21 days of culture); b — control sample after 24 hrs; c — algae and bacterium after 24 hrs; d — filtrate of algae — culture inoculated with bacterium after 24 hrs

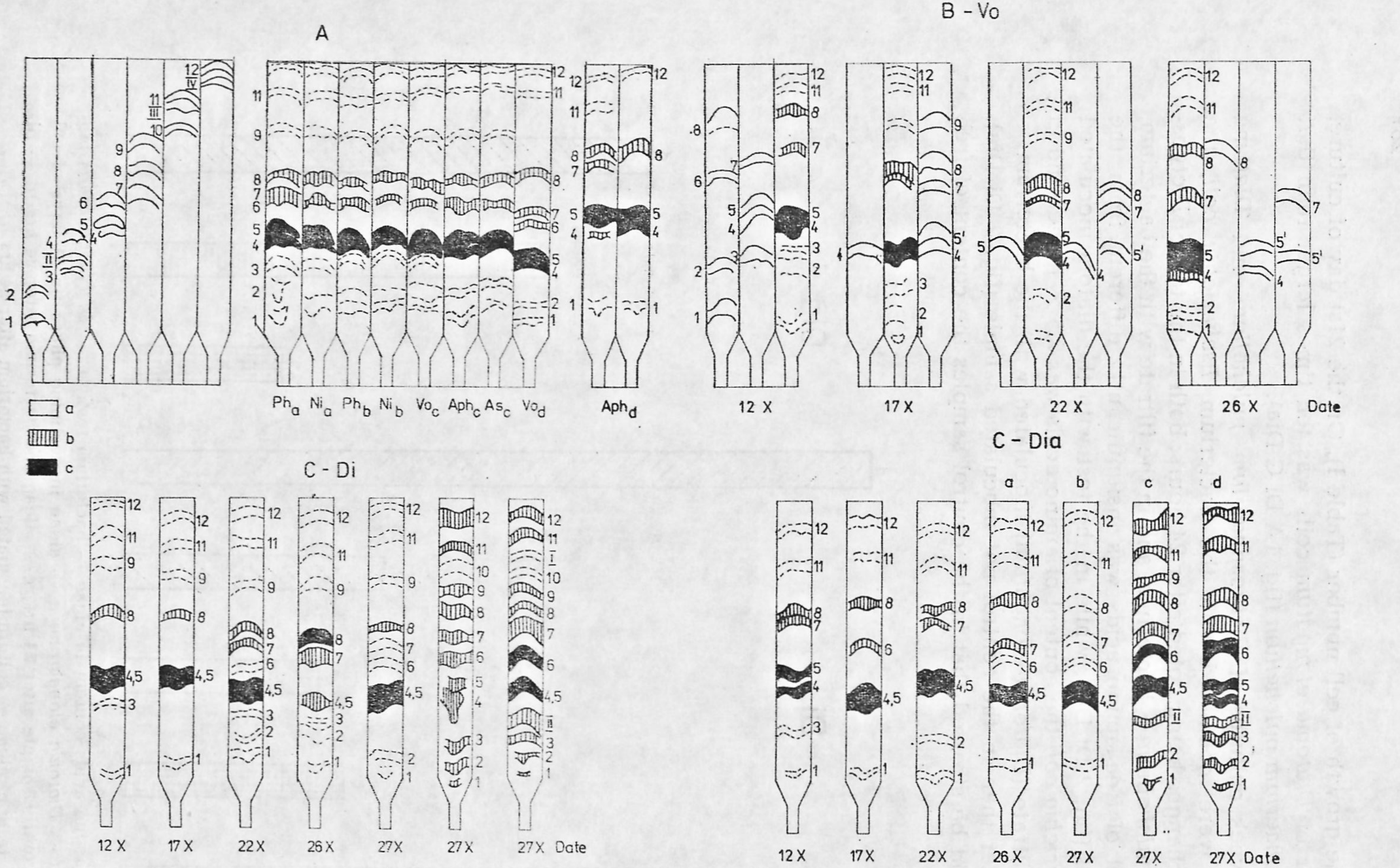

Fig. 3. Composition of free amino acids in algae cultures: 1 — $(cys)_2$, 2 — lys, 3 — his, 4 — asp, 5 — ser, gly, 6 — glu, 7 — thr, 8 — ala, 9 — tyr, 10 — trp, 11 — val, 12 — leu. I — unidentified amino acids; II — arg; III — met; IV — phe. a — traces; b — mean values; c — big quantities. A: Ph — *Phormidium favosum;* Ni — *Nitzschia palea;* Vo — *Volvox aureus;* Aph — *Aphanizomenon flos aquae;* As — *Asterionella formosa.* B: Vo — *Volvox aureus,* 5' — gly. C: Di — *Dictyosphaerium pulchellum;* Dia — *Diatoma elongatum.* a — algae culture on 21th day of growth; b — control after 24 hrs; c — algae and bacterium *Escherichia coli* after 24 hrs; d — filtrate of algae inoculated with bacterium after 24 hrs

amino acids was markedly lower after 24 hours: in the green alga culture by 0.596 mg and in the diatom culture by 0.858 mg isoleucine · 0.1 dm^{-3} of suspension (fig. 2).

The results of qualitative analyses of free amino acids in algal filtrates showed great amounts of aspartic acid and serine with glycine, medium content of alanine, and in most cases of threonine, and more rarely of glutamic acid. Apart from the above-mentioned compounds, trace amounts of the following components were also noted: cystine, lysine, histidine, tyrosine, valine, and leucine (fig. 3A B C).

In tests with *Dictyosphaerium pulchellum* and *Diatoma elongatum* inoculated with bacterium *Escherichia coli* after a 3-week period of growth, the content of free amino acids which previously appeared in very small amounts, was increased to a medium value (fig. 3C), while the amino acids whose content was large or average, remained almost unchanged. A different situation was found in the *Dictyosphaerium pulchellum — Escherichia coli* test where the high content of aspartic acid and serine with glycine was lowered to an average level after the inoculation (fig. 3C Di c d). This was revealed by the intensity of spots on chromatograms. Moreover, in the *Dictyosphaerium pulchellum* test inoculated with bacterium arginine, tryptophan, and an unidentified amino acid (fig. 3C Di c d), were noted, while in an analogical *Diatoma elongatum* test arginine appeared (fig. 3C Dia c d).

4. Discussion

4.1. Factors affecting the release of amino acids in algal cultures

It was found that the increased density of inoculum — double in *Volvox aureus* and threefold in *Diatoma elongatum* in relation to *Asterionella formosa* (according to S i e m i ń s k a 1974, the cells of these two algae are similar in size), brought about an increase in the amount of amino acids released to the medium, the increase being proportional to the number of cells (fig. 1A Vo, fig. 1C As Dia). Thus, the change in the initial density of cells did not affect the amount of extracellular free amino acids per unit of dry weight of algae. The concentration of these substances in the medium was conditioned by the specific physiology of the given organism. An identical dependence was observed by F o g g et al. (1965).

It should be stressed that, like the density of the inoculum, the size of cells which affects the surface of the whole community, was not decisive for the amount of amino acids released by algae. *Nitzschia palea* which was characterized among the investigated diatoms, by the smallest size of cells (S i e m i ń s k a 1974), released the least amount of amino acids per unit of alga dry weight, as compared with other diatoms (Table I). On the other hand, great amounts of amino acids in relation to the alga dry weight were noted in the culture of *Aphanizomenon flos aquae* (Table I) whose cells were smaller than those of *Phormidium favosum* (S t a r m a c h 1965) as well. The results obtained in *Aphanizomenon flos aquae* are in agreement with other authors' observations (N a l e w a j k o, L e a n 1972, C h r ó s t, B r z e s k a 1978) that blue-green algae release considerable amounts of peptides and amino acids.

4.2. The content of free amino acids in algae cultures in relation to phase of their physiological phase of growth

Organic substances released by algae in the initial phase of growth are intermediates in metabolism of their cells (F o g g 1971). As it was already mentioned, in the cultures of green algae *Dictyosphaerium pulchellum* (fig. 1A Di) and *Volvox aureus* (fig. 1A Vo), and in blue-green algae *Phormidium favosum* and *Aphanizomenon flos aquae* (fig. 1b), the phase of the logarithmic growth of cell number began when the content of amino acids in the media was considerably increased.

An analogical dependence of an increase in the numbers of the green alga *Chlorella* upon the content of organic substances was found by N a l e w a j k o (quoted after F o g g 1966). In that case glycollate added to the medium shortened the adaptation phase (the log phase) and increased the division of algal cells. According to F o g g (1971), the growth of cultures occurs when the quasi-equilibrium between intra- and extracellular metabolites has been reached.

With regard to the concentration of free amino acids in log phase of diatoms, and particularly of *Diatoma elongatum* and *Nitzschia palea*, was different. As mentioned above, the logarithmic growth of cell number began when the excretion of these metabolites was not increased yet.

The obtained results of green algae and diatoms correspond to test investigations on the effect of municipal sewage on rivers (B o m b ó w n a et al. 1978). It was found that green algae grown in natural water polluted with municipal sewage containing large amounts of organic matter, grew much faster than diatoms. The latter group developed most rapidly in natural water without organic components.

Among the investigated algae, *Nitzschia palea* presented an extreme example of the wide ratio of cell number to free amino acids (fig. 1 CNi). The constantly low level of these compounds which occurred simultaneously with the very intense growth of cell number seemed to support the opinion that the development of diatoms did not depend upon the content of organic matter in the environment. The published date concerning this diatom did not agree with the above hypothesis. Huber-Pestalozzi (1938) reported that this alga was frequently found in the mucilaginous envelope of *Microcystic*. Täuscher's (1980) observations also showed that the diatoms excellently developed in *Microcystis* environment. It seems that this blue-green alga releases some amounts of free amino acids, similarly as *Aphanizomenon flos aquae* in present experiments. This alga was found to release relatively great amounts of amino acids per unit of dry weight (Table I). On the other hand, Turoboyski (1970) claimed that *Nitzschia palea* preferred media of rich organic matter content. These controversial findings show that with regard to the content of organic matter in the environment, the investigated diatom can grow under extreme conditions.

As it was described in chapter 3, the next stage, between the 12th and 14th day of growth, was characterized by a considerable decrease in the concentration of free amino acids in most cultures (fig. 1A Di Vo_2, fig 1B Ph, fig. 1C Dia). Then it was probably brought about by the development of bacteria, this being supported by other authors. In the culture of *Scenedesmus quadricauda* inoculated with bacteria from a lake, an increase in the consumption of extracellular protein to 89% was noted by Steinberg (1977) on the 14th day of growth. In the culture of *Anabaena cylindrica* a certain decrease of dissolved organic matter was noted between the 8th—12th day of growth (Chróst, Brzeska 1978) while the total number of bacteria cells simultaneously increased. Also an indirect evidence of the role of bacteria in reducing organic matter just in this stage of growth was given by the results of Pimenova et al. (1970), as quoted in chapter 1.

According to some authors, the rate of organic matter release by the algae is intensified as these organisms grow older (Nalewajko, Lean 1972). The present results showed that the content of these compounds (free amino acids) resulted not only from the metabolism of cells but also from their autolysis. This phenomenon was manifested by a decrease in cell number during the 3rd week of culture, simultaneously suggesting the ageing of cultures. It should be stressed that contrary to green algae *Dictyosphaerium pulchellum* and *Volvox aureus*, the diatom *Diatoma elongatum* released a fivefold amount of amino acids per 1 cell just at that time and not in the log phase. However, in the medium of *Volvox aureus*, the content of free amino acids per 1 colony was only 1.5 × greater in the log phase than in the 3rd week of growth. Actually, it was many times greater, if Prescott's assumption (1951) that the number of cells increases as the colony grows older, is taken into consideration. In the discussed case, this is also supported by the number of *Volvox aureus* colonies which was maintained at the same level in the 3rd week of culture (fig. 1A Vo_2).

4.3. The effect of bacterium *Escherichia coli* on the content of free amino acids in *Dictyosphaerium pulchellum* and *Diatoma elongatum* cultures

In control cultures, 24 hours after the *Dictyosphaerium pulchellum* and *Diatoma elongatum* cultures were transferred to flasks, a decrease in the concentration of free amino acids was observed. It amounted to 64% in the medium of the green alga and to 74% in that of the diatom (fig. 2 Di Dia). The aeration of cultures, bringing about desamination, resulted in the loss of these compounds. The effect of oxygen on protein decomposition was also found in the investigations of Krause (1964) and Rjabov et al. (1974).

The inoculation test with *Dictyosphaerium pulchellum* culture and bacterium *Escherichia coli* showed that after 24 hours the amount of free amino acids in the control (without bacterium inoculation) did not significantly differ from their content in the alga-bacterium and algae filtrate-bacterium tests (fig. 2 Di). In this case the inoculation did not cause an increased excretion by the algae or a release of greater amounts of amino acids by way of autolysis. On the other hand, after the alga *Diatoma elongatum* was inoculated with the bacterium *Escherichia coli,* the content of free amino acids was a few times increased both in the mixture of algae and bacteria and in the alga filtrate inoculated with bacterium (fig. 2 Dia), as compared to the control.

Twenty-four hours after the algae cultures were inoculated with bacteria, Meffert and Zimmerman-Telschov (1979) observed an increase in the extracellular protein content without an increase in the bacteria number. At the next stage, also after a 24-hours period, the number of bacteria increased while the protein content was eliminated. An analogical situation manifested by a marked increase in the concentration of free amino acids, was noted in the diatom + bacterium and diatom filtrate + bacterium tests.

Contrary to *Diatoma elongatum*, the green alga *Dictyosphaerium pulchellum* inoculated with *Escherichia coli* did not release increased amounts of amino acids to the medium and in the 3rd week of growth when the accompanying bacteria developed, the number of cells was insignificantly decreased as it was already said above, this probably suggesting lower sensibility of green algae to environmental factors, i.e., in this case to bacteria (Starmach et al. 1976). It should be mentioned here that a substance called chlorelina was isolated from *Chlorella* cultures by Pratt et al. (quoted after Starmach 1963). The substance was found to have an antibiotic effect also on the bacterium *Escherichia coli.* The results of the test probably show that similarly as *Chlorella,* the green alga *Dictyosphaerium pulchellum* markedly limited the growth of the bacterium used in the test, this indicating a dependence between the development of this bacterium and a given alga environment.

The test of diatoms with bacterium differed from the green alga. In the medium of *Diatoma elongatum*, great amounts of free amino acids did not appear until the death phase, after the bacterium *Escherichia coli* was introduced. In these two cases the decomposition of algae cells probably occurred (fig. 1C Dia, fig. 2 Dia). Thus, if the development of diatoms is not accompanied by the release of great amounts of amino acids, it is possible that in natural conditions the presence of dissolved organic compounds originating from polluted environment inhibits the development of *Diatoma elongatum*. This would explain the disappearance of most diatoms under the influence of pollution (Bombówna et al. 1978).

4.4. Changes in the composition of free amino acids in algae cultures inoculated with bacterium *Escherichia coli*

As it was already said analyses of the composition of free amino acids in algae cultures showed the presence of aspartic acid, serine with glycine, alanine, and in many cases of threonine and more rarely of glutamic acid. These amino acids probably originated from the cell metabolic pool. Apart from the above-mentioned usually low-molecular amino acids a considerably lower content of cystine, lysine, histidine, tyrosine, valine and leucine (fig. 3A B C) was also noted.

An analogical composition of amino acids, particularly of those occurring in greater amounts, both in cultures and in natural conditions was found in the investigations of Brehm (1967), Paluch, Stangret (1969), Gocke (1970), Zygmuntowa (1972), and Steinberg (1977).

On the other hand, the bacterium inoculation brought about changes in the proportion of different amino acids both in green alga and diatom tests. The concentration of amino acids involved in cell metabolism did not change significantly, while that of cystine, lysine, histidine, tyrosine, valine, and leucine whose content had been lower before inoculation, grew. This was probably caused by the bacterial hydrolysis of protein (fig. 3C a b c d). Similar changes in amino acids composition in algae cultures inoculated with bacteria were observed by Steinberg (1977).

5. Conclusions

1. The accumulation of free amino acids in most algae cultures occurred in the log phase of growth of cell number and in the phase of cell death, the amount of free amino acids being characteristic of the given species.

2. In diatom cultures the largest content of free amino acids occurred in the period of cell death.

3. The release of amino acids in diatom cultures depended more on bacterial decomposition processes than on cells excretion. This is indicated by larger amounts of these compounds in the media, observed in the phase of cell death when the accompanying bacteria were developed, than after the bacterium *Escherichia coli* was introduced.

The author is much indebted to Professor Stanisław Wróbel for his valuable help in preparing the publication of the obtained results. The help of Dr. Aleksandra Starzecka in making the *Escherichia coli* cultures available is very much appreciated.

6. Polish summary

Wolne aminokwasy w kulturach różnych gatunków glonów

Przeprowadzono badania laboratoryjne kultur sinic: *Aphanizomenon flos aquae*, *Phormidium favosum* (ryc. 1B), okrzemek: *Asterionella formosa*, *Diatoma elongatum*, *Nitzschia palea* (ryc. 1C) i zielenic: *Dictyosphaerium pulchellum*, *Volvox aureus* (ryc. 1A).

W próbach pobieranych co kilka dni liczono komórki glonów oraz określano ich biomasę metodą wagową. Oznaczano również ogólną koncentrację wolnych aminokwasów metodą ninhydrynową Moore'a i Steina i ich skład jakościowy metodą chromatografii cienkowarstwowej na celulozie MN.

Wykonano ponadto test, szczepiąc kultury glonów *Dictyosphaerium pulchellum* i *Diatoma elongatum* w stadium starzenia kultury (po trzech tygodniach hodowli) bakterią *Escherichia coli* (ryc. 2).

Stwierdzono akumulację tych związków w fazie logarytmicznego wzrostu liczby komórek, ich ubytek w fazie stacjonarnej i ponowne gromadzenie w stadium obumierania komórek (tabela I). Ilość uwalnianych aminokwasów w przeliczeniu na mg suchej masy glonu w poszczególnych fazach była charakterystyczna dla danego gatunku glonu (tabela I). Nie zależała ona od gęstości inokulatu, jak również od wielkości komórek.

Spośród badanych glonów, okrzemka *Nitzschia palea* wydzielała najmniej wolnych aminokwasów w stosunku do suchej masy glonu (tabela I).

W pierwszym etapie (faza wzrostu liczby komórek), wolne aminokwasy pochodziły przede wszystkim z pulli metabolicznej komórek, w drugim zaś (stadium starzenia kultury) — dodatkowo jeszcze z bakteryjnego rozkładu białka glonów po ich autolizie. Ilustrowały to chromatogramy aminokwasów z kultur glonów (ryc. 3A B C) i z ich testów z bakterią *Escherichia coli* (ryc. 3C cd).

Wzrostowi okrzemek *Diatoma elongatum* i *Nitzschia palea* (ryc. 1C Dia Ni) nie towarzyszyło w przeciwieństwie do zielenic *Dictyosphaerium pulchellum* i *Volvox aureus* (ryc. 1A Di Vo) znaczne wydzielanie wolnych aminokwasów. U tych ostatnich glonów ilość wolnych aminokwasów przypadająca na komórkę w fazie wzrostu była kilka razy większa, aniżeli w fazie obumierania komórek. W kulturach *Diatoma elongatum* w trzecim tygodniu hodowli (death phase) stwierdzono pięciokrotnie większą, aniżeli w fazie

łog koncentrację tych związków, przypadającą na jedną komórkę, przy równocześnie dziesięciokrotnie większym, aniżeli u *Dictyosphaerium pulchellum* ubytku liczby komórek. W pożywce tej okrzemki zanotowano również znaczny wzrost (w porównaniu z zielenicą) koncentracji wolnych aminokwasów po zaszczepieniu jej bakterią *Escherichia coli* (ryc. 2 Dị Dia c d). Wskazywałoby to, że badana okrzemka jest bardziej od zielenicy podatna na działanie bakterii zarówno towarzyszących (w trzecim tygodniu hodowli), jak i wprowadzonych do pożywki.

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