Tiscia (Szeged) Vol. XVIII, pp. 23—31 (1983)

MICROCYSTIS TYPE PLANOCOCCUS STATE OF ANABAENA IN THE TRANSITORILY ALKALINIZED TISZA RIVER

I. KISS

Department of Botany, Attila József University, Szeged
(Received June 25, 1981)

Abstract

During mass productions of *Anabaena spiroides* — owing mainly to anaerobic conditions and the accumulation of metabolic products — the trichomes broke up into so-called planococcus cells. The clumps of these cells were very similar to the colonies of *Microcystis*. The trichomes could also be induced to break up under experimental conditions. Such examinations were performed by author earlier, too, in the case of *Spirulina platensis*. He also observed such phenomena in connection with *Aphanizomenon* and *Oscillatoria*. These phenomena may have important bearings in the field of taxonomy, ecology and physiology.

Introduction

In standing waters I have observed on several occasions the breaking up of the trichomes of the species of *Anabaena* or their hormogoniums into independent cells. These cells remained together in the mucilaginous envelope of the trichomes, propagated there, and the clumps of them were very remindful of the thallus formations of varying shape of the genus *Microcystis*. If the origin of these cell clumps were unknown, we might easily take them for *Microcystis* thalli and this would cause trouble in their determination. I have raised this question also earlier, in the course of the investigations performed for a longer period on the trichomes of *Spirulina platensis* (NORDST.) GEITLER during the mass growth of this species (KISS 1957). It was also observed in connection with species of *Aphanizomenon* and *Oscillatoria*. Under unfavourable conditions this phenomenon is not infrequent.

This question deserves our attention also because the real existence of certain species belonging to genus *Microcystis* have been called in question by several authors. HUBER—PESTALOZZI characterized this genus as follows: "Eine systematisch sehr schwierige Gattung, da die Abtrennung gegenüber den Nachbargruppen *Aphanocapsa* und *Aphanothece* unscharf ist", and further: "Aber innerhalb der Gattung ist wiederum die Abgrenzung der Arten voneinander ebenso schwierig wie die Umgränzung der Gattung überhaupt". — "Die Schwierigkeit liegt darin, dass Merkmale, welche für eine Art charakteristisch sein sollten, auch als Stadien anderer Arten auftreten: auf diese Weise gibt es zahlreiche Übergänge und Zwischenformen" (GEITLER) — ("It is a very difficult genus systematically, for its uncertain separation from related groups *Aphanocapsa* and *Aphanothece*". — "But inside the genus the

separation of species from one another is also as difficult as the delimitation of the genus itself" — "The source of the difficulty is to be found in that features to be regarded as characteristic of one species appear also as stadiums of other ones and in this way numerous transitions in form occur" —). According to GEITLER and HUBER—PESTALOZZI *Microcystis aeruginosa* is probably identical with *M. flos-aquae*. HUBER—PESTALOZZI does even disbelieve the existence of *Microcystis scripta, M. ochracea* and *M. pseudofilamentosa* (GEITLER 1925, HUBER—PESTALOZZI 1938). In the form group of *Microcystis aeruginosa* STARMACH separated the f. *aeruginosa* (f. typica) ELENKIN and f. *flos-aquae* (WITTR.) ELENKIN (STARMACH 1966). FELFÖLDY (1972) claims that *Microcystis aeruginosa* and *M. flos-aquae* are independent species.

Our objective cannot be to negate the existence of the genus *Microcystis,* nevertheless it should also be emphasized that in this regard examinations on the formation and further fate of planococcus cells seem to be of key importance. In the judgement of the morphological characteristics of the single species ecological physiological methods should also be considered and the use of genetical methods is also urgent. Further experiments must be performed to elucidate as to which environmental conditions are necessary for the planococcus cells developing from trichomes to produce new trichomes. Namely, this has not been observed yet either under natural conditions or in laboratory cultures. In spite of that it is likely that every planococcus cell is able to produce a new trichome. In the following the planococcus formation of Anabaena spiroides Kleb. observed in the backwater of the Tisza river at town Csongrád will be reported.

Materials and Methods

The breaking up of the trichomes of *Anabaena spiroides* into planococcus cells took place in most cases in periods of lasting mass production. Such a mass production had been observed before in sodaic lakes (e.g. Fehér lake at Szeged) and in four backwaters of the Tisza river (Csongrád, Cibakháza, Rakamaz, Tiszaluc). Here the examinations performed in Csongrád backwater will be presented, since the algal flora of this water body was studied also in the period 1976—79, and this phenomenon could be observed there on several occasions. The transitory transformation of the water into alkaline (sodaic) one is likely to have also played a role in the breaking up of trichomes into planococcus cells, since in this time this phenomenon was generally observable in waters of 8—8.5 pH. The cause of alkalinization is due to the circumstance that the zone of sodaic soils between the Danube and the Tisza extends as far as here. Mass production of *Anabaena spiroides* lasted here for several months during summer 1978, and the surface of the blueish-green water layer of some cm thickness was covered by floating sausage-shaped algal clots. These were 1—2 cm in length and 0.5—1 cm in thickness, and in their inside parts the trichomes were tightly pressed together. Due to the unfavourable conditions of life, large-scale breaking up of trichomes into planococcus cells was observable.

Samples of mass production taken from various places were examined in living and fixed condition. For the fixation of samples 2—3% formaldehyde in water proded to be the best preservative. Experiments of culturing were performed in the laboratory both with clumped and nonclumped parts of the living material. The nonclumped sample of mass production was filtered to remove the planococcus cells from among the trichomes. This could ve accomplished only in part. The further fate of the sausage-shaped living bioseston clumps was studied so that one part of them was left unchanged in clumps of different sizes, the other portion was separated to constituent parts as much as possible and in the course of that care was taken to produce as little pressure as possible on the clumps. Live preparations were made from this material and the breaking up of trichomes into planococcus cells was examined at intervals. The forms of the living preparations were the following: 1. Aerobic preparations in Petri dishes, 2. Hermetically sealed material in glass tubes, 3. Preparations on excavated slide with air bubble (a greater or smaller air bubble was left over the material placed into the excavated slide before the sealing of its cover with wax), 4. Anaerobic preparation on nonexcavated slide without air bubble and sealed with wax.

Results

The enormous mass production extending over the whole area of the backwater during summer and autumn 1978 was inspected on four occasions: July 23, August 4, September 3 and October 24. Superficial and underwater bioseston samples were collected from various places in the littoral and open water. The samples showed that Anabaena spiroides was most variable morphologically and in regard of size. The number of the convolutions of the trichomes coiled in a spiral fashion varied between 2 and 10. The width of the convolution of the type form of the species was 40—50 μm, the lead of the convolutions of it the same of less. The cells were sometimes spherical, ranging from 7 to 9 μm in diameter, their width being generally greater than the length of cells. The cells always contained gas vacuoles, in summer in greater numbers, causing the trichomes of the bioseston to float entangled in the upper few cm thick layer of water during August and September. The heterocysts were spherical measuring 7 μm in diameter, the spores were elongated and slightly bent, 11—13 μm in width. Such a type form from an open water bioseston is seen in micrograph 1 of Table I. Trichomes with convolutions of 23—27 μm width and with lead of 18—20 μm also occurred in a minor amount. They may be ranged among var. contracta Klebahn (micrograph 2, Table I). More seldom trichomes with longitudinally compressed cells were also seen. The width of these cells were 7—8 μm, the length of them only 3—4 μm. They may have belonged into the form group of var. Talyschensis Wor.

Concerning structural condition of the mass production, the following observations could be made:

1. In the littoral, the overwhelming majority of trichomes produced sausage-shaped clumps, while in the open water the bioseston was rather made up of non-clumped, individual trichomes, exhibiting in some places syrup-like density. In this latter case, the stronger movement of water surface may have also had a role. In the open water, *Aphanizomenon flos-aquae* (L.) RALFS was also observed, but characteristic colonies of *Microcystis* were nowhere to be found.

2. In the bioseston clot, the trichomes of *Anabaena spiroides* exhibited various forms of breaking up into planococcus cells, especially from August. Inside the mucilaginous envelope of the trichomes the planococcus cells remained in groups, occasionally divided producing planococcus clumps of spiral shape. They simulated *Microcystis* colonies, and had we not known their origin, we might have mixed them up with real *Microcystis* colonies. This situation is illustrated in micrographs 4, 6, 7. The trichome seen in micrograph 6 belonged into the form group of var. *contracta* Klebahn. The breaking up into planococcus cells had just begun. The arrow points to a solitary heterocyst. Micrograph 7 shows a more advanced stage of planococcus formation of a trichome similar to the previous one. Here the cells had several cell divisions and formed spirally coiled clusters in the mucilaginous envelope. The clumps of trichomes were gradually entangled during summer to form a floating layer of 1—2 cm thickness at the water surface. Among the entangled trichome clots, however, solitary spiral trichomes still occurred and in the increasingly worsening environment they gradually broke up into individual cells. Micrograph 4 illustrates the formation of the planococcus colony. It is visible that in the mucilaginous envelope of sharp contour the cells are already arranged in rows of 4—5, and the cell clump is not only in the state of loosing its spiral character, but its division into 4—5 smaller cell clumps has also started.

3. Spherical bodies 1—2 μm in diameter, produced by the disintegration of cells into granules always occurred in the clumps consisting of planococcus cells, particularly in the spaces encircled by the entangled masses of clumped trichomes. The arrow in the upper left side of the colony of micrograph 4 points to such a disintegration. It is visible that the small granules are located in a group inside the mucilaginous envelope, showing that they are the products of the disintegration of a single cell. These cells are some 1 μm in diameter. Lower another arrow points to two small bodies which are in the process of releasing from the mucilaginous sheath. Their diameters are somewhat greater than those of the former ones: 1.5—2 μm. It was generally observed that this disintegration into granules in the trichomes or in the planococcus cells intensified with the increase of gas vacuoles in cells. The disintegration of this uniform cell structure seemed to be enhanced by strong vacuolization. The tigher the clustering of hormogoniums and planococcus cells, the more intensive will be the gaseous vacuolization and granule formation.

Experimental examinations

The objective of these examinations was to obtain more information about the ecological conditions of planococcus formation. The results of these experiments will be presented according to the four groups mentioned in Materials and Methods.

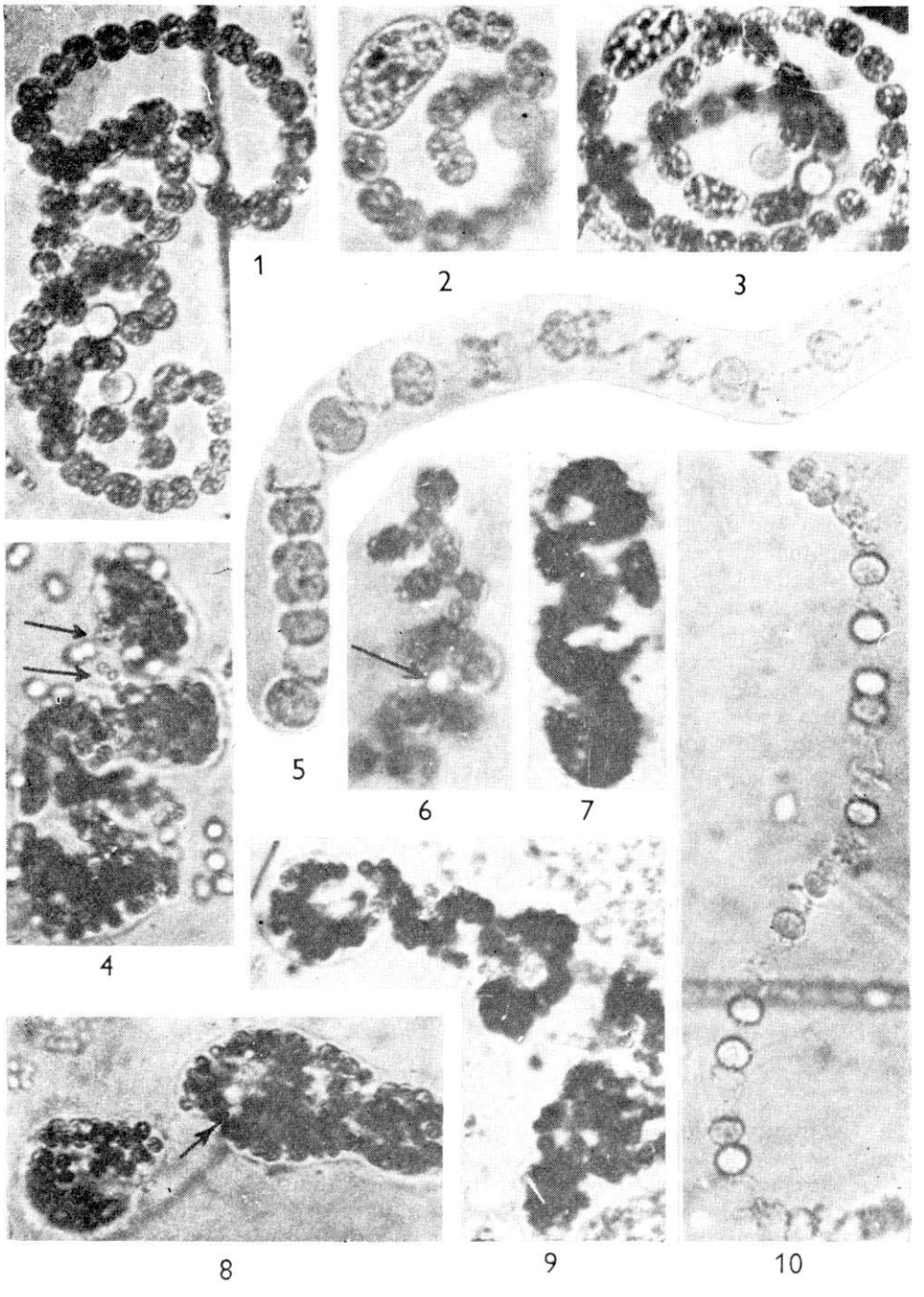
1. *Anabaena* trichomes collected from open water plankton and kept in Petri dishes under aerobic conditions seemed to remain undamaged for a longer period. E.g. micrograph 3 in Table I was taken of a material kept in a Petri dish for 3 months. The only change in that case was that the width of the convolutions of the sedimenting spiral trichomes decreased, the trichomes were nearly pressed in length into the same plain. Under such aerobic conditions the entangled masses of trichomes were less damaged, their hormogonium production and their breaking up into planococcus cells were also less intensive, and their disintegration into granules was also insignificant.

2. In glass tubes sealed air-tight, the braking up of trichomes into hormogoniums and planococcus cells was surprisingly frequent. Even the solitary trichomes

Plate I

1. Type form of *Anabaena spiroides* KLEBAHN from the bioseston of open water algal bloom, 700:1
2. *A. spiroides* var. *contracta* KLEBAHN from open water mass production, 900:1
3. Undamaged trichome of *A. spiroides* from 3-month-old aerobic culture, 700:1
4. Formation of a spiral planococcus mass by several divisions of *A. spiroides* cells in clotted bioseston, 400:1
5., 10. Some cells of *A. spiroides* broke up into small granules without the clustering of planococcus cells in sealed anaerobic culture on nonexcavated slide within three weeks. 5. = 1000:1, 10. = 500:1
6. Trichome of *A. spiroides* var. *contracta* from clotted bioseston at the beginning of its breaking up into planococcus cells, 600:1
7. An older planococcus cluster from the trichome of *A. spiroides* var. *contracta*. The cells in the cluster had divided more than once, 200:1
8. Trichomes of *A. spiroides* broke up into planococcus clusters in two months in a culture with air bubble on excavated slide and sealed, 300:1
9. The trichome mass of *A. spiroides* closed into a glass tube broke up into planococcus clusters within a few weeks in anaerobic environment. Disintegration of planococcus cells into granules in also visible, 300:1

Plate I



clustered closely together within a short time, and the single trichome spirals often piled transversally one upon the other were entangled in a net-like fashion. In such cases planococcus formation took place very soon. Such a case is shown in micrograph 9, Table I. It is seen that the breaking up of the single cells into granules has begun. If trichome masses formerly clumped together got under similar conditions, this phenomenon took place faster.

3. A similar process could be observed in preparations on excavated slides with air bubbles under the sealed cover slide. Trichomes nearer to the bubble broke up more slowly into planococcus cells and those remote from it faster. Micrograph 8 of Table I was taken of a clump more remote from the bubble, in the second month following setting in. Here the separation into smaller cell clumps is already visible. In the greater clump on the right, the clustering of trichomes took place also transversally, causing a very unequal grouping of planococcus cells. The arrow points to two light spots. These were heterocysts, which fell a little below the optical plane of the micrograph. One of them must have belonged to a transversal trichome. If clumped trichomes were placed into excavated slide, not only planococcus formation but also disintegration of cells into granules occurred.

4. In preparations on nonexcavated slides sealed without air bubble the trichomes of *Anabaena* were in the most unfavourable conditions. Here was planococcus formation and the disintegration into granules the most intensive and observed firstly. The spirals of solitary trichomes flattened, i.e. became laterally compressed and longitudinally elongated, the lead of the spirals increased, and their damages assumed extreme dimensions. Micrographs 5, 10 of Table I taken in the same time of solitary trichomes of the open water bioseston originated from preparations set in in the same time. Micrograph was taken of filtered material, micrograph 10 of an unfiltered, planococcus-containing material. The picture is the same in both cases: The disintegration of trichomes into small granules had begun before their breaking up, though 3 weeks before the setting in of preparations the trichomes were generally undamaged. Most trichomes exhibited the same picture. It is visible that the single cells resp. sections of trichomes were not the same in regard of their physiological conditions. It is likely that a so-called inequal division was also involved in that. The genetical inheritance of cells was obviously the same, but the hormonal dividedness between the young cells, the small local differences in the environmental factors produced differences also in the vitality of the single cells resp. cell groups.

Later the spiral planococcus clumps of *Anabaena spiroides* broke up into smaller colonies, or remained entagled in greater net-like, so-called "open-work" groups and having vegetated for a longer time became deceptively reminiscent of the genus *Microcystis*.

Discussion

The interpretation of the surprising morphological phenomena described in the foregoing may raise several questions. Of them two are waiting for an answer: 1. Are the clumps produced by the breaking up of trichomes viable?, 2. What induced the trichomes to break up into planococcus cells?

The first question is adressed to the future, since today we can only state both from the aspect of morphology and taxonomy that the cell clumps with mucilaginous envelope are similar to Microcystis colonies, and in a favourable medium remain undamaged for several months. The main point in this question is whether viability

and vitality mean also perfectibility. This latter begets, however, another question: Are these planococcus cells able in isolated condition to produce new trichomes? Even if we could realize this today under suitable conditions still unknown, we could not negate with complete certainty the existence and biological reality of the genus *Microcystis*. For the negation of this genus it would be necessary to grow trichomes from the isolated cells of an admittedly "real" *Microcystis* species tp be regarded as constant. As long as we fail in doing this only circumspect examination is recommended, e.g. we should avoid identifying clumps of planococcus cells as *Microcystis* colonies. In the mass productions of undamaged *Spirulina* or other Cyanophyta possessing trichomes, *Microcystis* colonies can occur, and it is also likely that with the worsening of conditions the trichomes will braek up into planococcus cells. In many cases, their clusters are indistinguishable from *Microcystis* colonies. Serological methods may perhaps be useful in the real interpretation of these formations. Of course, the possible existence of serotypes can even here cause difficulties.

It is easier to answer the second question. It was observed both in natural mass productions of *Anabaena spiroides* and in labortory cultures that the clustering into clumps of trichomes, the crowdedness resulted in the formation of planococcus cells. I have observed this previously, too, when studying the enormous mass production of *Spirulina platensis* (NORDST.) GEITLER. In that case I could establish the following: "The breaking up into spherical cells of trichomes is certainly a useful process since due to the increased plasma surface, the release of metabolic products resp. aeration can take place easier". Further "... In this case planococcus formation is not a direct process of propagation, but a transformation provoked by unfavourable conditions into a state in which the organism is still able to eyist" (KISS 1957).

This breaking up of cells was observed also earlier in the case of Spirulina platensis. The trichomes of this organism started breaking up at the beginning of the second month in the sealed slide preparations, and during the third month following setting in all trichomes broke up into planococcus cells. In the 6th month the picture changed completely, the marginal cells of the planococcus clusters were still normal, they had retained their colour, but the cells located lower than the 5th—6th cell layer became colourless, lost their cellular structure and underwent complete autolysis (KISS 1957). It seems that for the retaining of the cellular structure a certain energy level is also necessary for the cells. This was observed also with other algal organisms.

The breaking up into planococcus cells was observed also with genera *Aphanizomenon* and *Oscillatoria,* in the case of the former under even more extreme conditions. In micrograph 10 of Table I, the trichome of *Aphanizomenon* is also dimly visible under the optical plane in an environment that caused the breaking up of the Anabaena trichomes into granules. The resistance of *Anabaena* seemed to be greater.

It can be stated on the basis of results that the single algal species may undergo great changes both morphologically and structurally and therefore their identification should be based on the full knowledge of their ontogeny.

References

FELFÖLDY, L. (1972): A guide for the identification of blue greens (Cyanophyta) in Hungary (in Hungarian only). — Vízügyi Hidrobiológia *1*, Vízdok, Budapest.

GEITLER, L. (1925): Cyanophyceae. In: PASCHER, A. (edit.): Süsswasser-Flora Deutschlands, Österreichs und der Schweiz. — Gustav Fischer, Jena, Heft *12*, 1—450.

GEITLER, L. (1930): Cyanophyceae (Blaualgen). In: RABENHORST L. (edit.): Kryptogamen Flora von Deutschland, Österreich und der Schweiz. — Akad. Verl. Leipzig. 2. Auflage, Bd. *14*, 1—1196.
HORTOBÁGYI, T. (1955): Die Rolle der Gasvakuole im System der Cyanophyceae (in Hungarian). — Bot. Közlem. *46*, 25—29.
HUBER-PESTALOZZI, G. (1938): Das Phytoplankton des Süsswassers. I. Teil: Allgemeiner Teil, Blaualgen, Bakterien, Pilze. In: THIENEMANN, A. (edit): Die Binnengewässer.—Schweizerbart'sche Verl., Stuttgart 1—342.
KISS, I. (1957): Über die Planococcus-Haufen der *Spirulina platensis* und die Frage des *Microcystis*-ähnlichen Zustandes (in Hungarian). — Szegedi Ped. Főisk. Évk. *2*, 35—65.
STARMACH, K. (1966): Cyanophyta — Sinice, Glaucophyta — Glaukofity. In: STARMACH, K. (red.): Flora slodkowodna Polski. Tom. *2*, 1—807. — Inst. Bot. Polska Akad. Nauk, Warszawa.

Az Anabaena Microcystis-jellegű planococcus állapota a Tisza folyó időnkénti alkalizálódó holtágában

I. KISS

Tiszakutató Munkacsoport Szeged

Kivonat

Az *Anabaena spiroides* tömegprodukciójában főként a levegőtlenség és az anyagcseretermékek halmozódására a trichomák ún. planococcus sejtekre estek szét. Ezek halmazai nagyon hasonlítottak a Microcystis kolóniákra. A trichomák szétesését kísérletekben is elő lehet idézni. Ilyen vizsgálatokat szerző korábban a *Spirulina platensis* esetében is végzett, de ilyen jelenségeket ritkán az *Aphanizomenon* és az *Oscillatoria* körében is észlelt. E jelenségek a taxonómia és a fiziológia terén jelentősek lehetnek.

Anabaena Microcystis-u slično planococcus-no stanje u povremeno alkalnim mrtvajama reke Tise

KISS I.

Radna grupa za istraživanje reke Tise, Szeged, Hungaria

Abstrakt

Pri masovnoj produkciji *Anabaena spiroides*, uglavnom usled anaerobnosti i nagomolavanja produkata metabolizma, trihome se raspadaju na tz. planococcus ćelije. Njihove su grupacije veoma slične Microcystis kolonijama. Raspadanje trihoma moguće je izvesti i eksperimentalnim putem. Autor je ranije vršio ovakve opite u slučaju *Spirulina platensis*, a slične pojave je redje primetio i na *Aphanizomenon*-u i *Oscillatoria*-ma. Ove pojave mogu biti od značaja za taxonomiju i oblasti fiziologije.

ХАРАКТЕРНОЕ СОСТОЯНИЕ В ПЕРИОДИЧЕСКИ ЩЕЛОЧИЗИРОВАННОЙ СТАРИЦЕ РЕКИ ТИСЫ

И. Кишш
Рабочая группа изучения Тисы, Сегед, ВНР

Резюме

Anabaena spiroides в своей массовой продукции, при недостатке воздуха и накопления продуктов обмена веществ распадается на трихомы — клетки planococcus.

Эти накопления очень похожие на колонии *Microcystis*.

Распад трихомов можно вызвать искусственно — с помощью опытов. Такие опыты автор проводил и раньше с *Spirulina pratensis*. Иногда Можно наблюдать такие явления также в среде *Aphanizomenon* и *Osscillatoria*. Все эти явления могут иметь важное значение в области таксономии и физиологии.