SENSITIVITY TO FUEL DIESEL OIL AND CELL WALL STRUCTURE OF SOME *SCENEDESMUS* (*CHLOROCOCCALES*) STRAINS

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ABSTRACT

Sensitivity of three *Scenedesmus* strains exposed to aqueous fuel-oil extract (AFOE) is strongly strain-dependent: *S. quadricauda* is the most resistant, *S. armatus* moderately tolerant whereas the most sensitive appears to be *S. microspina*. The sensitivity of tested species increases parallel with decreasing of cell size and cell number in coenobium. The values of the cell surface/cell volumes ratios only partly explain the above relationships. Electron microscope investigations reveal that the sensitivity may depend on cell wall structure of the strains. Cell wall of all here investigated strains is built of two layers: the inner so-called cellulosic layer and the outer one showing a three-laminar structure (TLS). The latter contains an acetolysis-resistant biopolymer (ARB). These two layers are similar in thickness in the three strains tested, but the surface of *Scenedesmus* is covered with various epistructures that are characteristic of strains. Some of them as the tightly fitting warty layer of *S. armatus* and especially the loosely fitting reticulate layer of *S. quadricauda* may contribute to lower permeability of cell wall. The structure of the rosettes also appears to be correlated with the sensitivity of strains. Presence of invaginations of plasmalemma in areas under rosettes indicates their role in transport processes inside/outside the cells.

KEY WORDS: *Scenedesmus*, growth, morphology, cell wall structure, sensitivity, fuel oil.

INTRODUCTION

The variations in sensitivity of different microalgal groups to oil pollutants in conventional tests are widely documented (Batterton et al. 1978, Hilmer and Bate 1983). It seems to be obvious phenomenon because microalgae differ markedly in phylogeny, ecology, morphology or pattern of reproduction. Fisher et al. (1973) for instance have found that clones of three species of diatoms isolated from ocean waters were more sensitive to PCBs than estuarine clones. It is also suggestion of Van Baalen and O'Donnell (1984) that psychrophilic algae are more sensitive to petroleum pollution than mesophilic algae. On the other hand, there is lack of physiological, biochemical or structural explanations for species-dependent sensitivity of algae belonging to the same genus (Kauss and Hutchinson 1975), or clones of the same species. (Mahoney and Haskin 1980, Østgaard et al. 1984).

Results obtained previously indicate the great diversity in growth susceptibility of *Scenedesmus* species to aqueous fuel oil extract (Tukaj 1994). Hydrocarbons effect on algae is probably related to their concentration inside the cell. It seems that organisms able to maintain this concentration at a low level should be less affected. The cell wall is the first, at least mechanical barrier for oil hydrocarbons penetrating the cell. Thus the morphological structure of the cell wall and its composition may be related to the tolerance of algae to oil hydrocarbons. Such opinion is supported by Karydis and Fogg (1980) who suggest the protective role of diatom's silica cell wall against crude oil hydrocarbons. However there exist essential differences between the cell wall structure and composition of *Scenedesmus* belonging to green algae and diatoms. On the other hand, many reports indicate that cell wall of Scenedesmus is not only unusually complex in structure and composition moreover it depends on the strain (Bisalputra and Weier 1963, Staehelin and Pickett-Heaps 1975, Hegewald 1978, Burczyk et al. 1981, Kalina et al. 1993). Therefore it seems that the structural character of cell wall may be an important factor determining the differences in phytotoxicity among *Scenedesmus* strains.

The aim of this paper was to test differences in morphology and cell wall structure of three *Scenedesmus* strains and their sensitivity to fuel oil. The cell wall structure was analysed by transmission electron microscopy (TEM). Beside the standard TEM procedure, the whole cells were subjected to acetolysis or treated with NaOH before ultrastructural examination. We tried to correlate the pattern of epistructures with resistance of algae to fuel oil. Previous observations indicated that only inner layer of the cell wall of *Scenedesmus armatus* was thickened after AFOE treatment (Tukaj 1989), without additional alternation noticeable under electron microscope. Thus, the

cell wall micrographs of the strains treated by AFOE were omitted in this work although recently Burczyk et al. (1994) found that hexane, cyclohexane and benzene show influence on cell wall composition stimulating the accumulation of cell wall carotenoids in *Scenedesmus obliquus*.

MATERIALS AND METHODS

Organisms

Three *Scenedesmus* strains were used in the experiments. These were: *S. armatus* and *S. microspina* isolated from southern Baltic Sea water at the Institute of Oceanology, Sopot (Poland). *S. quadricauda* strain G-15 was obtained from the Institute of Botany Collection in Třeboň (Czech Republic).

Cultures and growth measurements

The algal strains were maintained on the agar (2%) slants containing BBM culture medium (Nichols and Bold 1965) enriched with 1% bactopeptone "Difco" and 2% glucose. Algae before the experiments were transferred into liquid BBM culture medium. Pre cultures of 5-7 day-old algal cells were used to set up batch cultures. Algae in both cultures were grown in 100 cm^3 E-flasks, each containing 50 cm^3 of suspension. The flasks stoppered with bacteriological plugs were shaken manually twice a day.

Pre cultures and batch cultures of all strains were maintained at the same growth conditions: 22-24°C, a 14:10 light-dark cycle of fluorescence illumination providing a photon flux density of 50 $\mu mol*m^{-2}*s^{-1}$.

The density of cultures was determined using a Fuchs-Rosenthal hemocytometer. Cell surface and volumes were calculated using techniques and formulas described by Edler (1979) assuming the circular cross-section of all organisms and cylinder shape for *S. quadricauda* and rotational ellipsoid for *S. armatus* and *S. microspina*.

Preparation of oil for tests

No. 2 diesel fuel oil (Polish Norm: PN-67, C-96048) obtained from Gdańsk Refinery was used in this work. The oil sample (50 cm^3) was vigorously stirred for 20h with a culture medium (1 dm^3). The extraction was curried out at room temperature in a vessel tightly closed with a Teflon plug. The extraction was curried out at room temperature in a vessel tightly closed with a Teflon plug. The mixture was transferred to a separating funnel for 4h. The lower aqueous phase (AFOE), containing dissolved hydrocarbons and a small amount of dispersed oil, was used in experiments. Qualitative characterisation of AFOE will be found elsewhere (Tukaj 1987). Total fuel-oil hydrocarbons in the AFOE (carbon tetrachloride extractable oil) were determined by the infrared spectrophotometer method (Gruenfeld 1975). The final concentration of hydrocarbons in culture medium containing AFOE measured by the above method was 49.8 ± 9.1 ppm (n=5).

Transmission electron microscopy (TEM)

The 7-day cultures were harvested by centrifugation, rinsed with BBM medium and fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.0, for 24 h at room temperature. They were next washed and post-fixed for 1.5 h in the same buffer containing 1% OsO_4.

The material dehydrated in acetone was embedded in Spurr's low-viscosity resin (Spurr 1969). Ultrathin sections were stained with uranyl acetate and lead citrate. Observations were carried out with a BS 500 microscope (Tesla).

Aceto- and alkalolysis

The acetolysis procedure was essentially that of Atkinson et al. (1972). The algae were collected by centrifugation (10 min * 1000g), and the pellets were several times washed with fresh BBM medium. The last pellet was subjected to acetolysis in small vials capped with Teflon plugs. A large excess of acetic anhydride/conc. sulphuric acid (9:1, v/v) was added to each vial.

After addition of reagent, the pellet was resuspended in the mixture and maintained at 95°C for 1h. After cooling and carefully mixing with water, the remaining material was pelleted by centrifugation and washed several times with water.

A small drop of this material was placed on the form grids and allowed to settle for a few min. Dried material was directly examined with electron microscope.

Another specimen of pellet was treated with 6N NaOH for 1h, at 95°C (alkalolysis). The remaining material, many times washed with water, was directly reviewed in electron microscope.

RESULTS

Changes in population densities of three *Scenedesmus* strains grown in the presence of aqueous fuel-oil extract (AFOE) are shown in Table 1.

The results indicate a broad range of sensitivity to AFOE. *S. quadricauda* appeared to be the most resistant strain. Cell growth of this strain was after exposition to AFOE reduced only about 10% in comparison to control, regardless the time of culture. *S. armatus* showed moderate tolerance to AFOE. The growth of treated cells in this case was reduced to 60% of control on the 3rd day and maintained constant to the end of culture. It means that after 3 days normal cell divisions we-

TABLE 1. Growth of three strains of *Scenedesmus* in BBM medium without (Control) and with aqueous fuel-oil extract (AFOE). Initial cell density was $1*10^4$ cells per 1 cm^3 of BBM medium. The algae were grown at 22-24°C, under light intensity 50 $\mu mol*m^{-2}*s^{-1}$ and light/dark photoperiod (14/10).

Days of culture	Number of cells in 1 cm^3 of suspension ($*10^4$)					
	S. microspina		*S. armatus*		*S. quadricauda*	
	Control	AFOE	Control	AFOE	Control	AFOE
1	1.2 (0.1)	1.0 (0.1)	1.6 (0.1)	1.4 (0.1)	1.7 (0.1)	1.5 (0.1)
3	2.6 (0.3)	1.6 (0.2)	10.5 (0.6)	6.3 (0.4)	6.0 (0.3)	5.6 (0.2)
7	46.6 (2.0)	6.9 (0.4)	95.7 (5.8)	57.1 (2.6)	28.0 (2.0)	24.8 (1.9)
14	150.4 (7.8)	9.4 (0.5)	251.1 (11.5)	152.4 (7.6)	140.5 (9.9)	129.8 (6.7)

Values in Table are the mean of three separate experiments; each, at least in three repetitions. In parentheses are given the standard errors of the mean.

re apparently resumed and growth rates were the same in the control and AFOE variants. *S. microspina* was found to be the most sensitive. Its growth was gradually reduced by AFOE resulting in 94% inhibition on the 14th day of culture. During this period the inoculum multiplied 150 times in control whereas in presence of AFOE only 9 times.

Results in Table 2 indicate that there exists a correlation between sensitivity of *Scenedesmus* to AFOE and cell size. *S. microspina* characterised by the smallest size of cells showed the highest sensitivity. Middle in size and sensitivity to AFOE was *S. armatus* whereas the biggest *S. quadricauda* was simultaneously the most resistant organism. However, the presented findings do not show a clear relationship between sensitivity of strains and the cell surface/cell volume (S/V) ratio. Values of this ratio to certain extent may be recognised as bioadsorption parameter. On this assumption the most sensitive organism should have the highest values of the ratios. This is the case of *S. microspina* cells as compared with the remaining strains, but the values of ratios for *S. armatus* and *S. quadricauda* are similar although both organisms markedly differ in the sensitivity to AFOE.

The cell wall of each investigated *Scenedesmus* strains consists of two layers. The inner, which is characterised by low electron density has been reported to contain microfibrillar component (Staehelin and Pickett-Heaps 1975). Figs 15-17 show that in the strains investigated in present study these layers are similar in thickness. The "cellulosic" layer is directly attached to the thin three-laminar structure (TLS) built of acetolysis-resistant biopolymer (ARB) (Atkinson et al. 1972, Burczyk and Dworzański 1988, Kalina et al. 1993). Each cell of *Scenedesmus* is surrounded by both mentioned layers. However, only some of *Scenedesmus* strains contain the so-called "epistructural layer", which is quite diverse in structure and composition (Komárek and Ludvík 1972, Hegewald 1989, Hegewald and Schnepf 1991).

The common features in epistructural ornamentation of strains studied are rosettes and spines of terminal cells. Spines of three strains are formed by the groups of close packed spikelets (Fig. 5) that cross-sectioned are rounded and empty (Fig. 2). They are usually crooked and tapering at the top. Basic structure of rosette in three strains is similar: it is ring-shaped in cross-section enclosing small mushroom-shaped structures, which are arranged in a few whorls (Figs 8, 11). The cell wall below rosettes is thickened, but loosely organised. The neighbouring plasmalemma frequently forms invaginations (Figs 13, 18, 19). Usually each cell contains a few rosettes situated near terminal spines as well as at the end of cell. There is a distinct relationship between the structure complexity of rosettes among the strains and their sensitivity to AFOE. The most simple and "open" form of rosettes seems to be the rosettes of *S. microspina* (Fig. 18). In contrast, the most complex structure and "close" form represents those of *S. quadricauda* (Fig. 20). Rosettes are usually situated in the places of marked hillocks of the "cellulosic" wall. Other epistructural elements as: warts, combs, props or spikelets appear to be strain-dependent.

The surface of *S. microspina* is the poorest in elements among the tested strains. Each coenobium is surrounded only by a three-dimensional structure resembling empty honeycomb (Figs 1, 3, 4). The cross-section of this structure is a network like of hexagonal meshes (Fig. 2). The meshes are formed of the top parts of small and thin spikelets, upraising from the cell surface.

Combs and warty layer are typical for *S. armatus*. The combs are formed by small tubular spikelets attached each other and arrayed in rows (Figs 6, 7). They run in a zigzag along each cell, parallel to its longitudinal axis (Fig. 8). The warty layer covers the coenobium contrary to TLS that bounds each cell of coenobium (Fig. 9). Thus the structure of warty layer could be especially seen in areas where the adjacent cells are joined. Skin-like basis of this layer covers subtle globular material being best seen at tangential sections to this places (Fig. 8). There are two types of warts:
a) single warts in bridging areas and whole cell, b) warts aggregated into large clumps overlaying the surface of each cell except bridging areas. The warts of both types circular in cross-section are partly empty near the ground and close on the top.

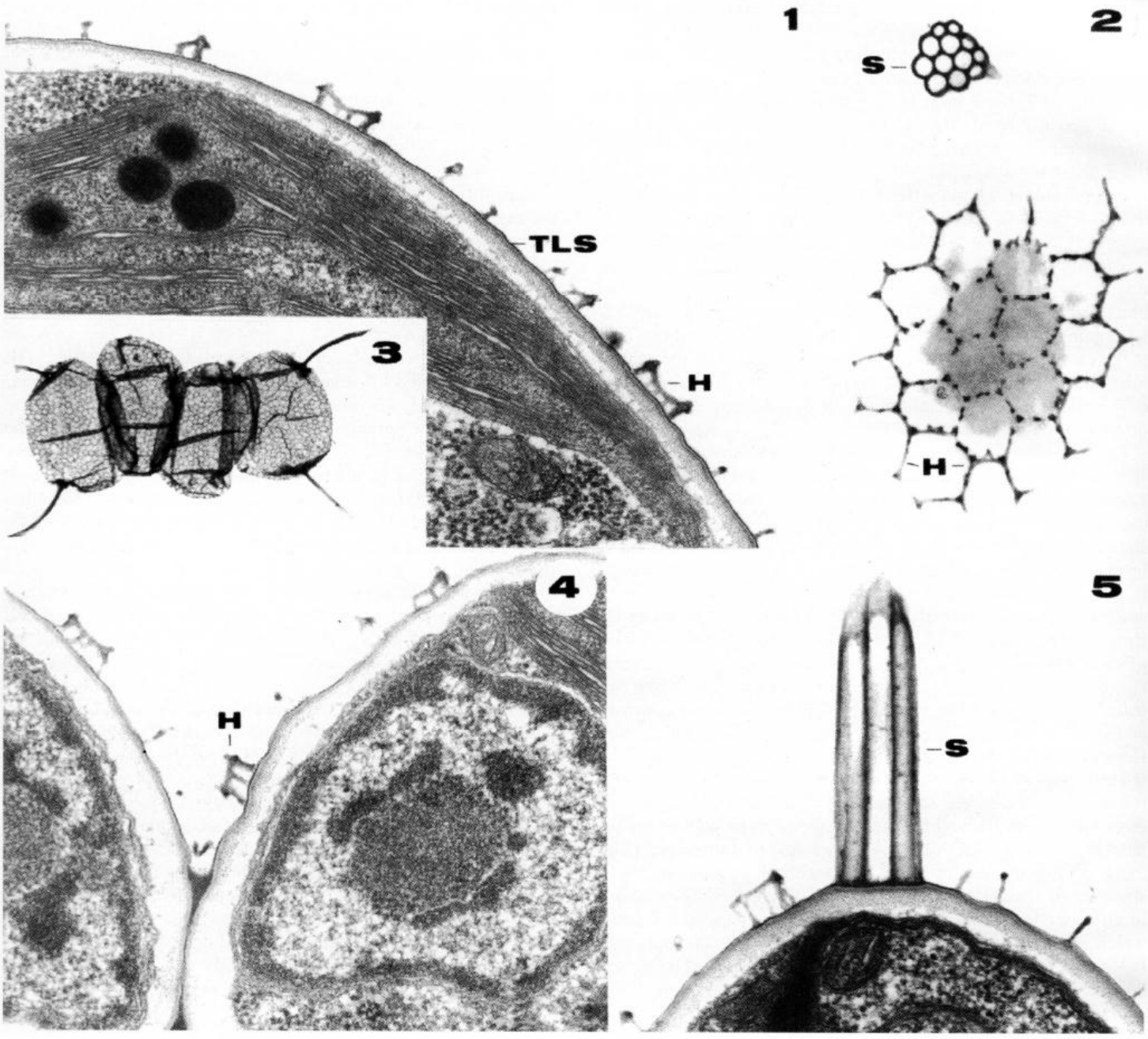
S. quadricauda shows the other type of ornamentation. The whole coenobium is enclosed with a network called reticulate layer (Figs. 14). This layer is kept separate from the TLS by propping spikelets, attached to the TLS at one end (Fig. 10). The rosettes also support the reticulate layer (Fig. 13). The network of this layer consists mainly of penta- and hexagonal meshes (Fig. 11). The rosette is divided into chambers (Fig. 11). Empty propping elements are tetra- or pentagonal in cross section (Figs 10, 11). Moreover numerous long bristles, not only emanating from openings (rosettes) but probably also from propping spikelets, were observed under light microscope.

Electron micrographs of whole cells exposed to acetolysis give evidence that their contents were completely dissolved

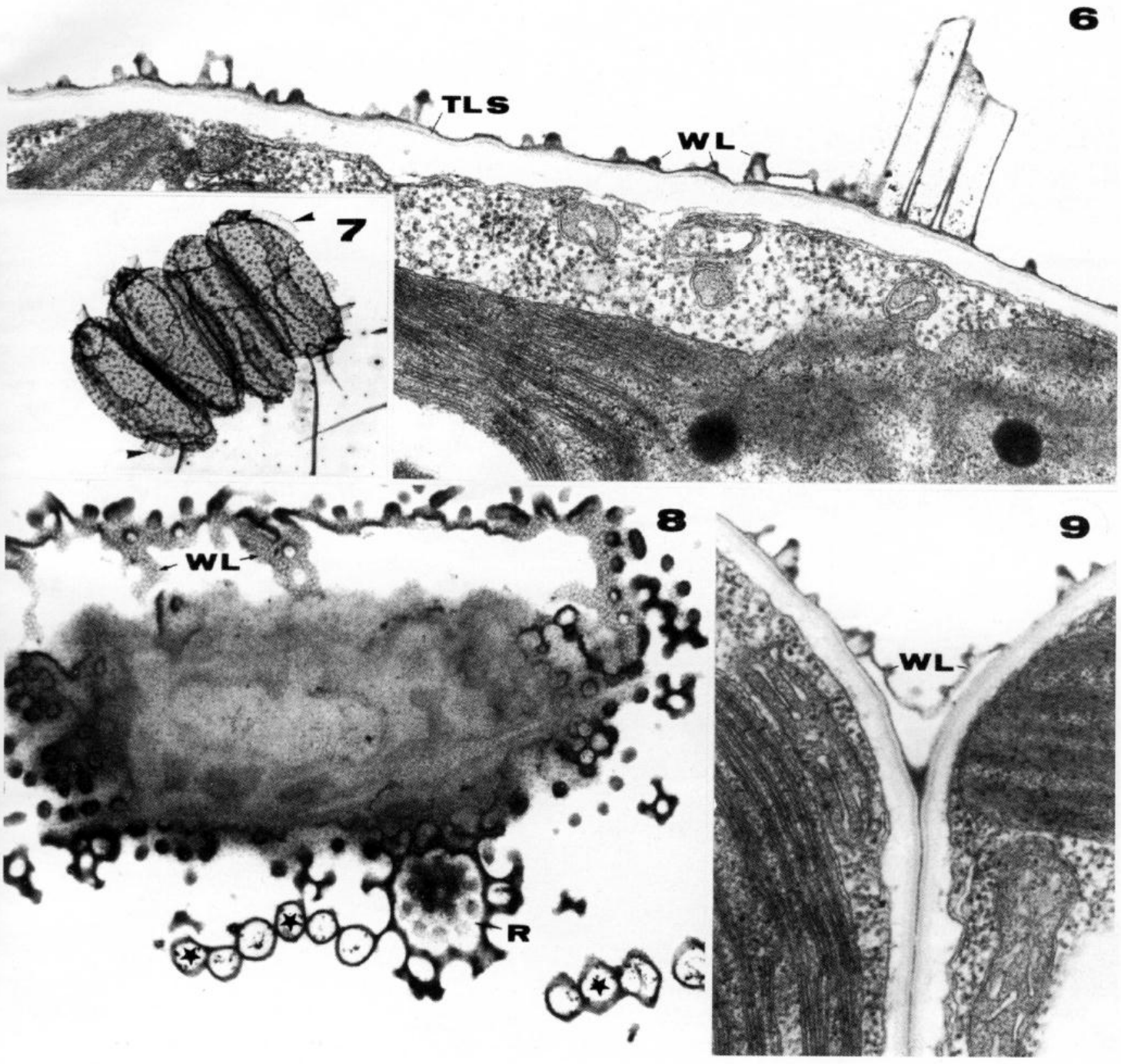
TABLE 2. Cell size, surface (S), volume (V) and S/V ratio of three *Scenedesmus* strains cultured 7 days in BBM medium. Values represent the means from duplicate cultures; in each 100 autospores were measured. Initial cell density was $1*10^5$ cells in 1 cm^3 of suspension. The growth conditions of algae are given in Table1.

Strains	Size (μm)		Surface	Volume	S/V
	length	width	(μm^2)	(μm^3)	
S. microspina	6.0 (0.82)	3.4 (0.44)	52.1	36.3	1.44
S. armatus	9.5 (0.82)	5.0 (0.78)	119.2	124.3	0.96
S. quadricauda	13.9 (1.98)	4.3 (0.8)	216.7	201.8	1.07

Values in parentheses indicate the standard error of the mean.



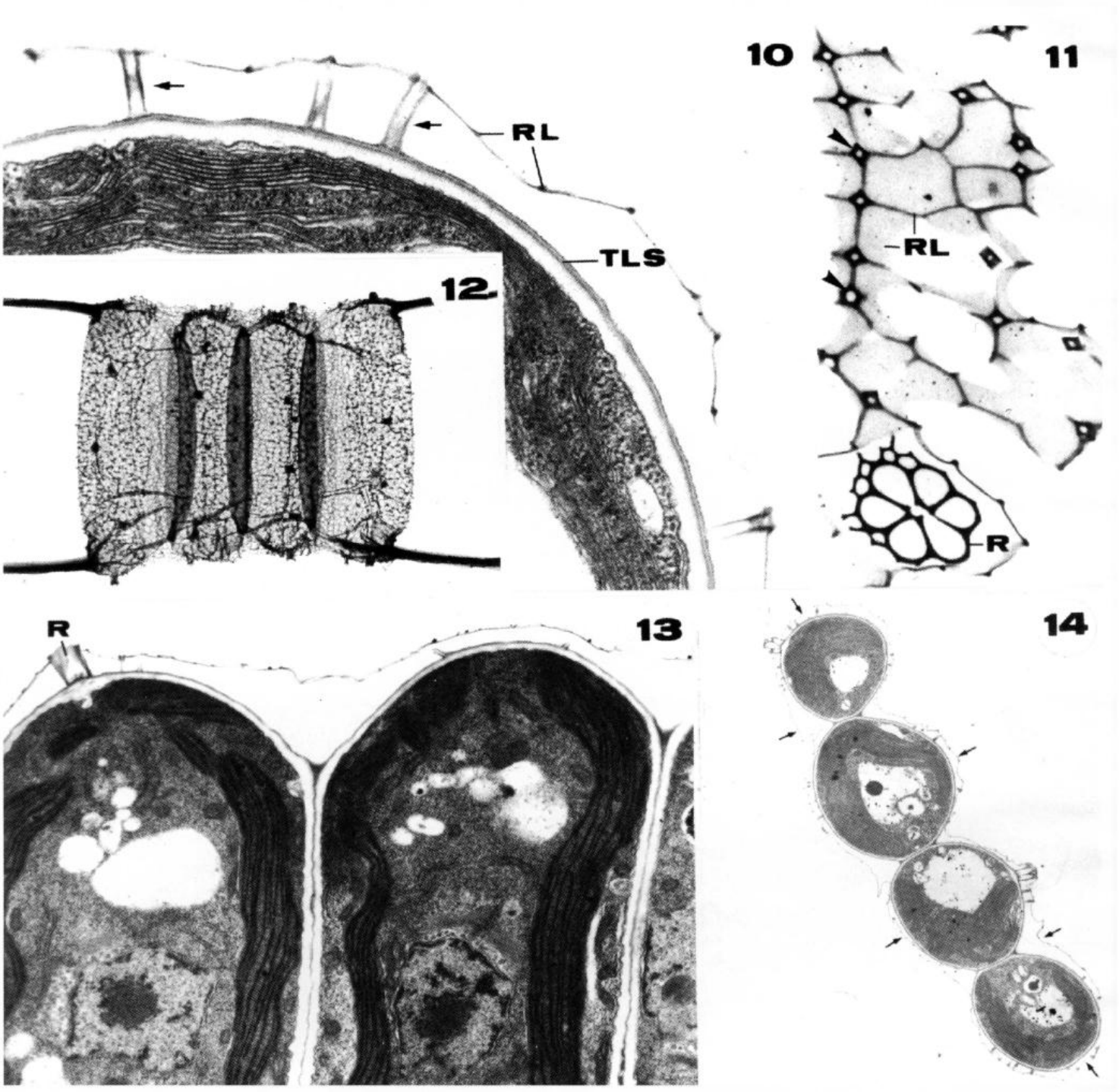
Figs 1-5. ***Scenedesmus microspina.***
Fig. 1. Honeycomb structure (H) is directly attached to three-laminar sheath (TLS); x 54 000.
Fig. 2. Tangentially sectioned honeycomb structure (H) and cross sectioned terminal spine (S). Spikelets, loosely arranged on the ground and compact at the top, build meshes of the honeycomb; x 40 000.
Fig. 3. Coenobium after acetolysis. TLS, honeycomb structure and terminal spines survive intact; x 3 200.
Fig. 4. The honeycomb structure (H) covers whole cenobium, also in places where cells join; x 54 000.
Fig. 5. In longitudinal section the terminal spine shows tubular structure (S); x 54 000.



Figs 6-9. ***S. armatus.*** Warty layer (WL) directly attaches to three-laminar sheath (TLS) (Fig. 6; x 54 000), excluding places of cells adhering (Fig. 9; x 54 000).

Fig. 7. Coenobium after acetolysis. TLS, warts, combs (arrowheads) and terminal spines survive intact; x 3 200.

Fig. 8. Tangential section of separating warty layer (WL) indicates fine perforations (arrows). Mushroom-shaped structures of rosette (R) are arranged in a few whorls. Tubular spikelets (stars) of a comb have a similar diameter and run zigzag along cell; x 54 000.

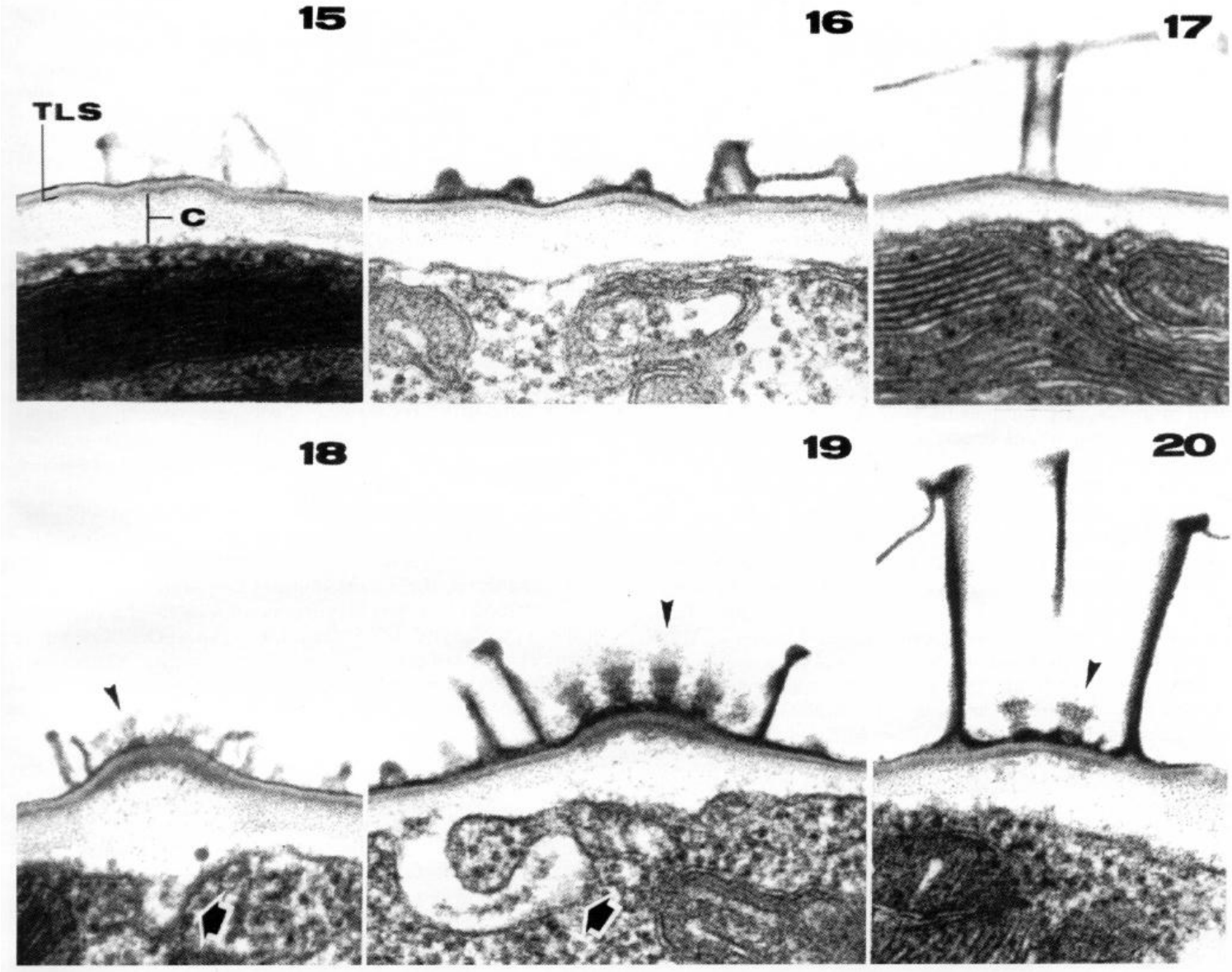


Figs 10-14. ***S. guadricauda.*** Reticulate layer (RL), spatially separated from three-laminar sheath (TLS), is supported by propping spikelets (arrows) (Fig. 10; x 54 000) and rosettes (R) (Fig. 13; x 13 800).

Fig. 11. In tangential section of the reticulate layer (RL), in corners of polygonal meshes of the layer, visible are tetra- and pentagonal profiles of cross-sectioned propping spikelets (arrowheads). Below rosette (R), cross-sectioned; x 40 000.

Fig. 12. Coenobium subjected to acetolysis. TLS, reticulate layer, spines and rosettes appear intact; x 3 500.

Fig. 14. Reticulate layer (small arrows) surrounds the whole, cross-sectioned cenobium; x 5 700.



Figs 15-17. Cell walls of *S. microspina* (Fig. 15), *S. armatus* (Fig. 16) and *S. quadricauda* (Fig. 17). Note presence of three-laminar sheath (TLS) and similar thickness of cellulosic layer (C) of the strains; all x 100 000.
Figs 18-20. Longitudinal sections through rosettes of *S. microspina* (Fig. 18), *S. armatus* (Fig. 19) and *S. quadricauda* (Fig. 20). Note presence of mushroom-shaped structures (arrowheads), loose structure of cellulosic wall underlying rosettes and plasmalemma invaginations (arrows); all x 100 000.

(Figs 3, 7, 12). Only the outer part of cell wall of algae, i.e. the TLS and epistructural elements including spines, propping spikelets, rosettes and combs survive the acetolysis. On the contrary, all types of epistructures disappeared after 6N NaOH treatment excluding TLS and small remnants of cellulosic layer (data not shown).

DISCUSSION

The obtained results indicate strain-dependent sensitivity of three tested strains of *Scenedesmus* to AFOE expressed by growth parameters. They are: sensitivity reflected in growth inhibition of algae caused by presence of AFOE as well as their cell size. It was found that the smallest *S. microspina* was the most sensitive to AFOE. Simultaneously this strain shows the most profitable S/V ratio for absorption of hydrocarbons among all tested strains. However, similarities of the S/V values for *S. quadricauda* and *S armatus* appear to exclude bioabsorption as an essential parameter suitable for characterising the sensitivity of algae. The more so that three strains used in this work form unicells as well as 2-, 4- and 8-celled coenobia in proportions depending on strains and the culture time. Unicell production in *S. quadricauda* has never been observed. In this strain in 7-day-old cultures 4-celled coenobia were observed as the dominant (95%) form under here described growth conditions (Tukaj and Bohdanowicz 1995 – in press). In contrast at the same time the population of *S. armatus* was composed of unicells (16%), 2-celled (65%) and 4-celled coenobia (19%). We assume that large multicellular coenobia of *S quadricauda* are better protected against AFOE then smaller 2- and 4-celled coenobia and especially unicells of *S. armatus*. Additional evidence of the above differences provide the fine structure analysis of cell walls of three strains examined, especially relating to "epistructural layers" of the walls.

In many reports reviewed by Lewis (1990) there were suppositions that the severity of the surfactants or detergent builders acts towards algae depend on the cell wall thickness and their chemical composition. Differences in the susceptibility of green microalgae to herbicides were also partly explained by different thickness of their cell walls (Kasai and Hatakeyama 1994). Sporopollenin is especially considered by the authors as a factor influencing the cell protection against pollutants. However, *Scenedesmus* cells are surrounded by both "cellulosic" layer and TLS containing acetolysis-resistant biopolymer (ARB). Moreover, "cellulosic" cell-wall layer of here used strains of *Scenedesmus* are similar in thickness. Despite this there are the great differences in sensitivity to AFOE between them. Biedlingmayer et al. (1987) found *S. armatus* 276-4a to be the most sensitive strain to LAS detergents comparing to the other species of the genus. Deficiency in continuity of TLS was speculated by the authors as enabling chemical attack of detergents on algal cells. Unfortunately, we have never observed any lack in TLS layer continuity within *Scenedesmus*. Therefore we assume that epistructure as for instance additional layers differ the properties of strains and influence on their susceptibility to AFOE.

Coenobia of *S. quadricauda* and *S. armatus* in contrast to coenobia of *S. microspina* are equipped by additional layers; the first with reticulate layer and the second with warty layer. These layers seem to protect cells against hydrocarbons increasing their resistance to AFOE in comparison with *S. microspina*. Moreover, cell wall ultrastructure of *S. quadricauda* G-15 strain resembles that of UTEX 614 and 77 (Bisalputra and Weier 1963) and *S. longus* (Staehelin and Pickett-Heaps 1975). The outer layer of both strains is described by the cited authors as containing pectin material. We couldn't recognise any pectin-like material on our electron micrographs. This can be explained by eventually disappearance of this material from algal cells as result of preparation to TEM using standard procedures. At present it is additionally known that "pectin layer" connotation applied to whole epistructure layer is misleading; some epistructures do not disappear after pectinase treatment (Trainor and Egan 1988). The other ones are solubilised by treatment with acids or alkali, as is obtained in this work. Moreover we confirm observation of Egan and Trainor (1989) that *S. quadricauda* possesses numerous bristles (70-130 µm long). Thus coenobial forms enclosed with spatially separated reticulate layer appear to make *S. quadricauda* the most tolerant organism among the examined strains. Some strains of *S. armatus* were found to be mucilage producers (Hegewald 1989), but it doesn't concern our strain (negative results with Chinese ink).

Another aspect that should be considered is the physiological function of rosettes. Lower electron densities of cell wall under these structures as well as accompanying invaginations of plasmalemma suggest the role of rosettes in transport outside/inside the cell. The arrangement and number of rosettes on the cell surface of three tested strains are similar but their structure is different. Our observations suggest that algae having the simpler rosette structure may be easier penetrated by hydrocarbons during incubation with AFOE.

In conclusion, the results indicate that both morphological and structural characters may be one of reasons of a strain-dependent sensitivity of *Scenedesmus* to AFOE. Coenobial forms appear to be less sensitive than unicells. Neither cell wall thickness nor ARB presence, but the epistructures seem to be strongly related to this sensitivity.

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LITERATURE CITED

ATKINSON A.W., GUNNING B.E.S., JOHN P.C.L., 1972. Sporopollenin in the cell wall of *Chlorella* and other algae: Ultrastructure, chemistry and incorporation of ^{14}C-acetate, studied in synchronous cultures. Planta 107: 1-32.

BATTERTON J.C., WINTERS K., VAN BAALEN C., 1978. Sensitivity of three microalgae to crude oils and fuel oils. Mar. Environ. Res. 1: 31-41.

BIEDLINGMAIER S., WANNER G., SCHMIDT A., 1987. A correlation between detergent tolerance and cell wall structure in green alga. Z. Naturforsch. 42c: 245-250.

BISALPUTRA T., WEIER T.E., 1963. The cell wall of *Scenedesmus quadricauda*. Amer. Jour. Bot. 50: 1011-1019.

BURCZYK J., SZKAWRAN H., ZONTEK I., CZYGAN F.CH., 1981. Carotenoids in the outer cell-wall layer of *Scenedesmus (Chlorophyceae)*. Planta 151: 247-250.

BURCZYK J., DWORZAŃSKI., 1988. Comparison of sporopollenin-like algal resistant polymer from cell wall of *Botryococcus, Scenedesmus* and *Lycopodium clavatum* by GC-pyrolysis. Phytochemistry 27: 2151-2153.

BURCZYK J., WARDAS W., TERMINSKA-PABIS K., SMIETANA B., LEBEK K., 1994. The influence of low molecular hydrocarbons on cell wal carotenoids of the alga *Scenedesmus obliquus*. Pol. Jour. Environ. Stud. 3: 19-22.

EDLER L., 1979. Recommendations of methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. The Baltic Marine Biologists (BMB) Publ. No. 5: 1-38.

EGAN P.F., TRAINOR F.R., 1989. The effects of media and inoculum size on the growth and morphological development of *Scenedesmus communis* HEGEW. (*Chlorophyceae*) in culture. Arch. Hydrobiol., 117: 77-95.

FISHER N.S., GRAHAM L.B., CARPENTER E.J., WURSTER C.F., 1973. Geographic differences in phytoplankton sensitivity to PCBs. Nature, Lond., 241: 548-9.

GRUENFELD M., 1975. Quantitative analysis of petroleum oil pollutants by infrared spectrophotometry. *Water Quality Parameters*, ASTM STP 573, American Society for Testing and Materials, 290-308.

HEGEWALD E., 1978. Eine neue Unterteilung der Gattung *Scenedesmus* Meyen. Nova Hedvigia 30: 343-376.

HEGEWALD E., 1989. The *Scenedesmus* strains of the Culture Collection of the University of Texas at Austin (UTEX). Algol. Stud. 55: 153-189.

HEGEWALD E., SCHNEPF E., 1991. *Scenedesmus abundans* (KIRCHN.) CHOD., an older name for *Chlorella fusca* SHIN. et KRAUSS. Arch. Protistenkd. 139: 133-176.

HILMER T., BATE G.C., 1983. Observations on the effect of outboard motor fuel oil on phytoplankton cultures. Environ. Pollut. 32: 1-10.

KALINA T., ZABOVÁ I., HILGARD S., 1993. The spectroscopy of the acetoresistant cell wall biopolymers of *Auxenochlorella protothecoides, Scenedesmus quadricauda and Chlamydomonas geitleri* zygospores (Chlorophyta). Algol. Stud. 68: 65-77.

KARYDIS M., FOGG G.E., 1980. Physiological effects of hydrocarbons on the marine diatom *Cyclotella cryptica*. Microb. Ecol. 6: 281-290.

KASAI F., HATAKEYAMA S., 1993. Herbicide susceptibility in green algae *Chlorella vulgaris* and *Selenastrum capricornutum*. Chemosphere 27: 899-904.

KAUSS P.B., HUTCHINSON T.C., 1975. Studies on the susceptibility of *Ankistrodesmus* species to crude oil components. Verh. Internat. Verein. Limnol. 19: 2155-2164.

KOMÁREK J., LUDVÍK J., 1972. Die Zellwandstruktur als taxonomisches Merkmal in der gattung *Scenedesmus*. 2. Taxonomische Auswertung der urtensuchten Arten. Archiv. Hydrobiol./Suppl. 41, Algol. Stud. 6: 11-47.

LEWIS M.A., 1990. Chronic toxicities of surfactants and detergent builders to algae: A review and risk assessment. Ecotox. Environ. Saf. 20: 123-140.

MAHONEY B.M., HASKIN H.H., 1980. The effects of petroleum hydrocarbons on the growth of phytoplankton recognised as food forms for the eastern oyster, *Crassostrea virginica* Gmelin. Environ. Pollut. (Ser. A) 22: 123-132.

NICHOLS W.H., BOLD H.C., 1965. *Trichosarcina polymorpha* Gen. et Sp. New. J. Phycol. 1: 34-38.

ØSTGAARD K., HEGSETH N.E., JENSEN E., 1984. Species-dependent sensitivity of marine planctonic algae to Ekofisk crude oil under different light conditions. Bot. Mar. 27: 309-318.

SPURR A.R., 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruc. Res. 26: 31-34.

STAEHELIN A.L., PICKETT-HEAPS J.D., 1975. The ultrastructure of Scenedesmus (Chlorophyceae). I. Species with the "reticulate" or "warty" type of ornamental layer. J. Phycol. 11: 163-185.

TRAINOR F.R., EGAN P.F., 1988. The role of bristles in the distribution of a *Scenedesmus* (Chlorophyceae). Br. phycol. J: 135-141.

TUKAJ Z., 1987. The effects of crude and fuel oils on the growth, chlorophyll a content and dry matter production of a green alga *Scenedesmus quadricauda* (TURP.) BRB. Environ. Pollut. 47: 9-24.

TUKAJ Z., 1989. The effect of fuel oil on the ultrastructure of the chlorococcal alga *Scenedesmus armatus*. Protoplasma 151: 47-56.

TUKAJ Z., 1994. The toxicity of three dispersed diesel fuel oils and dispersants towards some *Scenedesmus (Chlorococcales)* species. Oceanologia 36: 175-186.

TUKAJ Z., BOHDANOWICZ J., 1995. Diesel-fuel-oil induced morphological changes in some *Scenedesmus (Chlorococcales)* species. Algol. Stud. 77: in press.

VAN BAALEN C., O'DONNELL R., 1984. Sensitivity of two psychrophilic diatoms to crude oil and fuel oil. Mar. Environ. Res. 12: 63-68.

ZACHLEDER V., TUKAJ Z., 1993. Effect of fuel oil and dispersant on cell cycle and macromolecular synthesis in the chlorococcal alga Scenedesmus armatus. Mar. Biol. 117: 347-353.

WRAŻLIWOŚĆ NA DZIAŁANIE OLEJU OPAŁOWEGO A BUDOWA ŚCIANY KOMÓRKOWEJ KILKU SZCZEPÓW *SCENEDESMUS (CHLOROCOCCALES)*

STRESZCZENIE

Badano wpływ wodnego ekstraktu lekkiego oleju opałowego (AFOE) na wzrost populacji trzech szczepów *Scenedesmus*. Stwierdzono, że *S. quadricauda* jest odporny, *S. armatus* pośrednio wrażliwy, a *S. microspina* najbardziej wrażliwy na działanie AFOE. Wrażliwość testowanych szczepów rośnie wraz ze spadkiem wielkości komórki oraz liczby komórek tworzących cenobium. Współczynniki wyrażajce stosunek powierzchni komórek do ich objętości jedynie częściowo tłumaczą powyższą zależność. Badania z użyciem mikroskopu elektronowego wykazały, że wrażliwość szczepów zależeć może od budowy ich ścian komórkowych. Każda komórka *Scenedesmus* ma ścianę zbudowaną z dwóch warstw: wewnętrznej „celulozowej" i zewnętrznej, tzw. trójlaminarnej struktury (TLS) zawierającej biopolimer odporny na aceto- i alkalolizę (ARB). Te dwie warstwy mają podobną grubość u trzech badanych szczepów, podczas gdy epistruktury pokrywające cenobia są charakterystyczne dla szczepów. Takie epistruktury jak bezpośrednio przylegająca do komórek warstwa brodawkowata (*S. armatus*), a zwaszcza siateczka luźno zamykająca cenobia (*S. quadricauda*) mogą dodatkowo ochraniać wnętrze komórek przed węglowodorami. Budowa rozet jest także skorelowana z wrażliwością szczepów względem AFOE. Obecność inwaginacji plazmalemmy pod rozetami wskazuje na ich udział w procesach transportu komórkowego.

SŁOWA KLUCZOWE: *Scenedesmus*, wzrost, morfologia, struktura ściany komórkowej, wrażliwość, olej opałowy.