The light scattering matrix of *Chlorella vulgaris* cells and its variability due to cell modification*

OCEANOLOGIA, No. 36 (1)
pp. 19–31, 1994.
PL ISSN 0078–3234

Light scattering
Phytoplankton
Müller matrix

Konrad Witkowski
Institute of Experimental Physics,
University of Gdańsk,
Gdańsk

Tadeusz Król, Maria Łotocka
Institute of Oceanology,
Polish Academy of Sciences,
Sopot

Manuscript received May 20, 1994, in final form September 13, 1994.

Abstract

Laboratory light scattering measurements were performed for suspensions of axenic cultures of the unicellular alga *Chlorella vulgaris*. 10 functions constituting the scattering Müller matrix were measured at three incident light wavelengths (λ = 633 nm, 514 nm and 488 nm) for live cells and for cells whose internal structures had been chemically or mechanically modified. For medium scattering angles ($30° \leq \theta \leq 120°$) the scattering process is strongly influenced by submicron intracellular structures which are both absorbent and optically anisotropic.

Some of light scattering characteristics display a distinct correlation with the physiological evolution stage of a live cell culture.

1. Introduction

The optical properties of sea waters are strongly influenced by absorption and light scattering by organic suspensions in the sea – particularly by phytoplankton cells.

The interaction between light and the single cell is an extremely complex phenomenon which is still not sufficiently well understood. In order to obtain information enabling this process to be better comprehended and

* Some of the results in this paper were presented at the symposium on The Functioning of Coastal Ecosystems in Various Geographical Regions, Gdańsk – September 1992.

mathematically modelled, precise laboratory measurements, performed for well known, model samples are desirable.

Moreover, it is presumed that the light scattering characteristics of various phytoplankton cells could be correlated with their internal structures and with the stage of the physiological evolution of the culture.

In this paper we present the results of such laboratory light scattering measurements for suspensions of cells of the unicellular green alga *Chlorella vulgaris*.

The spherical shape of the cells of this species should facilitate mathematical modelling of the scattering process (Zieliński *et al.*, 1986), and the well-developed internal structures (nucleus, chromatophore, pyrenoid) should allow their influence on the scattering characteristics to be investigated.

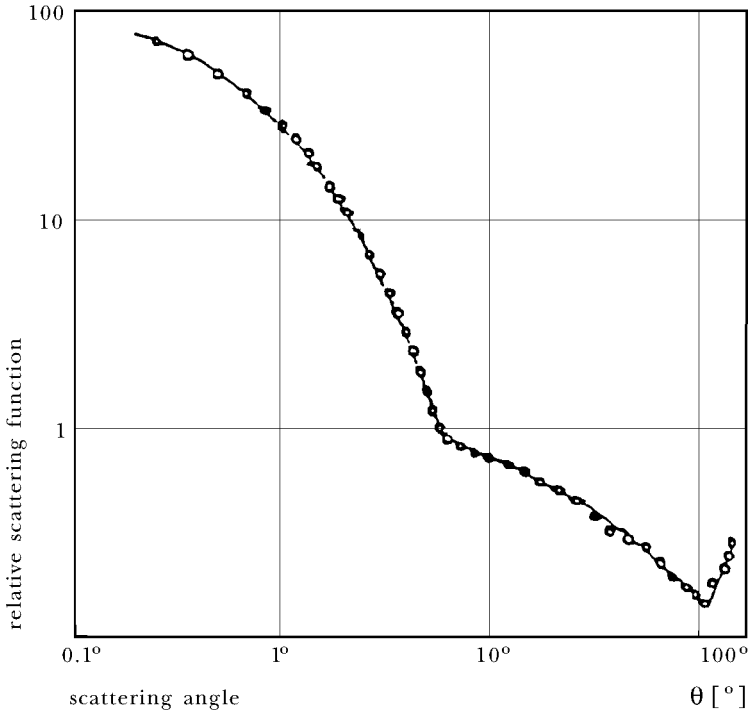

Fig. 1. Dependence of the relative intensity of the scattered light on the scattering angle for a *Chlorella vulgaris* suspension at wavelength $\lambda = 633$ nm, plotted on the bilogarithmic scale (after Król *et al.*, 1992)

The angular distribution of the intensity of light scattered from *Chlorella vulgaris* suspensions measured over a wide range of scattering angles taken from Król *et al.* (1992) is given in Fig. 1. The characteristic feature of this

scattering function is the very rapid increase in the intensity scattered in the forward direction, that is, for small angles ($\theta < 10°$). The theoretical interpretation of this scattering function, given in Zieliński *et al.* (1987), suggests that in this low scattering angle region, the scattering from the phytoplankton cell could be quite well described as Mie scattering from spheres whose diameter is equal to the external diameter of the algal cells.

The interpretation of the scattering function in the range of medium scattering angles ($\theta > 10°$) is, however, much less obvious. Results of theoretical analysis suggest that light scattering in this range is determined by intramolecular structures with dimensions comparable to the wavelength of the incident light. In such conditions the scattering and absorption cross-sections of such structures are particularly large – see *e.g.* Król (1991).

It is also possible, as was suggested in Zieliński *et al.* (1987), that molecular scattering from biomolecules and their aggregates plays an important role in the scattering process under consideration here.

In order to elucidate this problem, we have measured the angular distributions of the intensity and polarisation of the scattered light for algal cell suspensions at three selected wavelengths. The measurements were done with samples containing live cells and with modified samples containing partially disintegrated internal structures.

2. Stokes parameters and the scattering matrix

The polarisation and intensity of an arbitrary light beam can be fully characterised by means of four real numbers – Stokes parameters (for their definition see, *e.g.* van der Hulst (1957).

This set of Stokes parameters will be designated by S_i for the scattered and S_j for the incident beam, where $i, j = 1...4$.

If nonlinear phenomena are neglected, light scattering in an arbitrary medium can be considered to be the linear transformation of the set of Stokes parameters $\{S_j\}$ to those of the scattered beam $\{S_i\}$. This transformation is usually described by means of a scattering matrix $P_{i,j}(\theta)$, which is a special case of the Müller matrix (Schurcliff, 1962) and is defined by the following equation:

$$S_i(\theta)d\omega_s = \sum P_{i,j}(\theta)\ S_j \frac{d\omega_s\ d\omega_o}{R^2}, \tag{1}$$

where $d\omega_s$ and $d\omega_o$ are solid angle elements in the directions of the scattered and the incident beam respectively, and R is the distance from the centre of the scattering volume to the scattered light detector. The numbers $P_{i,j}(\theta)$ represent the differential scattering cross-sections of the medium and form a 4×4 matrix.

Both $P_{i,j}(\theta)$ and $S_i(\theta)$ are functions of the scattering angle.

Perrin (1942) has shown that if scattering can be considered a reversible process, then functions $P_{i,j}(\theta)$ have to be symmetrical and hence only 10 $P_{i,j}(\theta)$ elements of the 16 are really independent.

Functions $P_{i,j}(\theta)$ can be measured as linear combinations of the relative intensities of the scattered light components with appropriately selected polarisation of both incident and scattered beams – for particulars, see Harris and McClain (1977) or Witkowski (1992).

3. Materials and methods

The polarisation components of the scattered light were measured at the Institute of Experimental Physics, University of Gdańsk, with the apparatus described in Witkowski (1986). The light sources were two lasers: a He-Ne laser for the wavelength 633 nm (power 35 mW) and an argon ion laser working at 80 mW for wavelengths 514 nm and 488 nm.

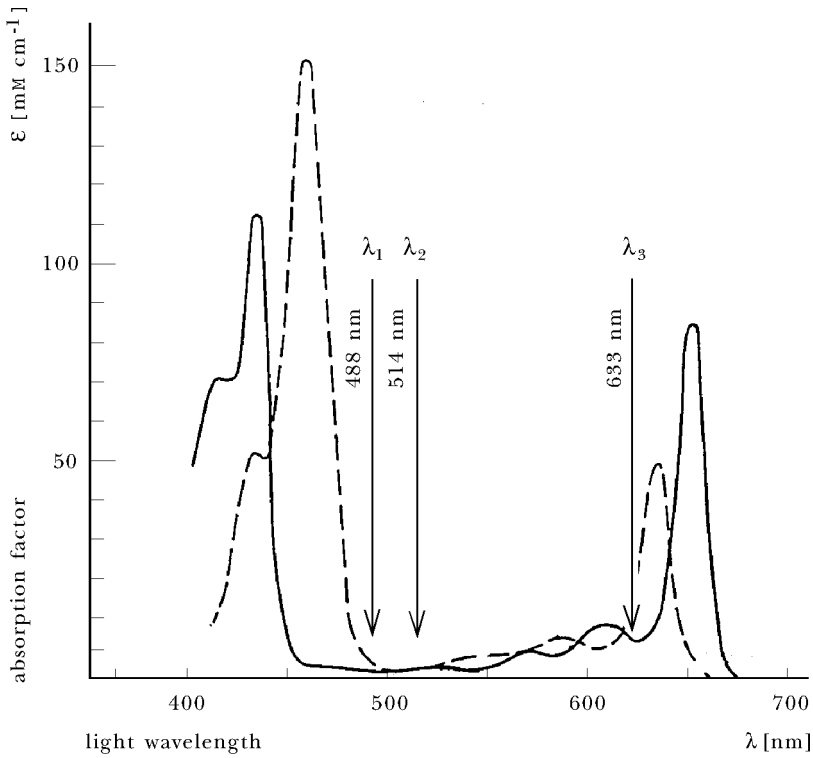

Fig. 2. Absorption spectra of chlorophyll *a* (—) and chlorophyll *b* (– –) (taken from Clayton, 1965), shown together with the laser wavelengths used in our light scattering measurements

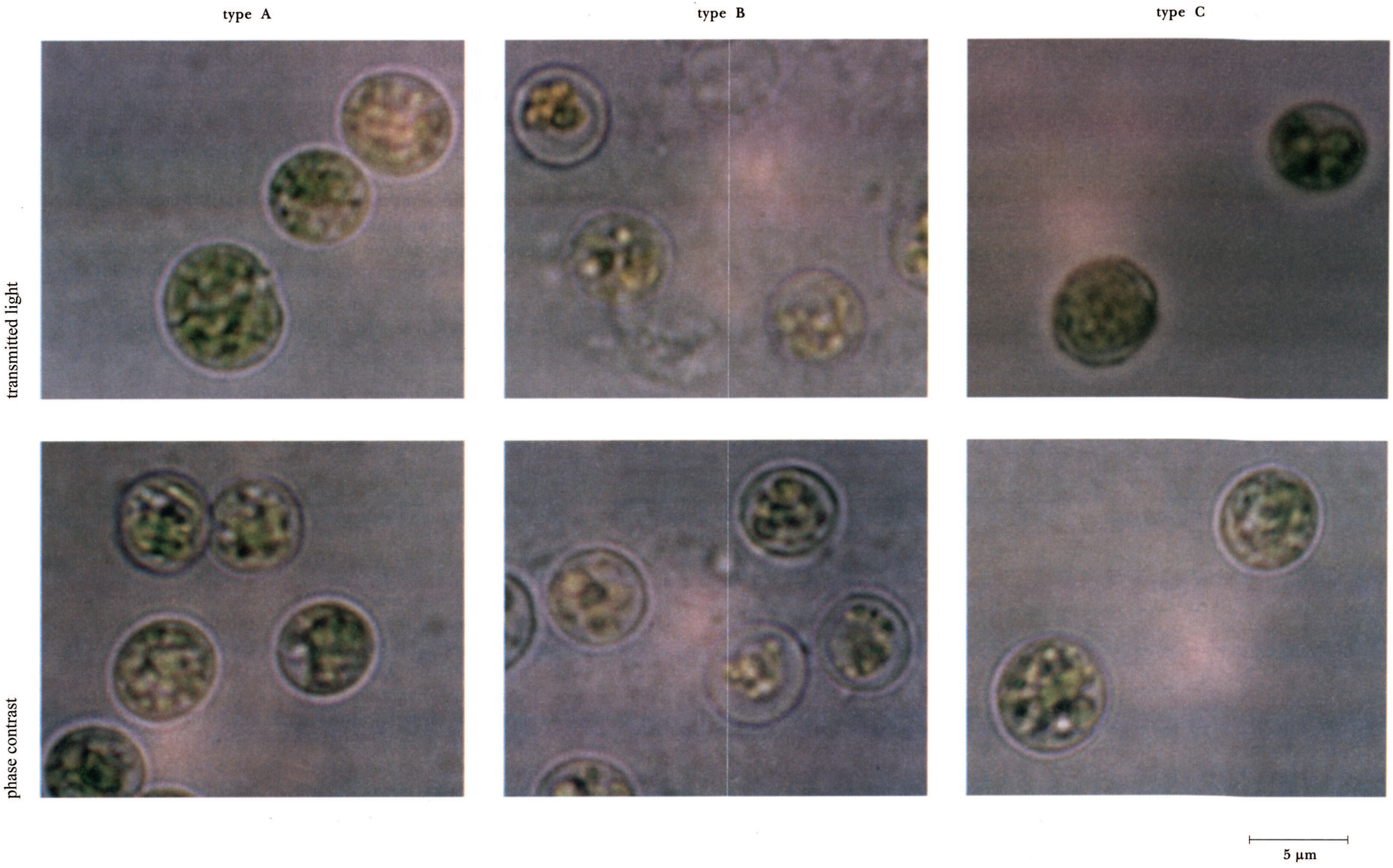

Fig. 3. Microphotographs of *Chlorella vulgaris* cells from live (type A) and modified (types B and C) samples

The light wavelengths used in our scattering experiments are shown in Fig. 2 together with the absorption spectra of chlorophyll. Wavelengths 488 and 514 nm are in the low absorption range, but the 633 nm line is at the edge of the strong absorption band of chlorophyll *a*.

The polarisation state of both incident and scattered beams was selected and modulated by means of rotating polarisation filters, as described in Witkowski (1992).

Measurements were performed for scattering angles over the range $5° < \theta < 170°$.

Axenic monocultures of *Chlorella vulgaris* cells were isolated from the southern Baltic Sea and sustained in a mineral medium under artificial illumination. Sample preparations have been described in Gędziorowska (1983) and Król *et al.* (1992). Three kinds of samples were prepared:

- type A – containing live cells suspended in a nutrient medium diluted to a concentration sufficiently low to avoid multiple scattering effects,
- type B – sample modified by boiling for 5 min. in 5% NaOH,
- type C – sample exposed to 7 W ultrasonic radiation of frequency 20 kHz.

Microphotographs of cells taken from samples of types A, B and C are shown in Fig. 3. Some decolouration of type B cells and some transformation or disintegration of the internal structures of cells of both types B and C can be seen. External cell walls were preserved in all cases.

4. Results

The results of our light scattering measurements are shown in the form of dependences of $P_{i,j}(\theta)$ functions on the light scattering angle. All functions $P_{i,j}(\theta)$ shown in Figs. 4–7 are normalised in such a way that they describe scattering of the light by a single (average) *Chlorella vulgaris* cell.

Dependences $P_{1,1}(\theta)$ and $P_{3,4}(\theta)$ obtained experimentally for live cells (sample A) are shown in Fig. 4 for the three wavelengths used in our measurements. $P_{1,1}(\theta)$ is simply the angular distribution of the total relative intensity of the scattered light measured with an unpolarised incident beam. The scattered light intensity increases with decreasing wavelength – a well-known effect in all scattering processes; the minimum of the scattering function $P_{1,1}(\theta)$ also shifts towards smaller angles with decreasing wavelength.

Function $P_{3,4}(\theta)$ which, as was shown by Harris and McClain (1985), depends on multiple scattering effects in complex intracellular structures as well as on absorption effects, exhibits a much stronger dependence on the wavelength of the incident light than the total scattered intensity $P_{1,1}(\theta)$.

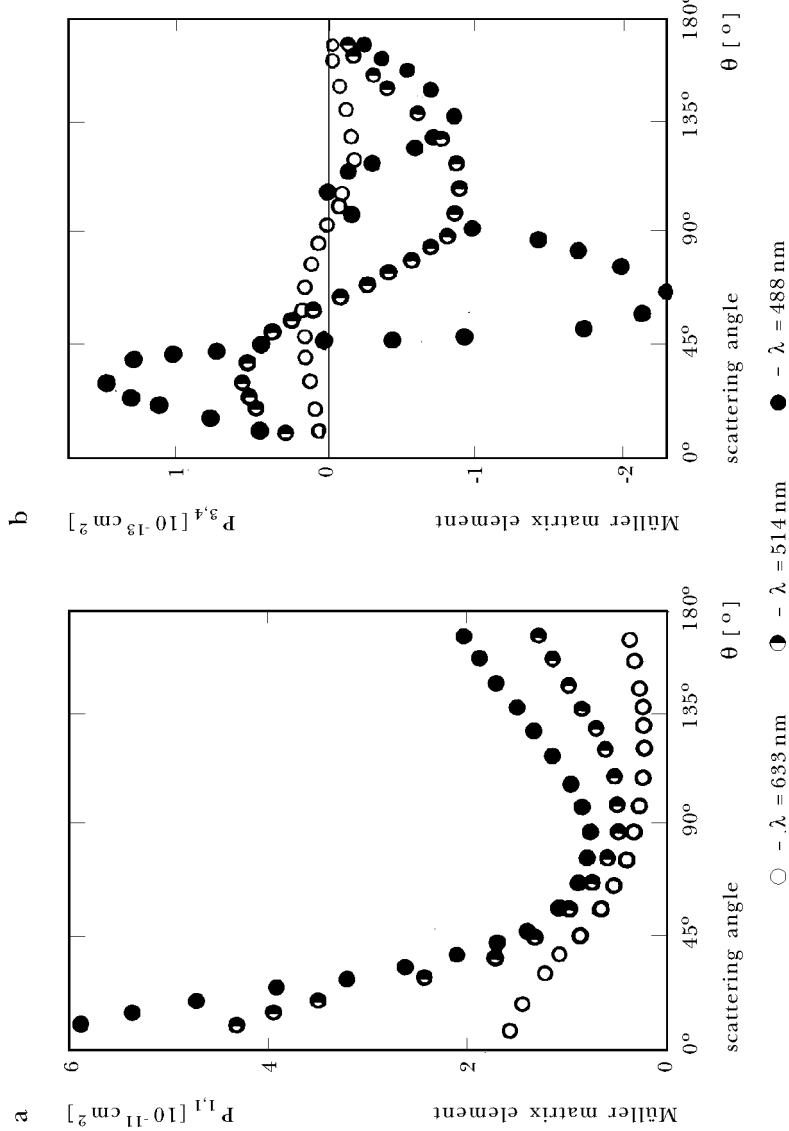

Fig. 4. Dependences of $P_{1,1}$ and $P_{3,4}$ scattering functions on the scattering angle for a suspension of live (type A) *Chlorella vulgaris* cells. Incident light wavelengths are 633 nm, 514 nm and 488 nm respectively

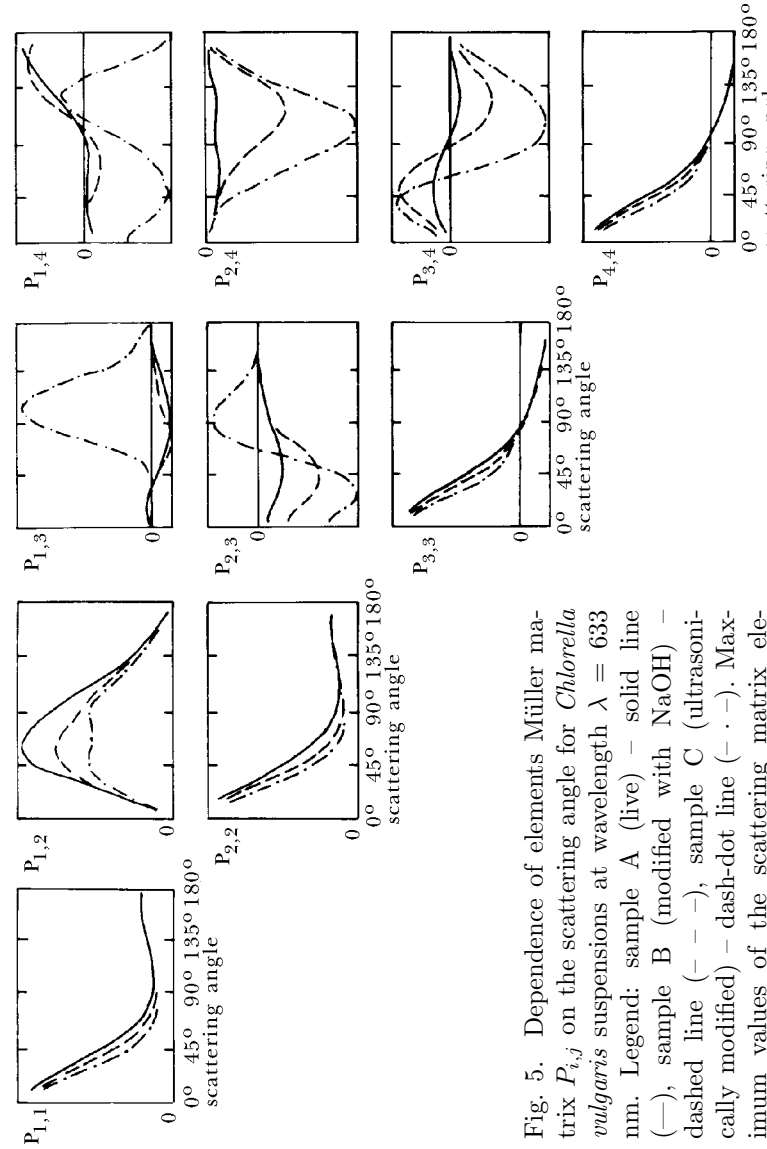

Fig. 5. Dependence of elements Müller matrix $P_{i,j}$ on the scattering angle for *Chlorella vulgaris* suspensions at wavelength $\lambda = 633$ nm. Legend: sample A (live) – solid line (—), sample B (modified with NaOH) – dashed line (– – –), sample C (ultrasonically modified) – dash-dot line (– · –). Maximum values of the scattering matrix elements are: $P_{1,1} = P_{2,2} = P_{3,3} = P_{4,4} = 1.6 \times 10^{-11}$ cm^2, $P_{1,2} = 3 \times 10^{-12}$ cm^2, $P_{1,3} = 1.9 \times 10^{-15}$ cm^2, $P_{1,4} = 2.2 \times 10^{-15}$ cm^2; minimum values are: $P_{2,3} = -2.6\ 10^{-15}$ cm^2, $P_{2,4} = -1.7\ 10^{-15}$ cm^2, $P_{3,4} = -2.9\ 10^{-15}$ cm^2

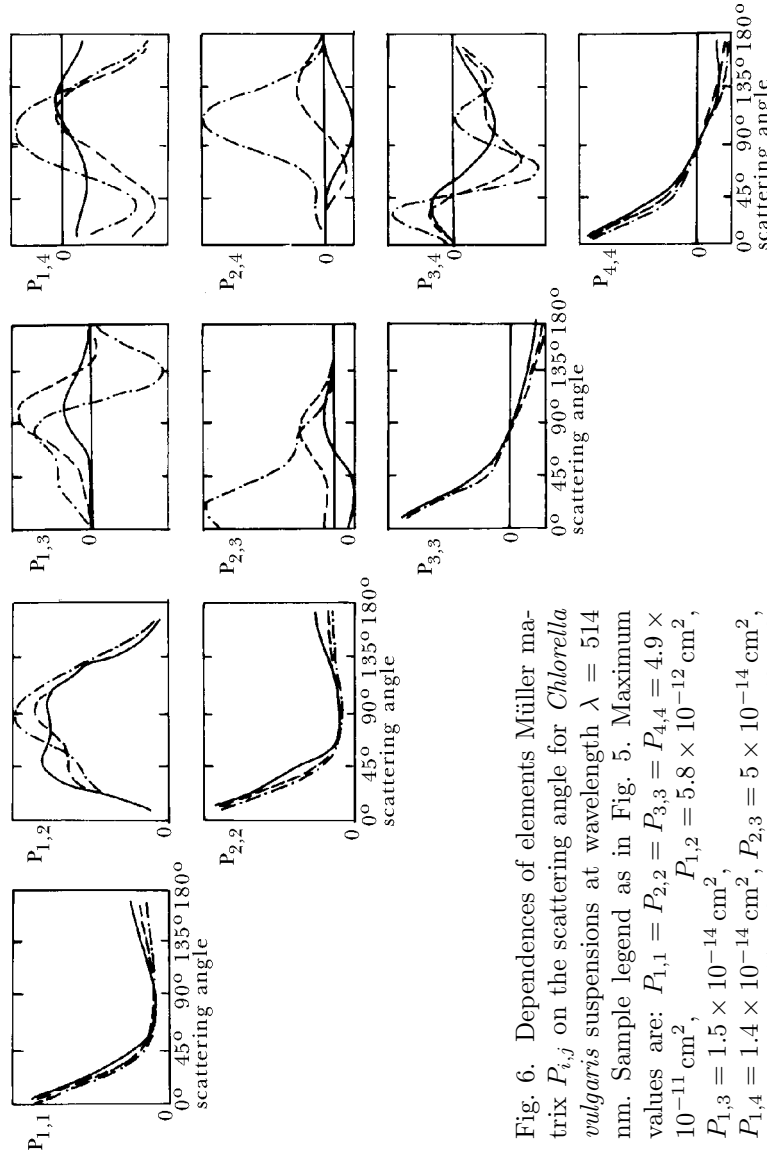

Fig. 6. Dependences of elements Müller matrix $P_{i,j}$ on the scattering angle for *Chlorella vulgaris* suspensions at wavelength $\lambda = 514$ nm. Sample legend as in Fig. 5. Maximum values are: $P_{1,1} = P_{2,2} = P_{3,3} = P_{4,4} = 4.9 \times 10^{-11}$ cm^2, $P_{1,2} = 5.8 \times 10^{-12}$ cm^2, $P_{1,3} = 1.5 \times 10^{-14}$ cm^2, $P_{1,4} = 1.4 \times 10^{-14}$ cm^2, $P_{2,3} = 5 \times 10^{-14}$ cm^2, $P_{2,4} = 2.2\ 10^{-14}$ cm^2, $P_{3,4} = 1.4 \times 10^{-13}$ cm^2; minimum values are: $P_{3,3} = p_{4,4} = -1.3 \times 10^{-11}$ cm^2, $p_{1,3} = -1.5 \times 10^{-14}$ cm^2, $p_{1,4} = -2.6 \times 10^{-14}$ cm^2, $p_{2,3} = -0.75 \times 10^{-14}$, $p_{2,4} = -0.6 \times 10^{-14}$, $p_{3,4} = -1.8 \times 10^{-13}$ cm^2

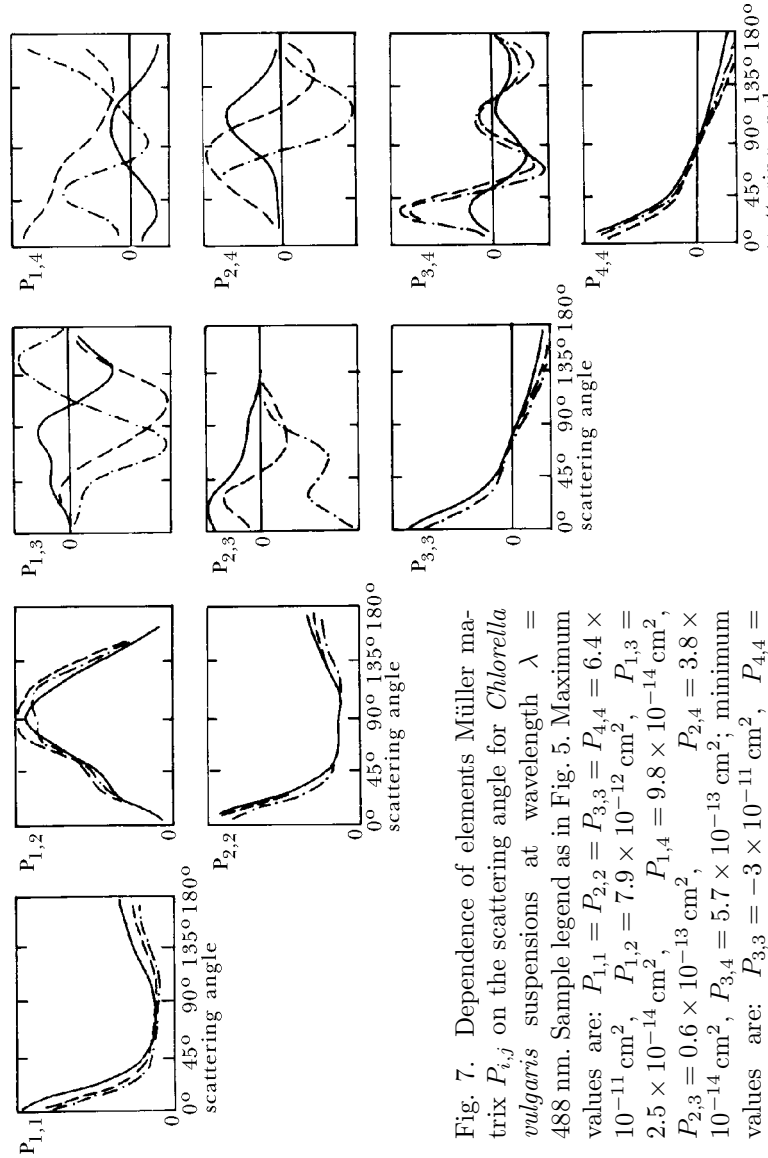

Fig. 7. Dependence of elements Müller matrix $P_{i,j}$ on the scattering angle for *Chlorella vulgaris* suspensions at wavelength $\lambda = 488$ nm. Sample legend as in Fig. 5. Maximum values are: $P_{1,1} = P_{2,2} = P_{3,3} = P_{4,4} = 6.4 \times 10^{-11}\,\mathrm{cm}^2$, $P_{1,2} = 7.9 \times 10^{-12}\,\mathrm{cm}^2$, $P_{1,3} = 2.5 \times 10^{-14}\,\mathrm{cm}^2$, $P_{1,4} = 9.8 \times 10^{-14}\,\mathrm{cm}^2$, $P_{2,3} = 0.6 \times 10^{-13}\,\mathrm{cm}^2$, $P_{2,4} = 3.8 \times 10^{-14}\,\mathrm{cm}^2$, $P_{3,4} = 5.7 \times 10^{-13}\,\mathrm{cm}^2$; minimum values are: $P_{3,3} = -3 \times 10^{-11}\,\mathrm{cm}^2$, $P_{4,4} = -2.3\ 10^{-11}\,\mathrm{cm}^2$, $P_{1,3} = -4.1 \times 10^{-14}\,\mathrm{cm}^2$, $P_{1,4} = -3.5 \times 10^{-14}\,\mathrm{cm}^2$, $P_{2,3} = -1.2 \times 10^{-13}\,\mathrm{cm}^2$, $P_{2,4} = -3.8 \times 10^{-14}\,\mathrm{cm}^2$, $P_{3,4} = -2.3 \times 10^{-13}\,\mathrm{cm}^2$

The sensitivity of the element $P_{3,4}(\theta)$ to changes in the ratio R/λ of the characteristic dimensions R of the scattering structure to the wavelength λ has been mentioned by several authors – see, *e.g.* Bickel (1976) or McClain *et al.* (1984).

The complete sets of measured scattering functions $P_{i,j}(\theta)$ for three types of samples and for three light wavelengths are given in Figs. 5–7. All the curves from these figures were obtained by interpolation from measurements performed in the 5° to 170° range, as shown in Fig. 4. Functions $P_{i,j}(\theta)$ are arranged according to the convention adopted in the literature (van der Hulst, 1957). Values of the off-diagonal elements are lower by 2 to 4 orders of magnitude than those on the main diagonal of the scattering matrix. This effect, predicted theoretically (Harris and McClain, 1977), results from the fact that off-diagonal elements $P_{i,j}(\theta)$ depend on the multipole radiation of higher orders.

The fact that the values of all off-diagonal $P_{i,j}(\theta)$ functions are nonzero proves that an important role in the scattering process under consideration is played by the intracellular structures, which are both geometrically and optically asymmetrical. It is a well-known fact that for Mie scattering from isotropically polarisable spheres or spherical shells, only $P_{3,4}(\theta)$ is nonzero, all other off-diagonal $P_{i,j}(\theta)$ being strictly equal to zero. Even for scattering from optically active spherical shells we obtain $P_{2,4}(\theta)$ and $P_{2,3}(\theta) = 0$, so we can conclude that we can observe light scattering effects from strongly non-spherical anisotropic structures (Bohren, 1975).

These off-diagonal scattering functions are particularly sensitive to the changes in intracellular structures caused by cells modification.

Comparing the scattering functions obtained for *Chlorella vulgaris* cells with similar functions measured for other scattering media, one can conclude that the values of off-diagonal functions $P_{i,j}(\theta)$ from Figs. 5–7 are rather small (but sufficiently large to be beyond the scope of experimental error). For example, aqueous suspensions of polystyrene latex spheres, whose diameter $d = 6\,\mu$m is approximately equal to the average diameter of the *Chlorella vulgaris* cell, exhibit $P_{3,4}(\theta)$ values about one order of magnitude greater than those reported here for the algal cells. In addition, for suspensions of polysaccharide macromolecules (Witkowski, 1992) – which can be considered a model of a medium which is optically anisotropic and rotates the polarisation plane of the light – the typically obtained values of $P_{2,3}(\theta)$ are ten or more times greater than those for *Chlorella* cells – see (Witkowski, 1992).

During our investigation we also found that the light scattering matrix elements depend on the stage of the physiological development of the

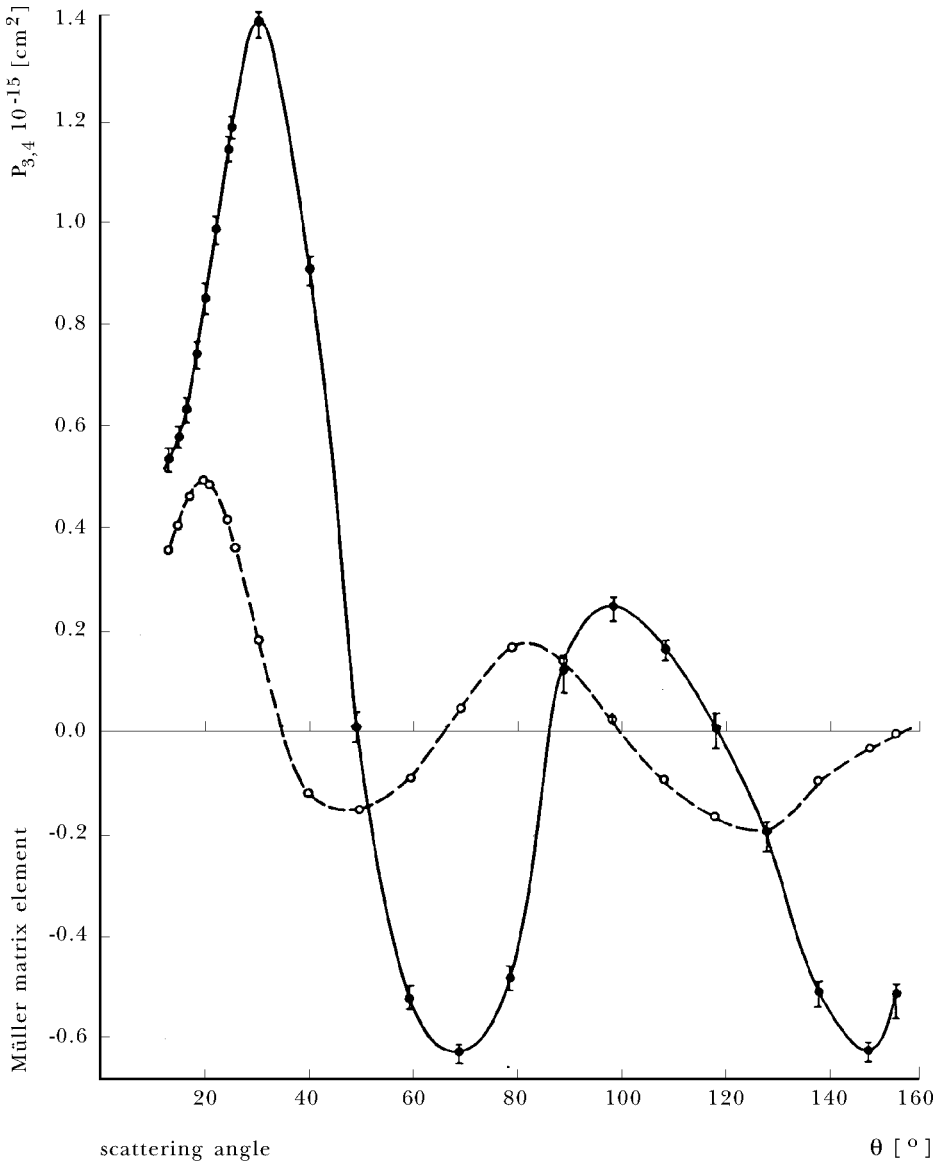

Fig. 8. Dependence of the scattering matrix elements $P_{i,j}$ on the incubation time of the live sample of *Chlorella vulgaris* cells. As an example, values of element $P_{3,4}$ are shown for incubation times $\tau < 5$ days (solid lines) and for periods from 7 to 10 days (broken line). Incident light wavelength $\lambda = 633$ nm

culture. This dependence is illustrated in Fig. 8, where values of the function $P_{3,4}(\theta)$ measured after one day are compared with those measured after seven days from the moment the culture was deposited in the fresh nutrient medium. Other $P_{i,j}(\theta)$ functions exhibit similar though less pronounced changes during the seven days of sample incubation. These results suggest that the optical activity of the intracellular scattering centres decreases with time, especially when cell development is inhibited after several days of incubation and when the fraction of young, frequently dividing cells decreases in the sample (Trainor, 1978).

5. Conclusions

Qualitative analysis of the experimental results shows that light scattering from phytoplankton cells should be considered a complex, multilevel phenomenon. Both absorption and light scattering from outer cell walls, intracellular structures and macromolecular complexes contained in the cell are involved in this process.

Scattering in the forward direction can be described by the Mie effect, *i.e.* scattering from the outer cellular wall treated as an isotropic spherical shell, but over the range of the medium scattering angles ($\theta > 10^\circ$), scattering from intracellular structures becomes dominant.

Though small, the off-diagonal scattering matrix elements determined for the investigated cell samples, seem to be particularly sensitive to changes in the intracellular structure.

Further quantitative analysis of the results obtained should enable this complex light scattering process to be modelled effectively.

References

Bickel W. S., Davidson J. F., Huffman D. R., Kilkerson R., 1976, *Measurements of circularly polarized components of the light scattered from suspensions of bacterial cells*, Proc. Natl. Acad. Sci. USA, 73, 486–488.

Bohren C. F., 1975, *Scattering of electromagnetic waves by an optically active spherical shell*, J. Chem. Phys., 62, 1566–1571.

McClain W. M., Scauerte J. A., Harris R. A., 1984, *Model calculations of intramolecular interference effects in Rayleigh scattering from solutions of macromolecules*, J. Chem. Phys., 80, 606–616.

Clayton R. K., 1965, *Modern physics in photosynthesis,* Blaisdell Publishing Company, Waltham, Mass.

Gędziorowska D., 1983, *Isolation of Baltic unicellular algae and obtaining axenic cultures for physiological and biochemical investigations*, Stud. i Mater. Oceanol., 41, 209–226, (in Polish).

Harris R. A., McClain W. M., 1977, *Toward a theory of the Perrin matrix for light scattering from dilute polymer solutions*, J. Chem. Phys., 67, 265–269.

Harris R. A., McClain W. M., 1985, *Elastic light scattering in diagonal polarization*, J. Chem. Phys., 82, 658–662.

van de Hulst H. C., 1957, *Light scattering by small particles*, Wiley, New York.

Król T., 1991, *The effect of the size of dispersed substances on absorption and scattering properties of dispersed media*, Stud. i Mater. Oceanol., 59, 175–181.

Król T., Zieliński A., Witkowski K., 1992, *Light scattering on Chlorella vulgaris cell*, Proc. Ocean Optics XI SPIE, 1750, 47–54.

Perrin F., 1942, *Theory of light scattering by macroscopically isotropic bodies*, J. Chem. Phys., 10, 415–421.

Schurcliff W. A., 1962, *Polarized light,* Harvard Univ. Press, Cambridge Mass.

Trainor F. R., 1978, *Introductory phytology*, Wiley, New York.

Witkowski K., 1986, *Simultaneous integral and quasi-elastic light scattering measurements for macromolecular solutions*, Optik (Stuttgart), 73, 91–96.

Witkowski K., 1992, *Ellipsometry of the light scattered from macromolecular solutions*, Optik (Stuttgart), 91, 54–65.

Zieliński A., Król T., Gędziorowska D., Woliński L., 1986, *Theoretical and experimental investigations into light scattering by Chlorella vulgaris cell*, Bull. Pol. Acad. Sci., Earth Sci., 34 (3), 313–319.

Zieliński A., Król T., Gędziorowska D., 1987, *The influence of the inner structure of the Chlorella vulgaris cell on light scattering properties*, Bull. Pol. Acad. Sci., Earth Sci., 35 (2), 119–125.