Optica Applicata, Vol. XXXII, No. 4, 2002

Changes of optical parameters in a suspension of *Scenedesmus obliquus* (chlorophyceae)

BARBARA PAWLAK

Institute of Physics, University of Szczecin, ul. Wielkopolska 15, 70–451 Szczecin, Poland.

Changes of optical parameters in a suspension of *Scenedesmus obliquus* are described. Size parameters were measured using a microscopic technique. Empirical distribution functions were approximated with the aid of φ-normal distributions. Spectral distributions of the light attenuation coefficient for *Scenedesmus obliquus* are illustrated and confidence intervals calculated. The form of the spectra is similar throughout all days and samples. Cell suspensions in culture evolve with respect to the dispersion distribution of the size parameter as well as the spectral distribution of the light attenuation coefficient. The evolution of these parameters was not unidirectional.

1. Introduction

PAWLAK and KOPEĆ [1] and PAWLAK [2] showed that vegetative suspensions of *Scenedesmus obliquus* undergo essential daily changes when grown in nutrient deficient conditions (in distilled water). Different indicators and methods can be used to examine the evolution of algal suspensions. The above authors analysed changes in dispersion distribution functions and found a good approximation with the help of φ-normal distributions. PAWLAK and KOPEĆ [1] analysed changes in the whole suspension, whereas PAWLAK [2] only looked at changes for some subsample of the suspension in which initial vegetation conditions were altered (different dilution degree of initial culture). Other important indicators of cell suspension status are optical properties, particularly the attenuation, scattering and absorption coefficients. These properties depend on the dispersion distribution, as well as on the shape and inner structure of the cell, the optical properties of cellular components (AAS [3]), and the content and concentration of dispersing solution. It is not necessary to consider all of these elements when comparing algal suspensions of the same species that differ only in storing time and initial concentration of dispersing solution. To evaluate changes in the suspension over time, aggregated parameters can be used (*e.g.*, the dispersion distribution of the size parameter aggregates geometrical properties and the spectral distribution of the attenuation coefficient aggregates optical properties of the cell material and the solution). Because the combined influence of these factors was analysed, attenuation coefficient was measured with respect to distilled water. The

purpose of this study is to use these two basic parameters to compare the temporal variability of algal suspensions as influenced by the initial concentration of cells.

2. Distribution of size parameter

The size parameter was measured using a microscopic technique described by GURGUL and KOPEĆ [4], DERA [5], GURGUL *et al.* [6], and PAWLAK and KOPEĆ [1]. A population of the alga *Scenedesmus obliquus* was prepared in distilled water and stored for five days. 3400 measurements of cell sizes were taken using the microscopic technique. The Ferret diameter was measured with accuracy of 1 μm. The number of cells in a given standard volume $v_0 = 0.45$ mm^3 was measured on a Bürcher table. Spectrophotometric measurements were performed using two beam spectrophotometer SPECORD UV-VIS in the range of wavelengths 330–750 nm. Distribution functions were approximated with the aid of φ-normal distributions using the following generating functions:

$$\varphi(x) = x - C^{1+1/\alpha}\,\frac{\mathrm{SD}}{D(\alpha)}\,\frac{1}{|x-B|^{\alpha}+C}, \quad C>0, \quad \alpha \geq 1, \quad -1 \leq \mathrm{SD} \leq 1, \tag{1}$$

$$\varphi(x) = x - \sqrt{C}\,\frac{\mathrm{SD}}{\mathrm{EMA}}\,\frac{z+p}{z^2+1}, \quad z = \frac{x-B}{\sqrt{C}}, \quad C>0, \quad \frac{\mathrm{EMA}}{\mathrm{EMI}} \leq \mathrm{SD} \leq 1 \tag{2}$$

where EMA and EMI are correspondingly maximum and minimum values of the function $E(z) = (1-2pz-z^2)/(z^2+1)^2$; p and B can be arbitrary, and SD – is a parameter defined in [7];

Table 1. Parameters of distribution functions.

Sample	Day	Number of function	$P(\alpha)$	SD	B	C	μ	σ	χ^2	Signal level for 5 degrees of freedom
0	1	2	0.3	–2.1601	10.2665	4.3491	10.5089	3.9840	1.3099	0.9339
	2	2	–0.5	1.0	13.3789	0.7648	9.9112	2.6468	0.6715	0.9845
	3	1	4.5	–0.7512	12.5168	9.9664	10.6143	2.662	1.2659	0.9384
	4	1	5.2	1.0	13.9565	14.5461	9.5389	1.9684	2.8367	0.7252
	5	2	–2.5	1.0	12.6275	0.2791	9.6267	2.5292	2.0744	0.8388
1	1	1	1	–0.9844	11.48	6.3442	14.5969	3.2607	3.8538	0.5707
	2	4		–2	10.8376	1.494	2.2562	0.296	3.2631	0.6595
	3	2	0.5	1.0	14.4564	4.3643	9.7469	2.0845	0.9825	0.9640
	4	4		0.9834	9.891	0.7951	2.2009	0.2745	0.9316	0.9679
	5	4		1.0	10.0908	1.9673	2.189	0.245	2.6222	0.8999
2	1	1	1.7	–0.6192	12.3306	13.3047	13.4626	3.1941	0.9261	0.9683
	2	2	–1.0	1.0	13.0003	2.6278	10.9	2.8419	0.9147	0.9691
	3	1	1.8	1.0	14.7669	3.6008	8.7443	1.617	2.6531	0.7533
	4	2	2.5	1.0	13.3911	0.9174	9.6009	2.3044	0.5232	0.9913
	5	1	7.5	1.0	15.042	10.5388	9.7805	3.0883	1.1253	0.9518

$$\varphi(x) = \ln x - \frac{SD}{BC}\exp(-C|x-B|), \quad C>0. \tag{3}$$

The distribution function is described by the formula

$$F(x) = \Phi\left(\frac{\varphi(x)-m}{\sigma}\right) \tag{4}$$

where Φ is a distribution function of standardised normal distribution. Parameters B, C, SD, p are determined by minimalisation of the statistics χ^2, and m and σ are determined from the sample using largest likelihood method. Inequalities for SD assure monotonicity of the function F. The parameters for functions that generate optimum approximations of the empirical distribution function and estimations of the accuracy of these approximations are given in Tab. 1.

3. Spectral distributions of attenuation coefficient

The applicability of the light attenuation coefficient to assess the degree of water contamination has been examined by many authors, *e.g.*, DERA *et al.* [8], JONASZ [9]–[11], KRĘŻEL [12], TOPLISS [13], BAKER and LAVELLE [14], BRICAUD and MOREL [15], BRICAUD, BEDHOMME and MOREL [16], BRICAUD, ROESLER and ZANEVELD [17], and STEEPER [18]. KOPEĆ and PAWLAK [19] and PAWLAK [2] showed that this method was particularly effective when the spectral distribution of the attenuation coefficient could be well approximated by the function

$$c(\lambda) = A\exp(-\alpha\lambda) + \frac{B}{|\lambda+\beta|^p} + D(\alpha)$$

where $D(\alpha) = (\alpha+1)^{1+1/\alpha}(\alpha-1)^{1-1/\alpha}/(4\alpha)$, $D(1) = 1$, $\alpha \geq 1$.

This function is applicable in artificial crude oil emulsions in water and in natural low salinity, slightly contaminated water areas. In areas where there is strong water contamination, however, a good approximation can usually only be obtained in some subintervals of the visible spectrum.

PAWLAK [2] analysed changes in the dispersion distribution of the size parameter to assess the diurnal variability of *Scenedesmus obliquus* suspensions differing in initial cell concentration. In the present study, this variability is examined by evaluating changes in the spectral distribution of the light attenuation coefficient. The sample 0 corresponds to concentration of suspensions in strongly contaminated water

T a b l e 2. Number of *Scenedesmus obliquus* cells on subsequent days in samples 0, 1 and 2.

Sample \ Day	1	2	3	4	5
0	390	350	346	448	342
1	126	110	92	93	119
2	74	43	68	76	56

Table 3. Experimental values of light attenuation coefficient (m^{-1}) for sample 0 on subsequent days of measurement.

Wavelength [nm]	Day 1	Day 2	Day 3	Day 4	Day 5
333.5	132.9	117.6	121.5	170.3	162.2
355.3	121.5	114.9	115.5	132.9	127.5
384.9	118.3	113.1	114.9	126.4	114.9
400.9	117.6	110.4	113.1	123.3	111.5
425.5	119.1	111.5	115.5	124.3	110.4
454.9	113.7	107.6	109.9	114.9	102.3
484.9	109.0	102.7	106.4	107.6	94.9
500.5	104.1	98.4	101.3	100.1	88.8
521.3	87.8	84.0	87.2	83.3	74.2
541.7	79.9	77.1	79.9	75.2	66.8
561.1	78.1	75.1	78.3	72.7	63.8
582.0	80.6	77.3	81.0	74.5	64.3
604.5	82.5	79.1	83.1	75.8	64.6
625.7	88.3	84.0	889.0	80.5	67.3
641.8	89.8	85.1	90.2	81.5	67.7
662.2	90.8	86.1	91.3	83.6	70.9
680.2	101.7	96.3	101.3	95.6	81.8
703.1	77.7	74.9	78.8	72.4	63.3
719.3	68.8	66.7	69.7	63.8	55.7
754.0	64.5	62.4	65.3	59.3	51.2

areas. Subsamples (samples 1 and 2) were obtained from the initial algal suspension (sample 0) and diluted with distilled water. Table 2 shows relative concentrations of cells as the number of cells in a given volume in subsequent samples and on subsequent days. The light attenuation coefficient was measured using a technique described by PAWLAK and KOPEĆ [1] and PAWLAK [2]. Daily attenuation coefficient measurements from all samples are given in Tabs. 3, 4, and 5. Diagrams of light attenuation as a function of wavelength are similar troughout all days and samples (taking into consideration the degree of dilution). In each diagram, the attenuation coefficient decreases considerably in the short wavelength part of the spectrum, and the wide transmission window lies in the 540–625 nm range. A relatively sharp local maximum exists at 680 nm followed by a rather rapid decrease in the long wavelength part of the spectrum (above 700 nm). On the first and the third days of observation, the attenuation coefficients are similar, especially for $\lambda > 500$ nm; however, the coefficients measured are quite different on the 2nd, 4th, and 5th days.

The smallest standard deviation (with respect to wavelength) among daily measurements of light attenuation (measurement error) is equal to 4.85, 2.58, and 1.36 in samples 0, 1, and 2, respectively. The error of a single measurement in suspensions and emulsions estimated by KOPEĆ and PAWLAK [19] was less than 0.2 m^{-1} for measured

T a b l e 4. Experimental values of light attenuation coefficient (m^{-1}) for sample 1 on subsequent days of measurement.

Wavelength [nm]	Day 1	Day 2	Day 3	Day 4	Day 5
333.5	43.1	39.8	43.4	37.0	32.0
355.3	36.9	33.6	38.1	31.6	27.4
384.9	33.7	30.7	34.7	28.7	24.5
400.9	32.8	29.9	33.9	27.9	23.7
425.5	33.2	30.3	34.5	27.8	23.4
454.9	31.8	29.1	33.0	26.3	22.1
484.9	29.8	27.3	31.0	24.5	20.6
500.5	28.5	26.2	29.8	23.6	19.6
521.3	24.9	23.2	26.2	21.0	17.3
541.7	22.7	21.3	24.1	19.3	15.7
561.1	21.8	20.4	23.1	18.5	14.8
582.0	21.8	20.3	23.2	18.5	16.6
604.5	21.8	20.3	23.2	18.4	14.5
625.7	22.9	21.3	24.3	19.1	15.0
641.8	23.2	21.7	24.7	19.3	15.0
662.2	23.0	21.5	24.6	19.3	15.3
680.2	25.9	24.3	27.7	21.5	17.3
703.1	21.7	20.5	23.4	18.4	14.7
719.3	19.6	18.6	21.2	16.7	13.2
754.0	18.2	17.2	19.6	15.5	12.1

values of the order of 10 m^{-1}. Comparison of these standard deviations shows that diurnal measurements of the light attenuation coefficient in all samples come from the same population, hence the final population is subjected to diurnal changes with all examined concentrations of cells.

More exact estimates for the suspension under examination can be provided based on formula (5) calculating the sum H of squared deviations of calculated and measured values, *e.g.*, for $333.5 \leq l \leq 541.7$ nm (10 measurements). The value of $\sigma^2 = H/(n-p)$ is an unbiased estimate of variance in the sample, where n is the number of measurements and p is the number of parameters determined from the sample using the least squares method (5 since the exponent was assumed to be $p = 1$) [19], [20]. The obtained values of H and σ (summary statistical and approximation error) are given in Tab. 6.

If diurnal measurements of the light attenuation coefficient in samples came from the same population, then for the lowest variance in different samples, confidence levels for $\alpha = 0.01$ would have the form: sample 0 <2.81; 23.81>, sample 1 <1.5; 12.71>, sample 2 <0.794; 6.667>.

The calculated value of σ lies outside these intervals in all samples, hence the hypothesis that measurements are from the same population must be rejected at the

T a b l e 5. Experimental values of light attenuation coefficient (m^{-1}) for sample 2 on subsequent days of measurement.

Wavelength [nm]	Day 1	Day 2	Day 3	Day 4	Day 5
333.5	28.7	24.9	23.3	20.3	17.8
355.3	25.6	23.4	19.6	17.2	15.0
384.9	24.2	22.2	17.8	15.6	13.4
400.9	23.3	21.2	17.4	15.3	13.0
425.5	21.0	19.0	17.5	15.3	12.8
454.9	18.4	16.2	16.9	14.6	12.0
484.9	16.5	15.3	15.9	13.6	11.2
500.5	15.7	14.4	15.3	13.1	10.8
521.3	14.6	13.4	13.7	11.7	9.5
541.7	13.5	12.3	12.5	10.8	8.7
561.1	12.9	11.7	12.0	10.3	8.3
582.0	12.3	11.1	12.0	10.3	8.1
604.5	11.9	12.0	12.9	10.2	8.1
625.7	11.6	10.5	12.5	10.6	8.3
641.8	11.4	10.4	12.7	10.7	8.2
662.2	11.3	10.3	12.6	10.6	8.4
680.2	11.1	10.2	14.3	11.9	9.5
703.1	11.0	10.2	12.2	10.2	8.1
719.3	11.0	10.0	11.0	9.3	7.3
754.0	11.1	10.1	9.9	8.6	6.8

T a b l e 6. Values of H (sums of deviations squared) and of σ (summary statistical and approximation error).

	Sample 0	Sample 1	Sample 2
H	25.03	1.052	0.2104
σ	2.237	0.458	0.205

significance level $\alpha = 0.01$ for sample 0 (and at significantly lower level for other samples).

In sample 0, mean values of light attenuation coefficient are quite different in sampling days. The largest mean value for light attenuation coefficient occurs on day 1, and the smallest value occurs on day 5. This decrease, however, is not uniform as the following data shows:

Mean value	96.32	95.66	94.66	91.20	85.21
Day	1	4	3	2	5

One can deduce that the above configuration of mean values results mainly from differences in distribution functions of dispersion distributions. From the data presented in the paper [2], it follows that the distribution functions differ significantly, and as Tab. 7 shows, the significance level differs between the fifth distribution and

T a b l e 7. Significance levels in subsequent pairs of days.

Significance level	8.6×10^{-5}	0.0085	0.0114	0.999	1
Pair of days	5 and 1	5 and 4	5 and 3	5 and 2	5 and 5

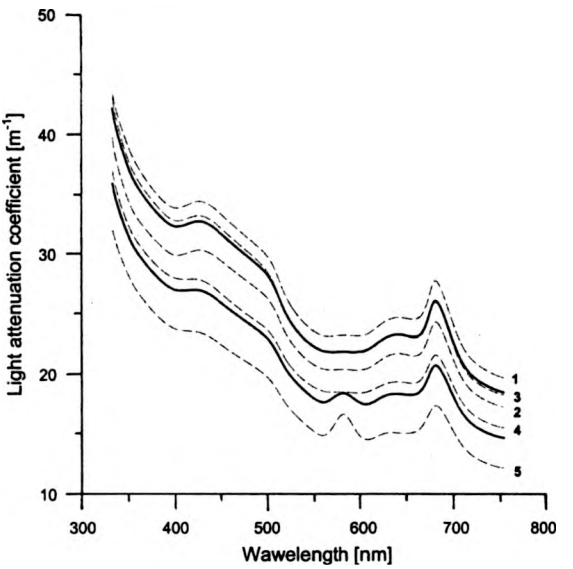

Figure. Spectra of experimentally obtained light attenuation coefficients for sample 1. Dashed lines show subsequent days of measurement and solid lines show confidence intervals and positions with respect to different measurements.

the other distributions. The identical order of days in both configurations confirms the above hypothesis.

In other samples distribution functions of dispersion distributions do not differ significantly over time, but attenuation coefficients differ at the significance level of 0.01 for all wavelengths. For sample 1, mean values for the distributions on day 4 and day 2 lie inside the confidence interval (at the level of 0.1), but the mean value for the distribution on day 5 lies to the left of the confidence interval, and values for the distribution on day 3 lie to the right of the confidence interval for all wavelengths. The Figure shows that the largest attenuation coefficient differences are between distributions 3 and 5.

In sample 2 the differences are less evident. However, the mean values for the distribution on day 5 always lie to the left of the interval, but the mean values for the distributions on days 1 and 3 are consistent to the right of the interval.

4. Conclusions

The influence of alga population on water ecosystem is well known. Algae are the main link of alimentary chain of water organisms as well as circulation of oxygen,

carbon dioxide and mineral substances. Natural development of alga populations was perturbed in the last decades, due to the human activity. Large quantities of mineral substances (nitrogen, phosphorus) introduced to the ecosystem, mainly as fertilisers, caused in some periods, substantial uncontrolled increase of these populations. This reduces the usefulness of water regions for municipal, industrial, fishing and recreation purposes. Therefore, in order to limit the resultant losses, it is necessary to observe the evolution of alga population, and investigate its dependence upon the initial conditions.

The most important optical methods allowing such an observation and investigation are examination of changes of size parameter of dispersion distribution with time (microscopic method) and investigation of spectral distribution of optical parameters (mainly light attenuation coefficient).

In our paper, we have used generalised normal distribution to describe dispersion distribution and statistical methods for spectral distribution. We have shown that employing both methods enables us to obtain better description of the evolution of alga and its dependence upon initial conditions, than using one of these methods separately.

Our conclusions are as follows:

– When suspensions of *Scenedesmus obliquus* were placed in nutrient deficient conditions with different initial cell concentrations, these suspensions underwent significant diurnal changes. In all samples the light attenuation coefficient changed over time for all wavelengths examined (significance level = 0.01), but in sample 0, the distribution function and the generating function (or its parameters for dispersion distribution of size parameter, Tab. 1) also changed over time.

– Patterns of the spectral distribution of the light attenuation coefficient were similar in all days and samples and were characterised by decreasing attenuation coefficients in the short wavelength part of the spectrum, a wide transmission window in the 540–625 nm range, and a sharp maximum at 680 nm followed by a progressive decrease in the attenuation coefficients in the long wavelength part of the spectrum. The values of the light attenuation coefficients for the first and the third days were similar at $\lambda > 500$ nm for samples 0 and 2 and throughout the spectrum for sample 1.

– With a large initial concentration of cells (sample 0), changes in the suspension over time were mainly observed in the variability of the distribution function of the size parameter dispersion distribution. The changes in the spectral distribution of the light attenuation coefficient were the consequence of this variability. "Optical activity" of the suspension (measured by the mean value of the light attenuation coefficient) was maximal on the first day, decreased on the second day, and returned to the initial level on the third and fourth days. Optical activity then decreased rapidly on the fifth day. The increase in light attenuation on the fourth day may not only have been caused by changes in the distribution function, but this increase may also have been due to maximal cell densities that occurred on the fourth day.

– As cell concentration decreased, the changes of the distribution functions of the dispersion distribution became stochastically unimportant; however, the light attenuation coefficient continued to show differentiation. Because the mean light

attenuation coefficient and mean cell number on subsequent days are weakly correlated in samples 1 and 2, the differentiation in light attenuation could indicate changes in optical properties of intracellular or extracellular fluids.

– We think that the present methods for analysing variability of an algal suspension over time as influenced by nutrient deprivation and initial cell concentration can be used to examine the influence of other factors on algal suspensions.

Acknowledgments – The author thanks Prof. J. Kopeć for help in writing numerical program for calculation of parameters of spectral distributions of the light attenuation coefficient.

References

[1] PAWLAK B., KOPEĆ J., Oceanologia 4 (1998), 345.
[2] PAWLAK B., Acta Physica US **11** (2000), 45.
[3] AAS E., J. Plankton Res. **18** (1996), 2223.
[4] GURGUL H., KOPEĆ J., *Appearance of crude oil substances in form of emulsions in Odra river estuary* (in Polish), [In] Studia i Materiały Oceanologiczne PAN (1986), No. 49.
[5] DERA J., *Marine Physics*, Elsevier, Amsterdam 1992, pp. 120–181.
[6] GURGUL H., KOPEĆ J., WIERZBICKA-KHARRAZ I., Acta Phys. **2** (1992), 71.
[7] PAWLAK B., Disserations and Studies T. (CDXL) 366, 1.
[8] DERA J., GOHS L., WOŹNIAK B., Oceanologia **10** (1978), 5.
[9] JONASZ M., *Using Light Scattering Phenomenon for Determination of Physical Properties of Sea Sediments* (in Polish), Ph.D. Thesis, Polish Academy of Sciences (PAN), Sopot 1980.
[10] JONASZ M., *The particle size distribution in the Baltic,* [In] *XIV CBO Conference*, Helsinki 1984.
[11] JONASZ M., J. Geophys. Res. C **13** (1987), 14637.
[12] KRĘŻEL A., Oceanography 7 (1980), 25 (in Polish).
[13] TOPLISS B. J., Mar. Environ. Res. **4** (1982), 295.
[14] BAKER E.T., LAVELLE J.W., J. Geophys. Res. C **89** (1984), 8197.
[15] BRICAUD A., MOREL A., Appl. Opt. **25** (1986), 571.
[16] BRICAUD A., BEDHOMME L., MOREL A., J. Plankton Res. **10** (1988), 851.
[17] BRICAUD A., ROESLER C., ZANEVELD J.R.V., Limnol. Oceanogr. **40** (1995), 393.
[18] STEEPER R.R., J. Phys. E **21**(1988), 378.
[19] KOPEĆ J., PAWLAK B., Oceanologica Acta. **21** (1998) 623.
[20] BARTOSZEWICZ J., *Lectures on Mathematical Statistics,* [Ed.] PWN, Warszawa 1989, pp. 205–207 (in Polish).

Received February 28, 2002
in reviced form July 15, 2002