ARCHIVES OF ENVIRONMENTAL PROTECTION

vol. 35 no. 1 pp. 65 - 73 2009

PL ISSN 0324-8461

CHANGES OF PHOTOSYNTHETIC PIGMENTS CONCENTRATION IN THE SYNCHRONOUS CULTURE OF *CHLORELLA VULGARIS* AS AN INDICATOR OF WATER QUALITY IN GOCZAŁKOWICE RESERVOIR

ANNA CZAPLICKA-KOTAS[1*], JOLANTA LODOWSKA[2], ADAM WILCZOK[3], ZBIGNIEW ŚLUSARCZYK[1]

[1] Cracow University of Technology, Institute of Water Supply and Environmental Protection
ul. Warszawska 24, 31-155 Kraków, Poland
[*] Corresponding author e-mail: aczapl@usk.pk.edu.pl
[2] Medical University of Silesia, Department of Biochemistry
ul. Narcyzów 1, 41-200 Sosnowiec, Poland
[3] Medical University of Silesia, Department of Biopharmacy
ul. Narcyzów 1, 41-200 Sosnowiec, Poland

Keywords: *Chlorella vulgaris*, synchronous culture, photosynthetic pigments, water quality control, Goczałkowice Reservoir.

Abstract: The aim of this study was to examine the possibility of use of synchronous culture of *Chlorella vulgaris* algae as a biotest in water quality control. In the experiment the samples of water collected from seven sampling points in Goczałkowice Reservoir were used. The criterion of changes was the concentration of photosynthetic pigments, from 24th hour of the cell life cycle, separated by HPLC technique. On the basis of changes taking place in the cells of the algae cultures it was possible to establish the timing of the flood period and autumnal changes in a water quality. It was also proved that the water quality in the main water current that fall into the western part of reservoir carried by the Wisła River after the flood period significantly differed from the water quality in the other parts of the reservoir, particularly in its eastern section.

INTRODUCTION

It is estimated that water is contaminated by about one million pollutants. In Poland the water quality is assessed by the physical, chemical and bacteriological analyses carried out according to the Polish standards, which do not include testing for all pollutants. The precise analysis of toxic substances in the water environment requires chemical analyses of a large number of samples. This is complicated and costly. Therefore, the use of biotest is a useful method of supporting the physicochemical analyses. Biotests are recommended in the USA and UE countries in the procedures of aquatic monitoring [17, 27]. In such biotests the general influence of contaminated environment on the growth of chosen living organisms is investigated. Since on the basis of biotests alone it is impossible to determine which components of complex mixture are responsible for the biological activity detected, it is necessary to combine these methods with the proper physicochemical analyses [20].

Asynchronous cultures of *Chlorella vulgaris* algae are often used and recommended by Polish [13] and European [27] directives. Published results of studies show that synchronous cultures of *Chlorella vulgaris* algae, in which the cells are at the same stage of life, give more uniform answers to the changes of toxicity than asynchronous cultures. Synchronous cultures allow investigating of processes at the all life stages of cells affected by the tested substances [8–10, 12, 16, 18–19, 22–23].

The first step of the investigations with synchronous cultures of *Chlorella vulgaris* algae in the environmental studies was undertaken in the Department of Molecular Biology, Biochemistry and Biopharmacy at Medical University of Silesia. The studies were focused on changes of culture absorbance and the rate of cellular division in synchronous culture of *Chlorella vulgaris* algae (cultivated in industrial wastewater from the Odra river watershed diluted with the distilled water) was determined [21]. Lodowska *et al.* [7] cultivated those algae in synchronous culture in tannery wastewater from Skoczów diluted with the distilled water. Czaplicka-Kotas [2], who investigated the water from the Goczałkowice Reservoir, showed that the changes of the concentration of photosynthetic pigments are the best criterion of water quality changes.

In the investigations mentioned above the following criteria were used:

- changes in the cell culture absorbance (per million cells) at 680 nm, spectrophotometric measurements were performed every hour during the first ten hours and at the 24^{th} hour of synchronous growth;
- changes in the number of algae cells in the given culture, the number of cells was counted in the Bürker's chamber, the results were used to establish the rate of cellular division;
- changes of the concentration of photosynthetic pigments (using HPLC).

The aim of this study was to examine the possibility of the use of synchronous culture of *Chlorella vulgaris* algae as a biotest in water quality control in the Goczałkowice water reservoir.

MATERIALS AND METHODS

Investigated *Chlorella vulgaris* (Beijerinck 1890; strain A-8) were synchronously cultured (Fig. 1) on the sterile mineral Kühl and Lorenzen medium [6] modified by Borns [1] ($5 \cdot 10^6 \pm 0.5 \cdot 10^6$ cells/cm^3). Every experiment was conducted in two measurement series. In the first, algae were cultivated in a medium prepared with clean water (control). In the second algae were cultivated in media prepared with water from sampling points from the Goczałkowice Reservoir. The algae cultures were carried under the stable conditions: temperature 30°C, illumination 15000 lux on the reactor surface during 10 hours of the light period followed by 14 hours of the dark period. During the light period, the cultures were aerated by air (30 dm^3/hour) supplemented with 2% CO_2, and during the dark period by air without CO_2. According to scheme of the cell life cycle of *Chlorella vulgaris* (Fig. 1) the development and growth of all stadium from G1 to cytokinesis is strongly dependent on illumination and culture conditions. From the very beginning of the dark phase, cytokinesis and sporulation take place [11].

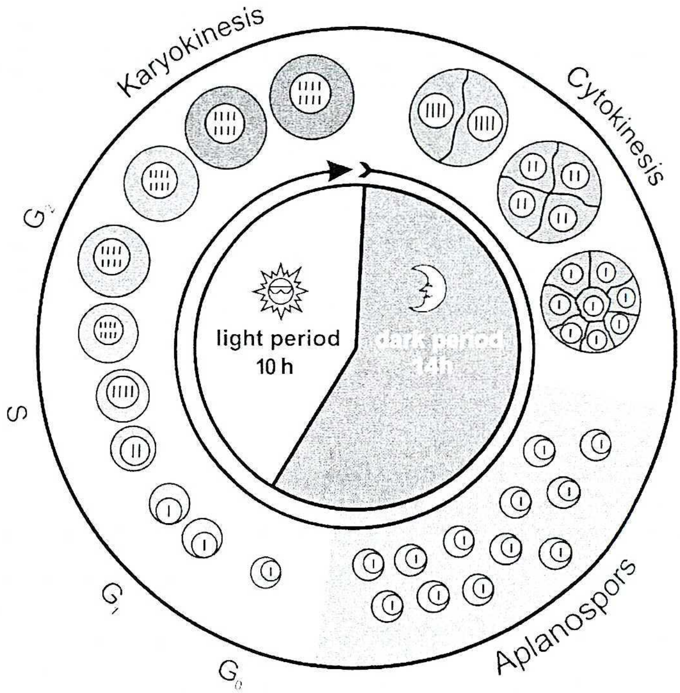

Fig. 1. Scheme of the cell life cycle of *Chlorella vulgaris*, Beijerinck 1890 [11]

Water samples, used in the experiment, were taken in the seven sampling points from the Goczałkowice Reservoir (Southern Poland, Upper Silesia region) (Fig. 2). Water samples were taken into acid clean polyethylene containers. Before the experiment water samples were filtered (0.45 m Millipore filters).

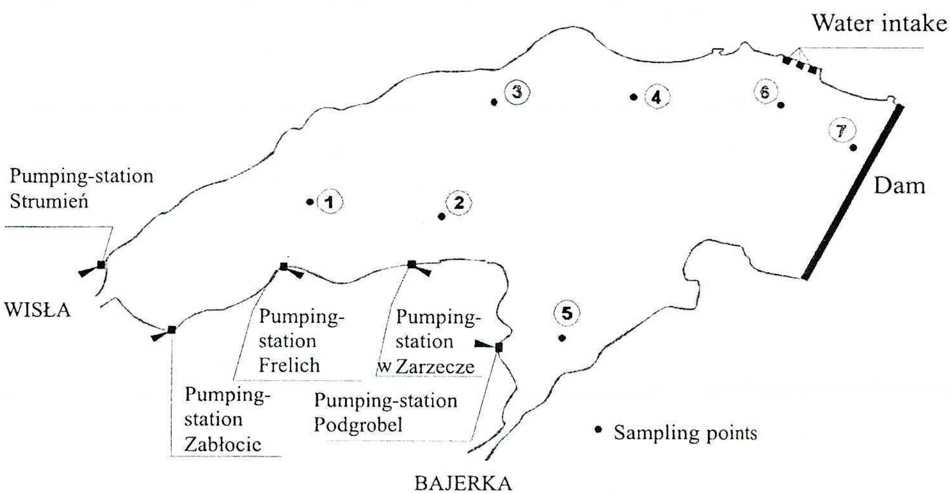

Fig. 2. Goczałkowice Reservoir with the localization of the water sampling points

Samples (20 cm^3) of *Chlorella vulgaris* suspension from the 24th hour of the life cycle were centrifuged (2400·g, 15 minutes), dried and resuspended in the pure methanol. Pigments were extracted from this mixture by boiling at 65 ± 2°C for 10 minutes. Extracts were brought up to 3 cm^3 with methanol and then centrifuged (2500·g, 3 minutes) [12].

Suspensions were filtered (0.22 m Millipore filters) and analyzed using 1050 Hewlett Packard liquid chromatograph equipped with an on-line degasser and variable wavelength detector UV/VIS (set at 440 nm). Aliquots ($20 \cdot 10^{-3}$ cm^3) were injected on C_{18} column Eurospher 100, Knauer (250 x 4 mm) using an autosampler.

Photosynthetic pigments were eluted by the gradient mixture of methanol – water (85–100% methanol in 15 minutes, followed by 100% methanol for 35 minutes). The flow rate was 1 cm^3/minute, the temperature 30°C and the total time of analysis 50 minutes. The chromatographic procedure was controlled by the HPLC ChemStation A.06.03 (Hewlett Packard). Photosynthetic pigments were identified by comparison with the commercially available standards and the literature references [14–15, 24–25].

To assess the impact of the Goczałkowice Reservoir water quality changes on the photosynthetic pigments synthesis in *Chlorella vulgaris* algae, the relative concentration factor (W_m) was used according to the equation:

$$W_m = \sqrt{\sum_{i=1}^{L_B} \sum_{j=1}^{L_P} (a_{ij}^m - 1)^2}$$

where: a_{ij}^m – relative value (to the control sample) of i photosynthetic pigment and j sampling point in m month,

m – month (VI – X),

L_B – number of photosynthetic pigments (nine),

L_P – number of sampling points (1–7).

This factor integrates the effect for the nine photosynthetic pigments. Each photosynthetic pigment can be described separately as was shown in the monograph of Czaplicka-Kotas [2].

The study of similarity profile of concentration changes of the nine identified photosynthetic pigments was performed using the cluster analysis. The applied similarity measure was the Euclidean distance. The distance among clusters was calculated by average between groups linkage method [4]. Statistic analysis ($p = 0.05$) was performed with the SPSS software.

RESULTS

On the basis of performed investigations it was concluded that the analyzed algae cells contain the following photosynthetic pigments: neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll a, chlorophyll b, α-carotene, β-carotene.

To compare synthesis changes of all photosynthetic pigments in *Chlorella vulgaris* algae from the seven sampling points localized in the Goczałkowice Reservoir the relative concentration factor of photosynthetic pigments was calculated. The relative concentrations of photosynthetic pigments were calculated on the basis of quotient for the concentration of photosynthetic pigment in the investigated and control cultures. The relative concentration in the control culture was equal 1. The Figure 3 shows that the relative concentration factor of photosynthetic pigments (W_m) was close to the control culture (factor equal 1) in the July and August. In the September and October the difference was the biggest.

To group profiles of the concentration changes of all photosynthetic pigments the clusters analysis with Euclidean distances as a criterion of grouping was used. In each profile the sum of the relative concentrations of all identified pigments in each sampling site in a particular month was taken into consideration. Results are presented as the dendrogram (Fig. 4).

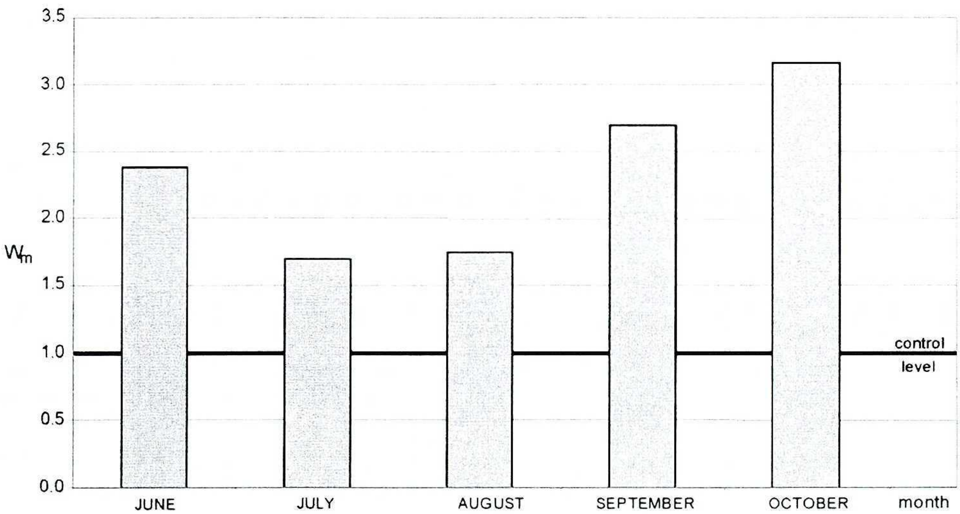

Fig. 3. Relative concentration factor of the photosynthetic pigments (W_m)

DISCUSSION AND CONCLUSIONS

In the investigated period (from June to October 1997) the water quality changes were affected by July flood and additional autumnal loading of contaminated waters from farm-fishing ponds.

The relative concentration factor of the photosynthetic pigments extracted from the algae cultivated on the water from the Goczałkowice Reservoir (Fig. 3) had the closest values to the factor for the control culture in the flood period and the following month (July and August). It is obvious that contaminants in the reservoir are diluted with water inflow from the watershed. The flood inflow is enriched with the rock material during the fall period. This material comes from the watershed and forms a new lake bed. As the result of natural processes in the lake the quantity of biogenic substances is increased [3]. Because of the decreased temperature and decreased algae activity these substances are not used by algae. Increased amount of biogenic substances is caused by the phosphorous and ammonia nitrogen from farm-fishing ponds in the Goczałkowice Reservoir water-

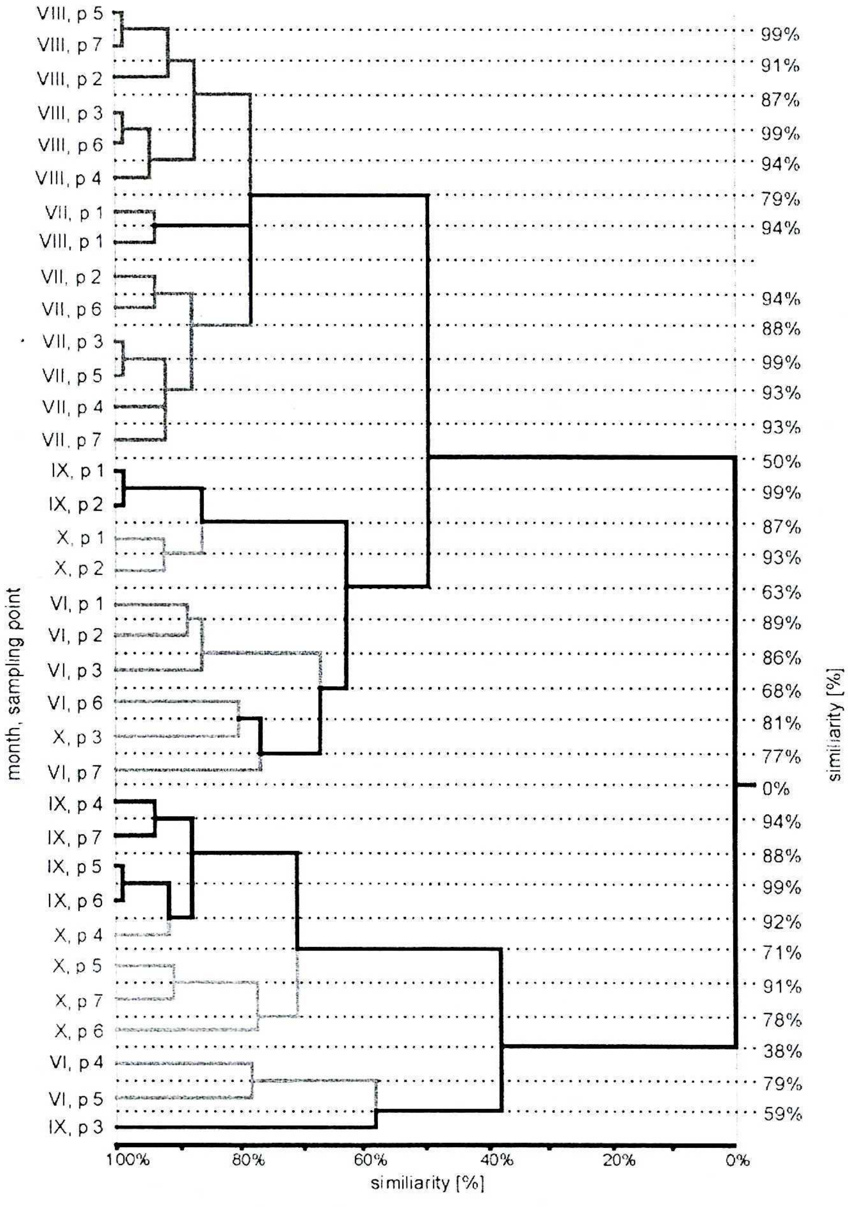

Fig. 4. Similarity dendrogram of the relative Euclidean distances calculated for profile of photosynthetic pigments changes (neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll b, chlorophyll a, α-carotene and β-carotene) in *Chlorella vulgaris* cells cultivated in the water from Goczałkowice Reservoir in June – October (VI–X) 1997

shed [5]. The farm-fishing ponds complex in this region is the biggest in Central Europe with the area of 900 ha [26]. *Chlorella vulgaris* algae cultivated on the Goczałkowice Reservoir water sampled in September and October synthesized the highest amounts of photosynthetic pigments. The relative factor values in this period were the most different from the factor for the control culture.

The dendrogram showing the similarity profiles of relative concentrations of all photosynthetic pigments changes in *Chlorella vulgaris* cells cultivated in the water from the Goczałkowice Reservoir is presented in the Figure 4. This dendrogram reflects the similarity and differences of water quality from June to October 1997. The Euclidean distances showed similarity for the pigments from algae cultivated on the water from July and August. Profiles of photosynthetic pigments from algae cultivated on water sampled in June and in sampling sites 1 and 2 (located in the western part of the reservoir close to the Wisła River inflow) in September and October created a separate cluster (similarity 63%). The significant difference (similarity 0%) was found among described clusters and the cluster of photosynthetic pigments profiles in algae cultivated on waters from sampling points 4, 5, 6, 7 (localized in the eastern part of the reservoir) from September and October. These profiles show that the water quality in the main water flow from the Wisła River was significantly different after the flood period, especially in the eastern part of the reservoir, what has not been documented by the results of the standard chemical and biological investigations [2].

The analysis of the results presented in the study allows for drawing the final conclusions:

1. On the ground of the photosynthetic pigments synthesis, it was shown that the *Chlorella vulgaris* algae Beijerinck 1890 of the A-8 strain are sensitive to water quality changes of the Goczałkowice Reservoir.
2. The analyses of concentration changes of photosynthetic pigments distinguish the July flood period and autumnal water quality changes.
3. Basing on the relative concentration factor of photosynthetic pigments in algae cultivated in water from July and August, it could be stated that concentrations of pigments are the closest to control, thus the water was the cleanest.
4. Concentration variations of photosynthetic pigments in *Chlorella vulgaris* cultivated synchronously in water collected from different points of the Goczałkowice Reservoir demonstrate that the water quality in the Wisła mainstream (western part of the reservoir) after the flood period differed greatly from the water quality in other parts of reservoir, particularly in the eastern section.

Acknowledgements

This work was a part of PhD thesis of Anna Czaplicka-Kotas entitled "Synchronic culture of Chlorella vulgaris algae in the water quality control" supervised by Professor Beata Cwalina. The authors would like to express cordial thanks to Professor Beata Cwalina for the revision of the manuscript.

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Received: May 28, 2008; accepted: December 17, 2008.

ZMIANY KONCENTRACJI BARWNIKÓW FOTOSYNTETYCZNYCH W SYNCHRONICZNEJ HODOWLI *CHLORELLA VULGARIS* JAKO WSKAŹNIK JAKOŚCI WÓD ZBIORNIKA GOCZAŁKOWICE

Celem niniejszej pracy było wykazanie możliwości wykorzystania synchronicznej hodowli *Chlorella vulgaris* jako biotestu w kontroli jakości wód. Próbki wody wykorzystane w eksperymencie pochodziły z siedmiu punktów pomiarowych zlokalizowanych w obrębie zbiornika Goczałkowice. Kryterium zmian była koncentracja barwników fotosyntetycznych pochodzących z dwudziestoczterogodzinnego cyklu życiowego komórek analizowanych techniką HPLC. Interpretując zmiany zachodzące w komórkach glonów hodowanych w wodach ze zbiornika Goczałkowice wyodrębniono okres lipcowej powodzi oraz jesiennych zmian jakości wód związanych z naturalnymi przemianami zachodzącymi w jeziorach. Wykazano również, że jakość wody w głównym nurcie strumienia wód wnoszonych do zbiornika przez Wisłę po okresie powodzi różniła się istotnie od jakości wód w innych miejscach zbiornika, zwłaszcza w jego wschodniej części.