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# Cell response of *Chlamydomonas actinochloris* culture to repeated microwave irradiation

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**Abstract**. *Grygorieva OO, Berezovsjka MA, Dacenko OI. 2015. Cell response of* Chlamydomonas actinochloris *culture to repeated microwave irradiation. Nusantara Bioscience 7: 38-42*. Two cultures of *Chlamydomonas actinochloris* Deason et Bold in the lag-phase were exposed to the microwave irradiation. One of them (culture 1) was not treated beforehand, whereas the other (culture 2) was irradiated by microwaves 2 years earlier. The measurement of cell quantity as well as measurement of change of intensities and spectra of cultures photoluminescence (PL) in the range of chlorophyll *a* emission was regularly conducted during the cell cultures development. Cell concentration of culture 1 exposed to the microwave irradiation for the first time has quickly restored while cell concentration of culture 2 which was irradiated repeatedly has fallen significantly. The following increasing of cell concentration of culture 2 is negligible. Cell concentration reaches the steady-state level that is about a half of the cell concentration of control culture. Initially the PL efficiency of cells of both cultures decreases noticeable as a result of irradiation. Then there is the monotonic increase to the values which are significantly higher than the corresponding values in the control cultures. The ratio of the intensities at the maxima of the main emission bands of chlorophyll for control samples of both cultures remained approximately at the same level. At the same time effect of irradiation on the cell PL spectrum appears as a temporary reduction of this magnitude.

**Key words**: *Chlamydomonas actinochloris*, microwave irradiation, photoluminescence

## INTRODUCTION

There is no doubt that microwave irradiation being the modern integral environmental factor, affects the living organisms in a varying degree. This influence can be single or continuous, remote or direct, that requires carrying out research on the responses of biological objects to the microwave irradiation providing different conditions of action, various doses, etc. In the course of such experiments with algae, it was established that even for supercritical irradiation doses, the individual cells remain viable and further intensively develop, overtaking the control culture on concentration. Here, the luminescent measurements show better functional condition of microwave-treated cells as compared to the control (Vakulenko et al. 2010b; Grigor'eva et al. 2012). It should be logical to suggest the microwave irradiation as a method of the start treatment of the industrially grown cultures.

In particular, the recent report about the unicellular algae of genus *Chlamydomonas* that can be used for cellulose preprocessing caused great scientific interest, in view of biofuel production possibility (Blifernez-Klassen et al. 2012. The purpose of this work is to investigate the susceptibility of the once irradiated cell suspension of *Chlamydomonas actinochloris* to the repeated microwave treatment.

## MATERIALS AND METHODS

ACKU 706-06 strain of *Chlamydomonas actinochloris* from the algological collection of the Botany Department of Institute of Biology of Taras Shevchenko National University of Kyiv, Ukraine was used in the work. This strain was extracted from the soil in State of Texas, USA. Before and during the experiment, the cultures were kept in the luminostate with LB-40 fluorescent lamps with a 12-hour alternating dark and light phases. The temperature stabilization in the room was not carried out.

Two cultures were exposed to the microwave irradiation. One of them (culture 1) was not treated beforehand, whereas the other (culture 2) was irradiated by microwaves 2 years before the start of this experiment within another investigation, the results of which were published elsewhere (Grigor'eva et al. 2012). Both cultures were kept in the liquid bold medium, and were diluted a week before the experiment so that they were exposed to microwaves in the process of their development. The week delay is needed for relaxation of the fluorescent parameters of culture, which are sharply changed as a result of dilution (Datsenko et al. 2012). Some parts of these suspensions remained not irradiated and thus considered as the control. Hereafter, the control samples of both cultures are marked with the letter C (1C and 2C) whereas the experimental ones are marked with the letter E, respectively (1E and 2E).

The household microwave oven magnetron with the frequency of 2450±50 MHz (the wavelength is about 12 cm) was used for irradiation. The irradiation dose was controlled by the suspension heating (Grygorieva 2005), where the temperature was measured with the "Prima long" digital thermometer by Amarell electronic. The initial temperature of the culture was about 25°C. Samples of 500 ml each were irradiated with the 100 ml batches for 30 seconds. The terminal temperature of the 1E culture was 49.8±0.8 °C. The 2E culture, previously irradiated, was heated up to 49.0±0.5°C. The irradiation doses for the cultures 1 and 2 were 100±3 and 100±2 J/g respectively.

The cell concentration of the developed cultures was regularly measured in the Gorjaev chamber by the standard method (Bilay 1982). To control the cells functional condition, the photoluminescence (PL) intensities and spectra of cultures were measured in the range of chlorophyll *a* emission. The PL was excited by 488 nm argon laser LGN-402. The excitation density in the beam was about 0.15 W/cm$^2$. In the luminescent measurements, the suspensions were continuously stirred in the cuvette to prevent settling of cells and to minimize the influence of fluorescence induction (Govindjee 1995) that can strongly distort not only the total intensity, but also the measured PL spectrum (Vakulenko et al. 2010a).

## RESULTS AND DISCUSSION

The temporal changes in the cell concentration in the studied cultures are shown in Figure 1. One can see that the control culture 1C concentration increases monotonously. As a result of irradiation, the culture cells (1E) lose their mobility, and their concentration is reduced, reaching a minimum in approximately 5 days after exposure. Further, normalization of their mobility as well as rapid increase in concentration is observed, so that by the end of the experiment, its concentration is even slightly higher than that of the control culture.

As seen the control concentration of the previously irradiated culture 2C is virtually unchanged from the beginning of experiment. After re-irradiation, the 2E cells temporarily lose their mobility as well, and their concentration decreases substantially, its time minimum substantially coincides with the minimum for culture 1E irradiated for the first time. However, subsequent growth of the concentration stops rapidly and after a week from the beginning of the experiment it reaches the steady-state level that is about a half of the control culture concentration.

The luminescence spectra of the *Ch. actinochloris* cultures consist of two bands peaked at 740 and 685 nm (Figure 2). The first one is associated with chlorophyll *a* of the photosystem I (Gulyajev and Tetenykin 1983). At the room temperature, the short-wave band dominates in the spectrum, where the molecules of the specified pigment in the light gathering complex of photosystem II are considered to be the source of this band.

The PL efficiency, i.e, the ratio of the emitted energy to the excitation energy absorbed by the suspension, can be considered as an indicator of the functional condition. This value gives a notion of the photosynthetic apparatus state. Indeed, at sufficiently high excitation levels, when the reaction centers are not able to assimilate the electronic excitation of the whole antenna in the light-gathering complexes, the fluorescence efficiency is high enough and proportional to the amount of chlorophyll in the thylakoids (Rubin and Krendelyeva 2003).

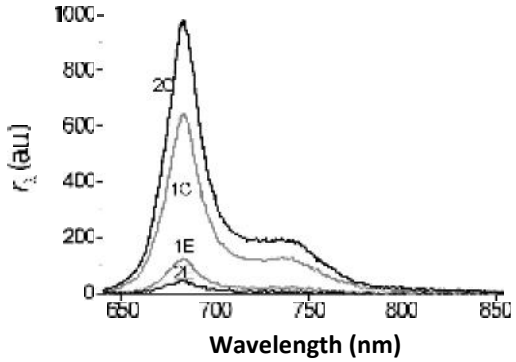



**Figure 2.** The luminescence spectra of the samples on the 5th day of the experiment. The value proportional to the energy emitted per spectral interval unit is given as ordinate.

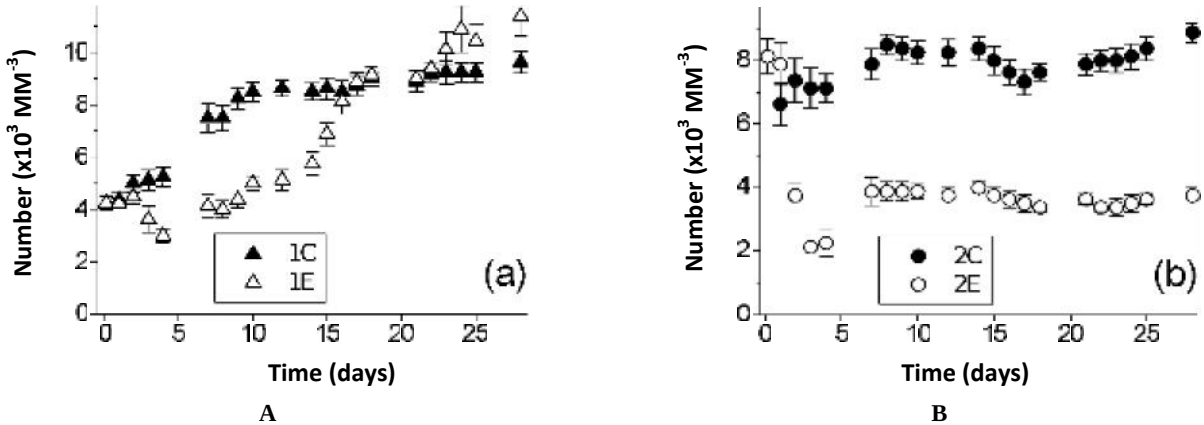
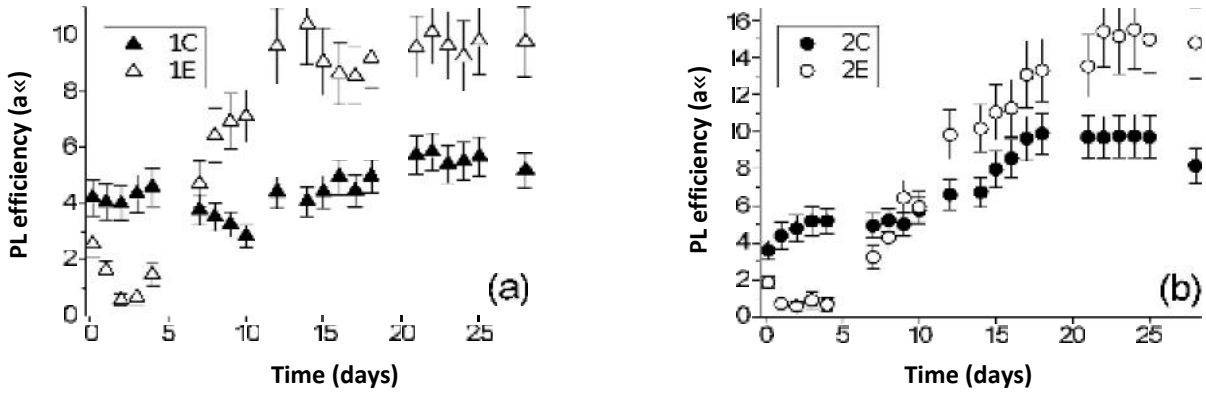
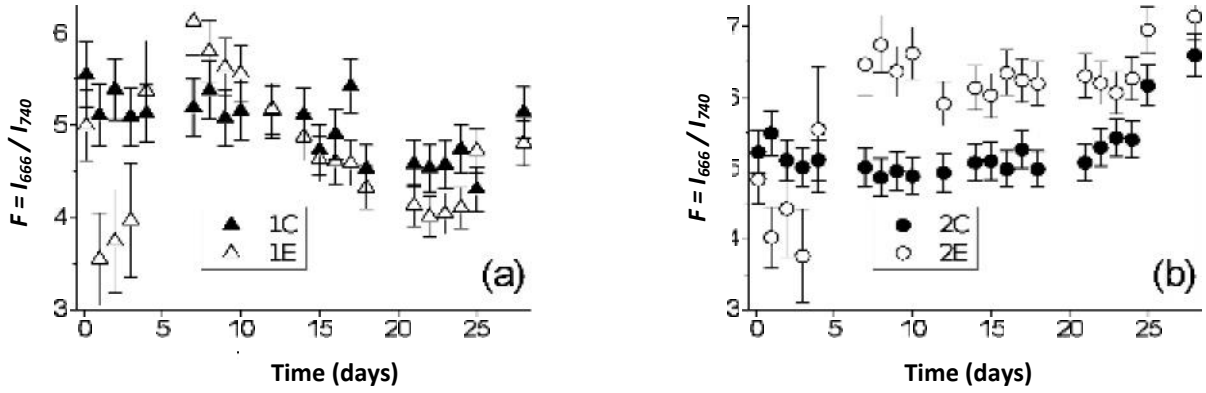



**Figure 1** The temporal changes in the concentration of cells in suspensions 1 (A) and 2 (B) after irradiation




A B

**Figure 3.** Change of PL efficiency of the suspensions 1 (A) and 2 (B) during the experiment




A B

**Figure 4.** Dynamics of the relative intensity of the bands in the PL spectra of cultures 1 (A) and 2 (B)

Another characteristic of the functional state is the total intensity of PL (area under the spectral curve). It would be possible to consider as the relative efficiency. But in its calculation, the amount of absorbed excitation light should be accounted for, and, meanwhile, the suspension transparency varies with time, decreasing with the growth of the cell concentration. It can be shown that for weak absorption, the total intensity of the luminescence is proportional to the cells concentration (Dacenko et al. 2014). Therefore, to get a notion of the luminescence efficiency, it is advisable to divide the total intensity of luminescence, i.e. the area under the spectral curve, by the concentration of cells in suspension. The resulting value is proportional to the PL efficiency of suspension and characterizes the average efficiency of emission of a single cell in culture. Its dynamics is presented in Figure 3.

The control cultures 1C and 2C after the week of ageing, as one can see, at the beginning of experiment have approximately the same PL efficiency and, respectively, the content of chlorophyll. However, the curve 1C at the beginning of study is already on the plateau, whereas the dependence of the re-irradiated culture 2C after dilution still monotonously rises for more than two weeks.

As a result of irradiation, the PL efficiency in both cell cultures noticeably drops down and continues to decrease, reaching a minimum in about two days after the microwave treatment, after which there is a monotonic increase to significantly higher values than the corresponding values for the control cultures. Another numerical indicator to control the plants functional condition, both higher (Fateyeva et al. 2004) and unicellular (Vakulenko et al. 2010b; Grigor'eva et al. 2012; Datsenko et al. 2012), is the ratio of the intensities at the peaks of the main emission bands of chlorophyll, the dynamics of which for different cultures can be seen in Figure 4.

For the control cultures, the value *f* remains during the experiment at approximately the same level, though for the non-irradiated culture 1C, the tendency to reduce this value

is noticeable. It should be noted, however, that the bands relative intensity change in the spectrum is a fairly natural phenomenon for developing culture and does not imply changes in the functional condition of its cells. As a rule, with the culture development, the value *f* initially increases, passes through a peak and then slowly decreases (Vakulenko et al. 2010b; Grigor'eva et al. 2012; Datsenko et al. 2012). The same is usually observed for the higher plants leaves during the vegetation period (Fateyeva et al. 2004).

Effect of irradiation on the PL spectrum of cells manifests itself as a temporary decrease in the *f* value, that is an increase in the contribution of the photosystem I emission band to the spectrum. It should be noted that the minimum of *f* coincides in time with the minimum of PL efficiency of the irradiated cultures in Figure 3. After irradiation, development of the culture 1E takes place qualitatively in the same way as in the earlier study (Grigor'eva et al. 2012) at doses near 120 J/g, the differences are, for the most part, only in the location of some characteristic features of the studied curves in the timeline. It would be natural to interpret the drop of the PL efficiency and *f* accompanied by the concentration reduction as deterioration in the functional condition of the cell culture. Obviously, only the most viable cells survive after the microwave treatment, they actively breed afterwards, occupying the vacant vital space. By the end of the experiment, the irradiated culture exceeds the control culture 1C both in concentration (Figure 1B) and in the PL (Figure 3B). The latter can be interpreted as better functional condition of the treated cells compared to control ones (Grigor'eva et al. 2012).

As for culture 2, the long-term increase in the luminescence efficiency of control cells after the start of the experiment (Figure 3B) already indicates that their functional state differs from that of control culture 1. The experimental data obtained in the initial stage of the experiment, also lead to the conclusion about functional condition deterioration after irradiation. The subsequent data are not so unambiguous. On the one hand, the PL efficiency of the irradiated culture increases all the time after the initial drop, and in the final stage exceeds the efficiency of any of the other cultures. The value of *f* behaves in the same way. This would seem to be a sign of good functional condition of the cells. On the other hand, although the concentration curve of 2E (Figure 1) has a minimum at about the same time interval as 1E, the subsequent increase in the concentration is very short, the concentration stabilizes in about 7 days after irradiation, and is near a half of the control (2C) concentration.

Taking into account the above, we can make some conclusions. After the first culture irradiation, all the signs of its functional condition general improvement are observed as a result of its development as compared to the control culture that can be explained by updating the populations by the most viable cells (Grigor'eva et al. 2012). However, this population becomes not less, and even more vulnerable to new irradiation. It should be taken into account that the culture luminescence characteristics (Figure 3 and 4) react to radiation quicker, and the minimal concentration of the re-irradiated culture 2E is only about 25% of the initial level, whereas at the first irradiation (culture 1E), the minimum concentration is only a quarter lower than the initial value (Figure 1).

And, moreover, one can conclude that the repeated irradiation worsens the final culture condition that is manifested in inhibition of cell reproduction. One can explain the abnormally high values of the luminescent parameters of culture 2E just by its low concentrations and, consequently, high transparency of suspension. This, in turn, implies higher average illumination for the culture cell and, hence, an increased content of chlorophyll. This explains high PL efficiency of this suspension and the increased value of *f* that, as shown in (Fateyeva et al. 2004; Saito et al. 2000), also correlates with the chlorophyll content.

However, there is another possible interpretation of the results obtained. Repeated exposure may actually improve the final functional condition of the cell culture, but negatively affect their reproductive capacity. In any case, one may conclude that microwave treatment as a method of treatment of industrially grown culture has its limitations and needs further investigation.

As a result of the microwave irradiation at a dose of about 100 J/g, the functional condition of the *Ch. actinochloris* culture cells in the lag-phase of development deteriorates, but eventually happens to be higher than that of the control culture, because of updating the population by descendants of its most viable cells. However, these cells become more susceptible to repeated exposure. The re-irradiation also worsens the functional condition of the culture that is manifested in its development termination at the early stage of the experiment.

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