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Influence of pH shift and salting on the energetics of microalgae *Chlorella vulgaris* and *Dunaliella maritima*

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Abstract The influence of pH shift and NaCl salting on the heat production, oxygen adsorption, and oxygen evolution rates of the unicellular green halotolerant microalga *Dunaliella maritima* and the freshwater microalga *Chlorella vulgaris* were investigated. In the growth process of both microalgae the alkalization of their culture medium was observed. And simultaneously it was shown that at increasing NaCl salting of cultural medium its acidification occurs. At alkalization and acidification of *Chlorella* medium the increase of heat production and respiration rates were observed. At alkalization and acidification of *Dunaliella* culture the adverse effect of decreasing heat production rate was observed. Acidification of culture medium of both algae led to short-term and sharp increase of photosynthesis measured by polarography and photomicrocalorimeter.

Keywords *Chlorella vulgaris* · *Dunaliella maritima* · Heat flow rate · Oxygen uptake and evolution rate · pH shift · Salinity changes

Introduction

The given work is a continuation of our work partially published in articles [1, 2]. In these works it has been shown that *Chlorella* was unable to adapt to concentrations of 500 mM NaCl and higher. This microalga has a metabolic rate which is significantly lower than that of *Dunaliella*. This halotolerant and cell wall-less microalga adapts to very high salinities. The tolerance is connected with the ability to maintain high rates of energy yielding processes. We showed that the heat production, O_2 uptake, and O_2 evolution rates increased in *Dunaliella* cells in conditions up to 2 M salt in the medium. The data support our opinion that an important feature of salt-sensitive and salt-tolerant algae is the increased rate of energy dissipation which ensures the quick and effective microalgal adaptation. We have concluded that the most important feature of salt-sensitive and salt-tolerant organisms is how they can adapt to stress situations without using all their energy capabilities to maintain the structural–functional integrity of the organism.

It has been also noticed that characteristic alkalization of the culture medium, which observed at growth of both cultures on light conditions, at addition of salt led to acidification of culture.

Also it has been noticed that photosynthetic allocation of oxygen in acidifies conditions also changed.

The present researches are devoted to the study of the action of alkalization and acidification on the energy yielding processes of this green single-celled alga. The unicellular microalga has been extensively used for biochemical and physiological work in many laboratories [3–8]. The authors consider that photosynthesis and respiration of these microalgae have a close resemblance with those in plants and thus they are valid models for them.

As is known, infringement of an ionic homeostasis as in a case of salting, and any ion stress, including changes of proton concentration causes the inclusion of adaptive mechanisms demanding the energy expense: (a) increasing rates of biopolymers and lipid catabolism; (b) changing in the rates of energy yielding processes; (c) change of membrane permeability with the interruption of ion homeostasis [9–12]. It is known that the energy demand of

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cells increases under adverse conditions [9–13]. According to [13, 14], the additional expenditure of metabolic energy under stress conditions is required for maintaining ion homeostasis and electrochemical gradients.

pH shift and salt stress cause a multitude of bioenergetic and biochemical changes in photosynthetic organisms. Thus, the aim of this research is to study the key energetic processes of the model systems of *Chlorella* and *Dunaliella* cells by measuring the rate of heat absorption and the light-dependent rate of energetic processes employing photo microcalorimetry [15–18], oxygen uptake, and evolution rates using polarography [19]. The study of the alteration of energy yielding processes of cells of both algae to pH shift may be fruitful to the understanding the mechanisms of its adaptation to unfavorable conditions and estimating of the energy costs of adaptation to pH shift by these algae.

Direct microcalorimetry was used to study changes in the heat production rate of microalgae depending on pH shift and salt in the culture medium. Its power as universal, integral, non-destructive, and highly sensitive tool for many environmental problems has been widely acknowledged as well as its value in providing thermodynamic information [20–23].

Experimental

The description of objects, methods, and the equipment is the same as in our previous articles, but a little reduced. In more details it is described in [1].

Biological materials

The unicellular green algae, *C. vulgaris* Beij. (from the collection of the Botany Institute, Saint Petersburg, Russia) and *D. maritima* (from the collection of the Timiryazev Institute of Plant Physiology, Russian Academy of Sciences) were the objects of the investigation.

Cells of both species were grown in Tamiya medium [24], pH 6.8–7.2, at 30 °C during the light period and at 22–24 °C in the night. The only difference between two cultures was that 500 mM NaCl was added to the control *Dunaliella* cells and the *Chlorella* cell suspensions were bubbled with 0.3% CO_2 in air. Both cultures were illuminated at 155 μmol photon m^{-2} s^{-1} with a light/dark photoperiod of 12/12. All the experiments described below were carried out at 30 °C. The cell concentration of *Chlorella* was maintained at 1–1.5 $\times$ 10^8 cells/mL and that of *Dunaliella* 1–2 $\times$ 10^7 cells/mL using an optical density method (photocolorimeter KFK-2MP; Zagorsk, Russia).

Salting and pH shift to the different level of pH was created by adding NaCl and solution of HCl or NaOH in cultivation medium of the investigated cultures.

The experimental samples were divided into aliquots to measure: (a) the rate of heat production; (b) the rate of oxygen uptake/evolution; (c) the rate of heat absorption.

Analytical methods

The rate of heat production was monitored by a heat conduction microcalorimeter (LKB Bio Activity Monitor BAM), manufactured by Thermometric AB, Järfalla, Sweden) [25].

The oxygen uptake and evolution rates were measured by polarography using a Clark-type electrode [19].

The rate of heat absorption was measured by photomicrocalorimetry. The necessary adaptations to a Tian-Calvet type microcalorimeter DAK-1-1A manufactured in Chernogolovka (Russia) were constructed in Kazan by Professor V.E. Petrov [15, 16].

Results and discussion

The concentration of H^+ and NaCl represent the important characteristics of an inhabitancy of water plants. The investigation of algae reaction to the environment change is of special interest since it allows to study a separate vegetative cells, and whole intact plants as well.

It is of interest to compare primary reaction of freshwater alga *Chlorella vulgaris* and halotolerant alga *Dunaliella maritima* to pH change and salting of the growth medium.

In the course of growth, *Chlorella* algae alkalinize the growth medium (from pH 7 till 10–11), a little less it is expressed for *D.* maritime (from 7 to 8.5–9.5 pH). This can be a consequence of extracellular transformation of HCO_3 in CO_2 (+H_2O) by carbonic anhydrase [26].

Freshwater alga *Chlorella* grows on the mineral environments which do not contain NaCl. At low concentration of NaCl the pH is shift in growing *Chlorella* culture a little raised during the first 20–25 min, then slowly decreased to the control level (Fig. 1). At concentration of 200 mM pH of culture in the first half of an hour practically did not differ from control, then it started slowly to decrease. At high concentration of salt after a short-term initial (3–5 min) decreasing pH slowly increased (Fig. 1). The osmotic increase in growth medium, in particular concentration of sodium chloride, results in the change the ions streams, and first of all H^+ [8], that is shown in pH changing of cultural medium.

Correlation of acidification of the culture medium with growth and heat production pointed that the H^+-exit is a primary adaptive reaction of microalgae for homeostasis maintenance at salting [8].

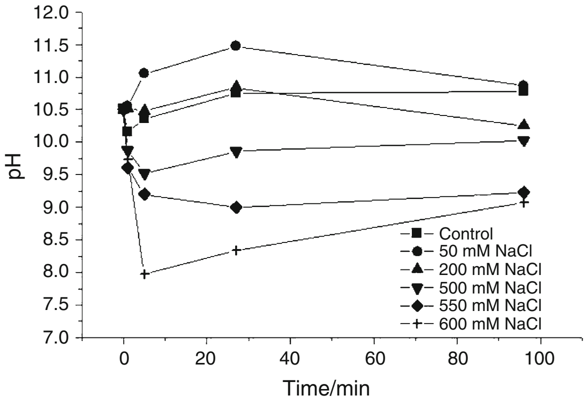

Fig. 1 Influence of increasing concentrations of NaCl on pH of *Chlorella* culture

pH shift of growing culture by addition of NaOH or HCl caused a response change of pH. After acidification of *Chlorella* there is a slow increase of pH to the control level, and after pH shift in more alkaline area the compensating change of pH does not occur (Fig. 2).

After pH shift in growing *D. maritima* culture (in both directions) the compensating change of pH (except for extreme shift lower than pH 4 which leads to death of culture) is not observed.

The pH shift essentially effects the heat production rate of both experimental cultures. At alkalinizing of *Chlorella* to pH level 12–13 the increase of heat production rate by 20–60% is observed, but at the decrease of pH level from 10 to 7–6.5 pH the heat production rate is by 15–20% lower than the control level (Fig. 3). The maximum of *D. maritima* heat production rate is observed at pH value close to neutral (real pH of control culture), the shift of pH to both acidic and alkaline direction essentially reduces the heat production of cultures (Fig. 4).

The increase of heat production rate by *Chlorella* observed in the experiment at pH shift after addition of hydrochloric acid corresponds to the standard representations about "the power price" to adaptation [27]. So the ambiguous heat production dynamics obviously points to the fact that the pH shift as a whole does not exert an essential effect on the respiration of the studied cultures estimated by oxygen consumption (Fig. 5), or this points to uncoupling of oxidation phosphorylation.

As a whole, the pH shift does not significantly influence the respiration rate of the studied cultures (Fig. 5) whereas the photosynthetic activity in the first 1–2 h considerably increased (Figs. 6, 7). The increase of oxygen evolution rates of both cultures at lighting (Figs. 6, 10, 11), and

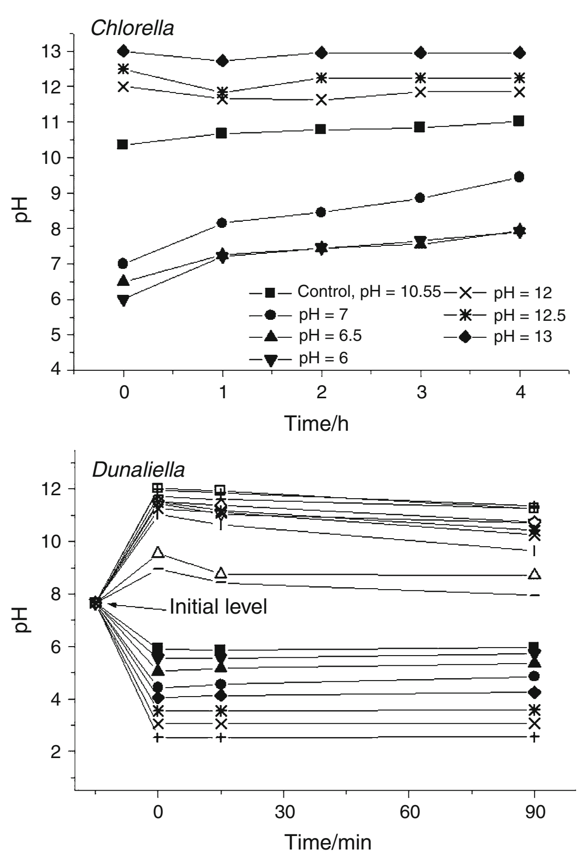

Fig. 2 Compensation of pH shift by growing cultures

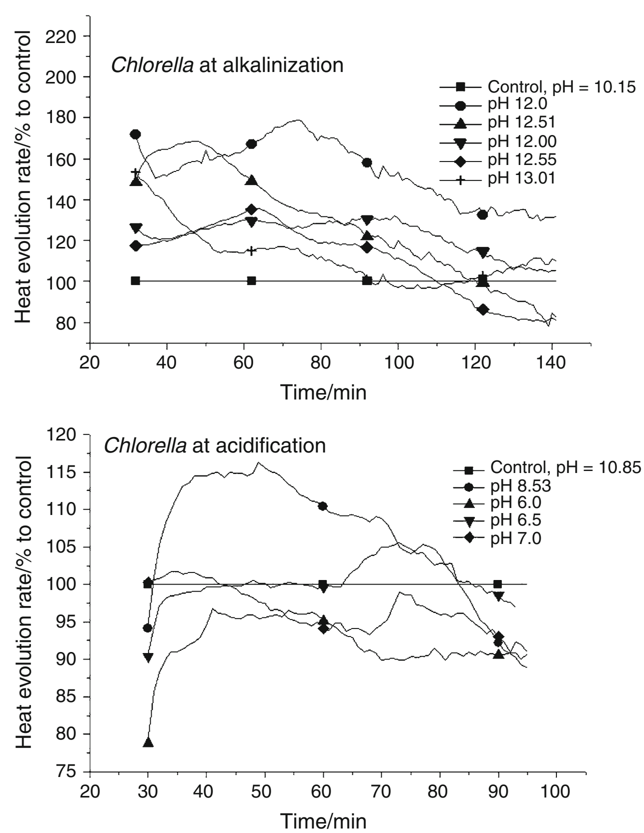

Fig. 3 Change of the heat evolution of *Chlorella* cells at alkalinization/acidification of cultivated medium

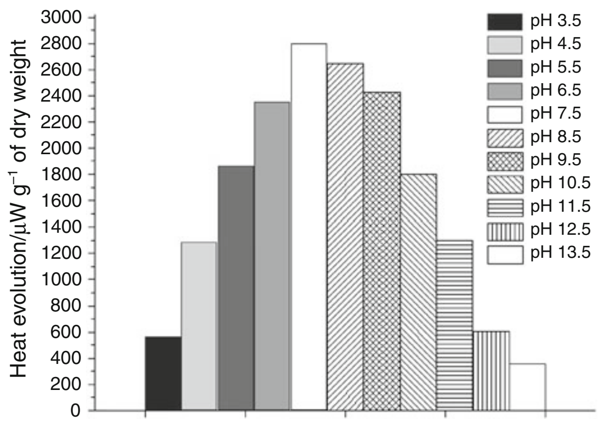

Fig. 4 Change of the heat evolution of *Dunaliella* cultures at alkalinization/acidification of cultivated medium

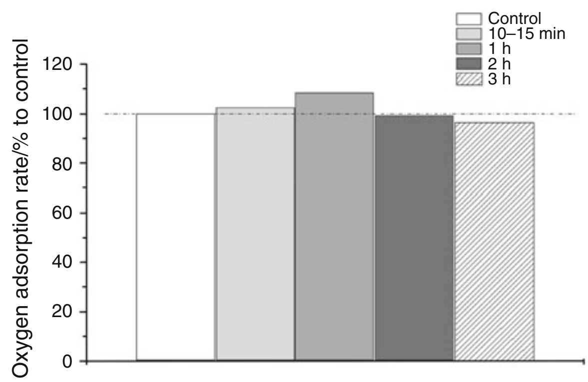

Fig. 5 Change of oxygen consumption rate of *Chlorella* cultures at pH shift to level 7

storage of the light energy, measured by photomicrocalorimetry point to that effect (Fig. 7).The pH shift influence on photosynthetic activity is very interesting. Photosynthesis of both cultures (measured by oxygen evolution) considerably increased after pH decrease caused by addition of HCl. The strengthening of oxygen allocation by cultures on light, and the storage of light energy rate, measured by photomicrocalorimeter point to this fact. This effect is observed on the average for 1–2 h. It is remarkable that both freshwater and halotolerant algae equally react to this influence. In literature there are data about the increase of photosynthetic O_2 allocation [26, 28], as well as about photosynthesis suppression [29] in response to the increase in concentration of protons. Probably, the reaction of organism to pH change depends from the experiment conditions, as well as from the physiological features of the studied organisms.

The additional oxygen allocated at pH shift is of a photosynthetic origin, and its allocation depends on functioning of both photosystems. Oxygen allocation was decreased by antimycine A, inhibitor of PS-1 and DCMU, inhibitor of PS-II (Fig. 8). The observed increase of

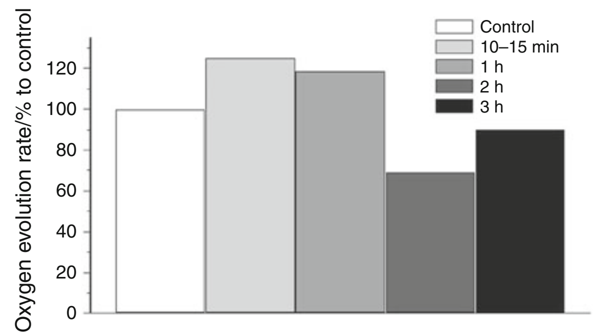

Fig. 6 Photosynthesis of *Chlorella* cells at acidification to pH = 7.0

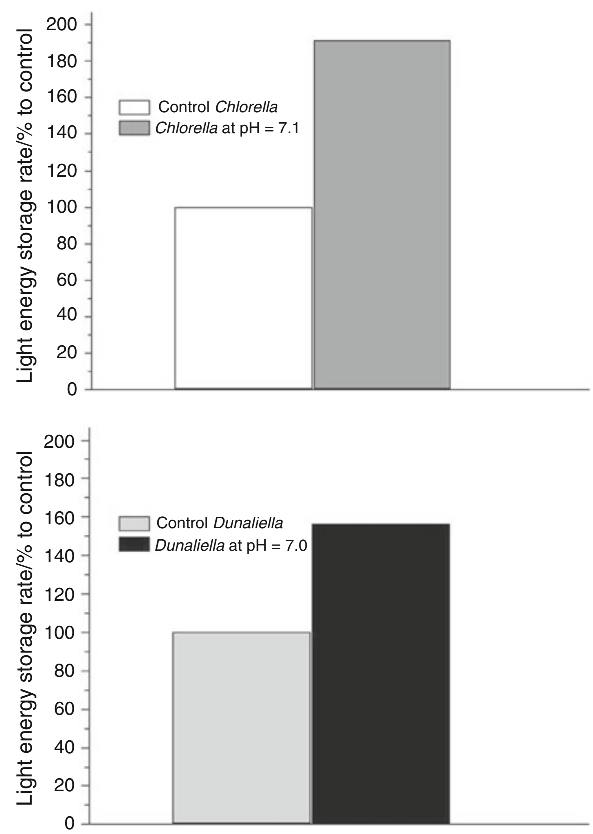

Fig. 7 Influence of acidification to pH 7 level on accumulation of light energy by *Chlorella* and *Dunaliella* measured by photomicrocalorimeter

photosynthesis does not finally result in the increase in a biomass growth.

It has been earlier shown by us that Na^+ ions influenced the photosynthesis activity of microalga [1, 2]. To examine a possible influence of Na^+ ions on the electron-transport chain of photosynthesis, we measured the action of (DCMU) on this process. DCMU was added to the cell suspension before it was placed in the calorimetric vessel. The inhibition reduced the light energy uptake to about one-third of the control. The additive action of NaCl

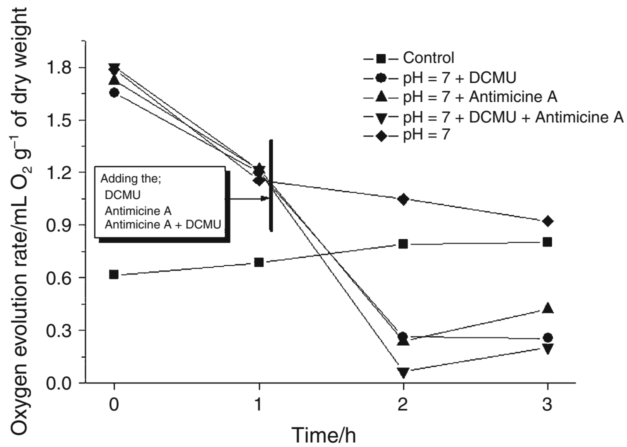

Fig. 8 Influence of antimycine A and DCMU, inhibitors of photosystems I and II, on additional oxygen allocating at pH shift to 7.0

(500 mM) and DCMU decreased the rate of heat absorption by cells by 27%. The site of DCMU action is known to be the non-cyclic electron transport between the first acceptor of PS-II and the incorporation in chain of plastoquinone [30]. Probably, rather high rates of the heat absorption under high salinity (500 mM) may be connected with the activation of cyclic photophosphorylation which is not directly associated with oxygen evolution. It could be argued that cyclic photophosphorylation might also play a role in the generation of extra ATP for the survival of the organism under salt stress [31–35]. Chen and Arnon [30] remarked that cyclic photophosphorylation might play a role in generation of ATP for the survival of the organisms under unfavorable conditions.

Joint action of NaCl and pH shift on *Chlorella* heat production was shown in Fig. 9. At the decrease of pH to neutral (pH 7) during the initial moment (the first hour) a little increase of heat production rate was observed, then it was stabilized at the level a little below the control. Entering of 0.2 M NaCl leads to a gradual decrease of the heat production.

In our opinion, the increase of the heat production rate and oxygen consumption results in intensification of the metabolic reaction directed at preservation of a homeostasis in salt conditions. The decrease of bioenergetics processes by *Chlorella* cells under the NaCl high concentration is connected with inhibition of the metabolic processes rate because Na^+ ions are toxic for plant cells. Targets of this ion can be proteins and nucleic acids [36]. The decrease of heat production of *Chlorella* at salting by 500 mM and above is accompanied by suppression of growth and possibly points to the exhaustion of adaptable possibilities of culture [1]. Change of the major physiological indicators of growing culture corresponds well to respiration and photosynthesis rates [1, 2]. At the action of NaCl and pH shift the heat production of *Chlorella* first essentially decreases, and then gradually raises, up to the level of 20% lower, than the control (Fig. 9). Entering of 0.2 M NaCl into *Chlorella* culture, which allocation of oxygen has been earlier intensified by acidification to pH 7, removed the stimulation effect of oxygen allocation.

After 1 h action of 0.2 M NaCl the pH shift to 7.0 leads to sharp increase of the oxygen evolution rate of both studied algae, as well as the pH shift to the level 7.0 in a control variant (Figs. 10, 11).

There are two mains explanations concerning the observed increase of photosynthetic activity. The most simple explanation proceeds from the increase of the quantity of the dissolved CO_2 in culture medium at acidification. [28]. On the other hand, acidification of environment increases transmembrane electrochemical potential of a cell, mainly, thanks to the increase of transmembrane pH gradient at the expense of which synthesis of additional ATP quantity occurs in cell [37, 38]. It is shown that the

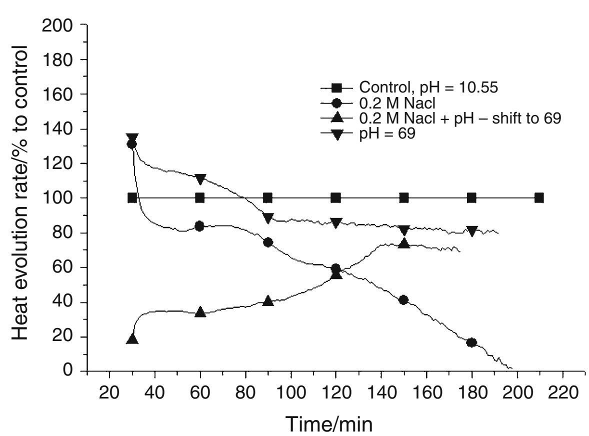

Fig. 9 Influence of NaCl and pH shift on heat production of *Chlorella* cells

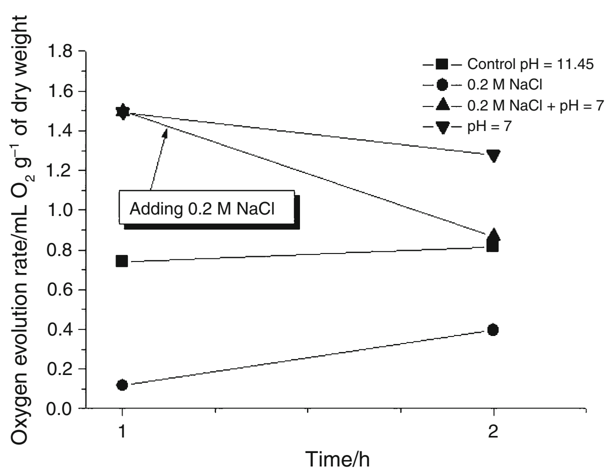

Fig. 10 Influence of pH = 7 and NaCl on photosynthesis of *Chlorella* cells

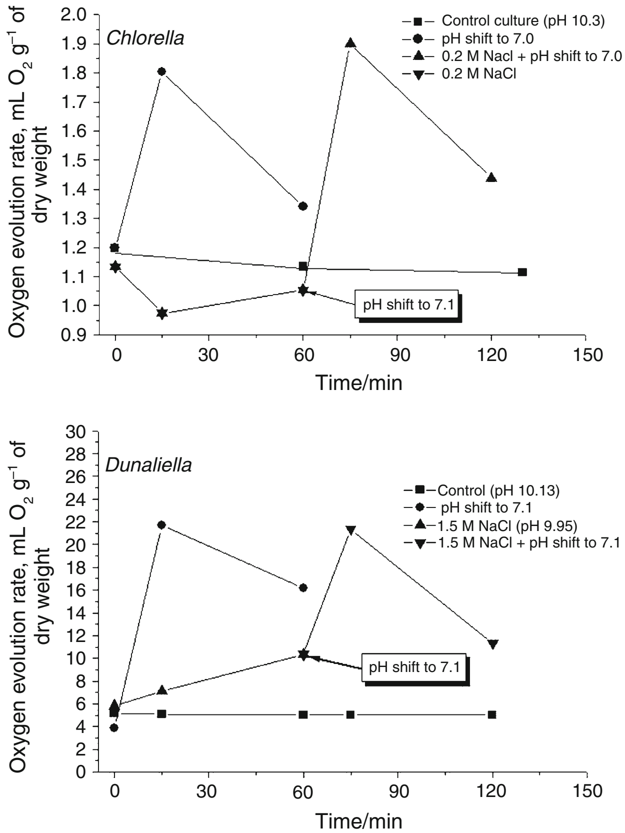

Fig. 11 Photosynthesis of *Chlorella* and *Dunaliella* at salting and pH shift to level 7.0

exogenic ATP stimulates photosynthesis on isolated chloroplasts [39–41]. Probably, both specified processes take place at pH shift. However, the analysis of joint action of pH shift and NaCl on photosynthetic oxygen allocation allows us to consider the second hypothesis as more perspective.

The alteration of ions and water streams caused by action of getting osmotic NaCl, changes the condition of a cytoplasmatic membrane, not allowing at present to synthesize additional ATP at the expense of transmembrane pH gradient. But in culture adapted to salting, the membrane was already stabilized at a certain level and a sharp pH shift resulted in the same effect, as in a control variant.

Conclusions

- pH shift causes different responses reactions of both cultures. So, alkalinization of *Chlorella* cultivation medium causes the increase of heat production rate, and acidification, the decrease of heat production rate to level below the control. In *Dunaliella* culture pH shift, both in acids and alkaline side, causes the decrease of heat production rate.
- At pH shift to level 7, by the photomicrocalorimetric measurement it is shown that the effect of the increase of light energy storage rate is observed in both cultures. This correlated with the increase of the oxygen evolution rate measured by polarographically.
- The duration of this effect is back-dependent on restoration rate of algae cultures to initial pH.
- Entering of 0.2 M NaCl into *Chlorella* culture, which allocation of oxygen has been earlier intensified by acidification to pH 7, removed the stimulation effect of oxygen allocation.
- After 1 h action of 0.2 M NaCl the pH shift to 7.0 leads to sharp increase of the oxygen evolution rate of both studied algae, as well as the pH shift to the level 7.0 in a control variant.
- The observed increase of photosynthesis does not finally result in the increase in a biomass growth that points to an adaptable orientation of use of the additionally stored light energy.

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