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## ДЕТЕКЦИЯ НЕКОТОРЫХ ВИРУСНЫХ ПАТОГЕНОВ КАКТУСОВ В БОТАНИЧЕСКИХ САДАХ УКРАИНЫ

*Коллекции кактусовых ведущих украинских ботанических садов были проанализированы на наличие вирусной инфекции. На кактусах были выявлены различные вирусоподобные симптомы, включая мозаики, хлороз и локальные некрозы. Биологические свойства детектированных вирусов определяли методами биологического тестирования, электронной микроскопии и косвенного иммуноферментного анализа.*

*Ключевые слова: иммуноферментный анализ, вирусы растений, кактусы.*

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# ETHANOL EFFECT ON BIOPRODUCTIVITY, PHOTOSYNTHESIS AND RESPIRATION OF MICROALGA *CHLAMYDOMONAS REINHARDTII*

***Some organic compounds may significantly stimulate the growth of unicellular green alga Chlamydomonas reinhardtii. Among them the most effective growth enhancers are acetate and monohydric alcohol methanol. The aim of the present work was studying the effect on the productivity of C. reinhardtii another alcohol – ethanol, which transforms into acetic acid in the process of intracellular oxidation. The results showed that in the presence of ethanol respiration was stimulated, photosynthesis inhibited and the growth of the culture stopped. We concluded that the cause of growth inhibition of C. reinhardtii was pH decline of the cultural medium due to oxidation of ethanol to acetic acid.***

***Keywords: Chlamydomonas reinhardtii, photosynthesis, respiration, ethanol, mixotrophy.***

**Introduction.** The green alga *Chlamydomonas reinhardtii* has importance as model for many biotechnological processes and algal biofuels [17]. Availability of a sequenced genome [10], a proteomic database [9], and metabolomics protocols [8] benefits the use of *Chlamydomonas* to establish many fundamental aspects of metabolic control in photoautotrophic organisms [8]. It can grow either photosynthetically in the light with atmospheric $CO_2$ as the sole carbon source, or under heterotrophic conditions in the dark using various exogenic carbon sources added to the growth medium or else mixotrophic conditions (light and carbon sources). Under all conditions, *C. reinhardtii* remains green and retains a normally developed chloroplast, which can thus metabolize a variety of carbon sources as located in the chloroplast (starch) or assimilated through the cytosol of the cultural medium [12].

Significant stimulation of microalgae growth by exogenic methanol at mixotrophic cultivation was shown for unicellular green algae *Chlorella minutissima, Scenedesmus obliquus* [16]*, Botryococcus braunii* [12] as well as *C. reinhardtii* [2]. Another alcohol – 2-carbon ethanol, following methanol in the homologous series of monohydric alcohols, is able to enhance the growth of microalga *Euglena gracilis,* being one of the most efficient carbon sources for this microalga [19]. Acetate, the product of ethanol oxidation, strongly stimulates C*. reinhardtii* growth [6]. *C. reinhardtii* is capable of heterotrophic and mixotrophic growth utilizing acetate as a source of carbon and energy.

In microanaerobiosis, which is naturally formed in habitat of microalgae when the respiration rate exceeds the rate of photosynthesis, the cells of *C. reinhardtii* excrete ethanol, formate and acetate [7]. Acetate can be metabolised to triose by an ATP-dependent entry into the glyoxylate or Krebs cycle to produce reducing equivalents, which can be used to reduce the plastoquinone pool [11]. It is incorporated into acetyl coenzyme A (acetyl-CoA) following two possible pathways: a direct conversion with acetyl-CoA synthetase or a two-step reaction involving acetate kinase and phosphate acetyltransferase. Acetyl-CoA enters into the glyoxylate cycle, where it is converted to succinate. Succinate is further utilized in the Krebs cycle. The carbon of ethanol, like in methanol, is oxidised to $CO_2$ at the final stage and may supplied as a substrate for photosynthesis.

From other hand, it was established that the addition of 0.3% v/v ethanol in the culture medium of *Dunaliella viridis* accompanied by cessation of culture growth and increased intracellular concentrations of DNA, RNA and total protein [1]. Ethanol increases ploidy of the cells and inhibits their metabolism. Microalgae pass from dormancy to intense growth after removal of ethanol from the cultural medium. It was found toxic effects of ethanol on the growth of *Chlorella vulgaris* and *Selenastrum capricornutum*, ethanol inhibited the growth of these algae at a concentration of 0.05% [4].

The ability of exogenic ethanol to regulate productivity of *C. reinhartdii* under aerobic conditions in the light and in the dark has not been investigated. The aim of our study was to determine the effect of exogenic ethanol on productivity of batch culture of *C. reinhartdii* and its effect on photosynthesis and respiration.

**Materials and methods.** Unicellular green alga *Chlamydomonas reinhartdii* was obtained from the microalgae collection of Kholodny Botany Institute of NAS of Ukraine (IBASU-B – 163). Batch autotrophic cultures were grown on liquid Kessler's medium [3] in 0.5 l flasks with magnetic stirrer agitation at room temperature. 24 h white fluorescent light with 100 μmol photons•$m^{-2}$•$s^{-1}$ on the surface of flasks was used. The ethanol effects were studied at the stage of exponential growth phase of batch culture. The packed cell volume (PCV) was determined as a measure for the biomass accumulation. The PCV, the volume of the cell pellet in μl, was measured by the centrifugation of a defined volume of the cell suspensions at 1400×g for 5 min in haematocrite tubes [13]. The chlorophylls (Chl) were determined spectrophotometerically in ethanolic extracts by the method of Wintermans and De-Mots [18]. The concentration of chlorophylls was calculated using the formulas: Chl *a* =13,70($A_{665}$-$A_{750}$)-5,76($A_{649}$-$A_{750}$); Chl *b* =25,80($A_{649}$-$A_{750}$)-7,60($A_{665}$-$A_{750}$); Chl*a*+*b* = 6,10($A_{665}$-$A_{750}$)+20,04($A_{649}$-$A_{750}$).

Intensity of visible photosynthesis (A) and dark respiration (R) was determined in the gas phase above the suspension of algae by IRGA method with QUBIT Systems S151 Carbon Dioxide Analyzer (Canada). Gas flow rate was 0.4 l/min and the concentration of carbon dioxide – 700-800 μM. Gas exchange measurements were carried out in a thermostated glass cell filled by 2 ml of concentrated suspension of microalgae (30-40 mg/l of chlorophyll). The rate of carbon dioxide uptake was determined under illumination with light intensity of 350 mol photons•$m^{-2}$•$s^{-1}$. Dark respiration measured with a low content $CO_2$ in the gas space above the suspension of microalgae after turn-

ing off the light. Before being introduced into the analyzer air was passed through the column with ascarite for $CO_2$ removing from the gas phase.

Each experiment was repeated several times (n) and the mean values (M) and standard deviation (m) calculated for each treatment. Mathematical-statistical data processing was performed using the software package Microsoft Excel 2010. Statistical difference between groups was determined by t-test, for statistically significant were taking changes with $P<0.05$.

**Results and discussion.** As an indicator of the growth intensity of *C. reinhartdii* culture, we have chosen the changing concentration of total chlorophyll in exponential growth phase. The rate of cell division is directly proportional to chlorophyll concentration in the culture [5]. By analogy with methanol [2], we examined the effects of 20 and 40 mM ethanol on the growth of *C. reinhardtii*. In the presence of this solvent, the accumulation of chlorophyll is reduced and accompanied by cell death both in the light and in the dark (Fig 1). Effects of 20 and 40 mM ethanol were the same.

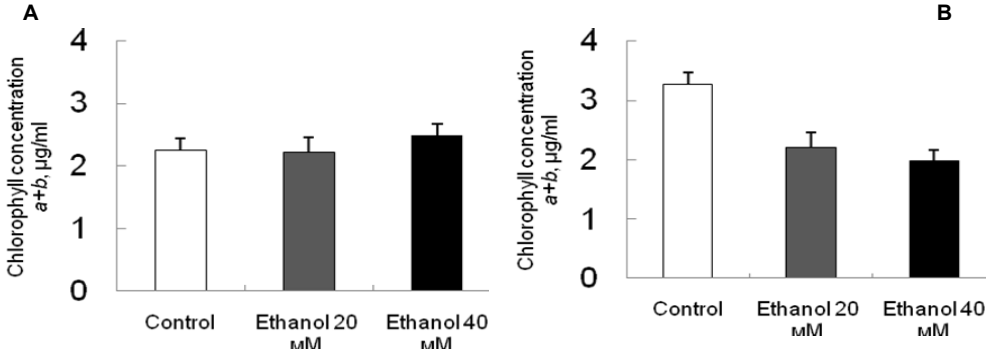


**Fig. 1. The concentration of total chlorophyll in suspension *C. reinhardtii* on the fourth day of cultivation, µg/ml.**
A – cultivation in the light, B – cultivation in the dark. (M+m, n=3)

Regardless of added ethanol concentration, the pH declines to 3.5 on the fourth day after ethanol application. The pH changes induced by ethanol are not depending on illumination. A gradual acidification of the medium takes place both in the light and in the dark. Apparently, acidification of the cultural medium in the presence of ethanol is due to its incomplete metabolization inside microalgal cells. Oxidation of ethanol occurs in several stages: 1. ethanol → acetaldehyde → acetic acid → $CO_2$. The result of the first stage of the oxidation is acetaldehyde, a toxic compound that can negatively affect the *C. reinhardtii* cells. Metabolization of ethanol by alcoholdehydrogenase occurs in both forward and backwards direction and depends on the ratio of substrates concentration – ethanol, acetaldehyde and nicotinamide coenzymes. The equilibrium concentration of acetaldehyde at the level of 1 mM may be established at addition of 40 mM exogenic ethanol [14]. However, under conditions of our experiments the formation of acetic acid proves that the enzymatic system involved in the detoxification of acetaldehyde to acetic acid are active. Since acetate is easily assimilated by the *C. reinhardtii* cells, its accumulation in the presence of exogenic ethanol may indicate a competition between acetate and ethanol at the level of the mitochondrial respiratory chain.

Microalgae begin secrete excess acetic acid in the culture medium when the rate of acetic acid formation exceeds the rate of complete intracellular ethanol utilization. As result of ethanol oxidation, acetic acid is accumulated in the liquid medium and induces its acidification. Toxic effect of ethanol can be explained by the formation of the equilibrium concentration of acetaldehyde in the culture medium, and its acidification due to secretion of excessive acetic acid from the cells.

In addition to the research culture growth by analyzing the content of chlorophyll, we determined the growth rate of the culture also by packed cell volume (PCV). PCV was determined on the third day after application of ethanol (10 µM, 100 µM, 1 mM, 10 mM) in the culture medium of *C. reinhardtii* and in control. Addition of ethanol at a concentration greater than 10 µM reduces the growth of *C. reinhardtii* biomass in in terms of PCV (Fig 2). Ethanol at a concentration of 100 µM inhibits the accumulation of biomass by 23% at a concentration of 1 mM and 10mM of ethanol inhibits the cellular growth by 33%. The presence of ethanol in the culture medium had a negative effect on the cells, resulting in a decrease in cell motility and increasing the proportion of fixed cells under a light microscope.

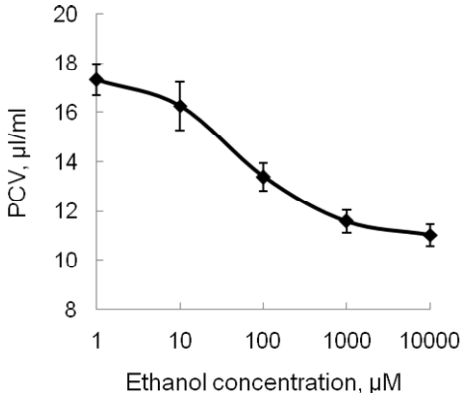


**Fig. 2. PCV in autotrophic culture of *C. reinhardtii* on the third day after application of different amounts of ethanol. (M+m, n=3)**

We analyzed the effect of ethanol (50 mM) on the rate of dark $CO_2$ release by *C. reinhardtii*, as a measure of dark mitochondrial respiration R. As seen in Fig 3, A, the rate of respiration in batch culture of *C. reinhardtii* increased twice after six hours of microalgae cultivation with ethanol compared with control. Increasing R can be explained by activation of turnover of the Krebs cycle as a result of utilization of excess acetyl-CoA.

The parameter of $V_{max}$ can indirectly characterize the intracellular pool of pyruvate and malate. Ethanol at a concentration of 50 mM increases $V_{max}$ by 29 % compared with control (Fig 3, B). Therefore, we can assume that ethanol increases the intracellular concentration of tricarboxylic acids. Ethanol oxidation lead to increasing of NADH content, which may lead to increasing intracellular concentrations of malate or pyruvate. Also R in culture with ethanol after six hours is equal to $V_{max}$, ie the rate of $CO_2$ release reaching $V_{max}$ after switching off the light remains at this level for 20 minutes. This can be explained by the fact that the addition of ethanol saturates Krebs cycle in the dark and in the light tricarboxylic acids are accumulated.

For further establishing the toxic effects of ethanol at concentration of 50 mM on the metabolism of *C. reinhardtii*, we investigated the change in apparent photosynthetic $CO_2$ uptake (A). Adding ethanol depressed photosynthesis by 25% compared with the control (Fig 3, C). Inhibition of apparent photosynthesis can occur as a result of activation of the light mitochondrial respiration, or due to toxic effects of acetaldehyde on pigment-protein complexes of photosynthetic membranes. Change A, R and $V_{max}$ with addition of ethanol in culture medium *C. reinhardtii* are similar to change these parameters during mixotrofic cultivation of *C. reinhardtii* in the medium with acetate [15]. However, unlike the cultivation with ethanol, acetate stimulates the growth of the alga. Therefore, we can assume that the toxic effects of ethanol on *C. reinhardtii* is not due to oxidation of acetate in Krebs cycle but rather due to oxidation of ethanol to acetaldehyde with participation of alcoholdehydrogenase.

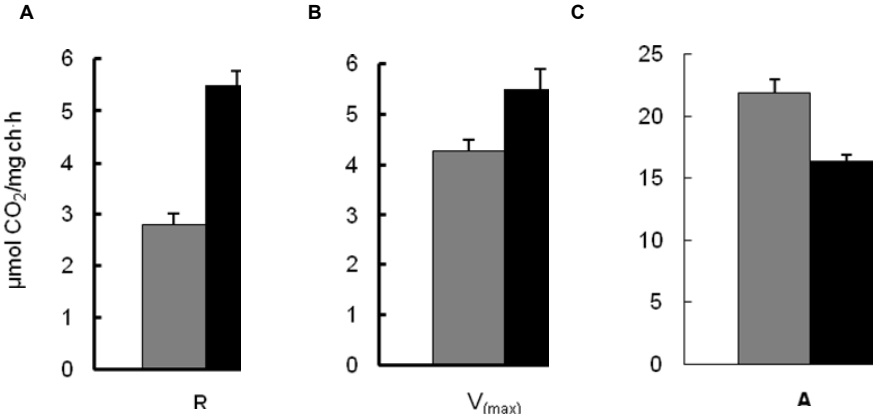


**Fig. 3. Indicators of $CO_2$ gas exchange *C. reinhardtii* (□ – control, ■ – in the presence of 50 mM methanol).**
A – dark respiration rate (R). B – the maximum rate of respiration ($V_{max}$). C – apparent photosynthetic rate (A).
**(M+m, n=5)**

**Conclusions.** Exogenic ethanol inhibits the growth of batch culture of *C. reinhardtii* in a concentration greater than 10 μM. Toxic effect of ethanol on the metabolism accompanied by a decrease in pH of cultivation medum both in the light and in the dark. The intensity of visible photosynthesis also decreases, and significantly increases the intensity of dark mitochondrial respiration of mixotrophic *C. reinhardtii* cultivated with ethanol.

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## ВПЛИВ ЕТАНОЛУ НА БІОПРОДУКТИВНІСТЬ, ФОТОСИНТЕЗ ТА ДИХАННЯ МІКРОВОДОРОСТІ *CHLAMYDOMONAS REINHARDTII*

***Деякі органічні сполуки здатні значно стимулювати ріст одноклітинної зеленої водорості Chlamydomonas reinhardtii. Серед них найбільш активними стимуляторами росту є ацетат і одноатомний спирт метанол. Метою даної роботи було вивчення впливу на продуктивність C. reinhartdii іншого спирту – етанолу, який при внутрішньоклітинному окиснені трансформується у ацетат. Отримані результати показали, що етанол стимулює дихання, пригнічує фотосинтез і зупиняє ріст культури. Зроблений висновок, що причиною гальмування росту C. reinhartdii було зниження pH середовища культивування внаслідок окиснення етанолу до оцтової кислоти.***

***Ключові слова : Chlamydomonas reinhardtii, фотосинтез, дихання, етанол, міксотрофія.***

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## ВЛИЯНИЕ ЭТАНОЛА НА БИОПРОДУКТИВНОСТЬ, ФОТОСИНТЕЗ И ДЫХАНИЕ МИКРОВОДОРОСЛИ *CHLAMYDOMONAS REINHARDTII*

***Некоторые органические соединения способны значительно стимулировать рост одноклеточной зеленой водоросли Chlamydomonas reinhardtii. Среди них наиболее активными стимуляторами роста являются ацетат и одноатомный спирт метанол. Целью данной работы было изучение влияния на продуктивность C. reinhartdii другого спирта – этанола, окисляющегося внутри клеток в ацетат. Результаты показали, что этанол стимулирует дыхание, подавляет фотосинтез и останавливает рост культуры. Сделан вывод, что причиной торможения роста C. reinhartdii было снижение pH среды культивирования в результате окисления этанола до уксусной кислоты.***

***Ключевые слова : Chlamydomonas reinhardtii, фотосинтез, дыхание, этанол, миксотрофия.***

**UDK: 616.33-002.44**

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# THE INFLUENCE OF LOW MOLECULAR WEIGHT ORGANIC COMPOUNDS ON ANTIOXIDANT DEFENSE SYSTEMOF THE GASTRIC MUCOSA UNDER ETHANOL-INDUCED GASTRIC LESIONS IN RATS

***It was investigated the preventive effect of low molecular weight organic compound (LMOC) on erosive and ulcerative lesions in the gastric mucosa of rats caused by ethanol. It was found that prophylactic injection of this substance at a dose of 1 mg/kg effectively protects the stomach from ethanol injuries. LMOC effectively restored the pro-/ antioxidant equilibrium by reducing the intensity of lipid peroxidation in the gastric mucosa of rats after ethanol injection and increase ofsuperoxidedismutase, catalase activityandactivity of glutathionesystem.***

***Keywords: ethanol-induced injuries, lipid peroxidation,low molecular weight organic compound.***

**Introduction.** Gastric ulcer is a common disease affecting many people worldwide [1]. The peptic ulcer, characterize by mucosal damage, is predominantly caused by *Helicobacter pylori*, antiplatelet agents such as acetylsalicylic acid [2], nonsteroidal anti-inflammatory drugs (NSAIDs) such as oral bisphosphonates, potassium chloride, immunosuppressive medications [3], serotonin reuptake inhibitors [4], alcohol consumption, and cigarette smoking [5]. The ulcer disease may lead to upper gastrointestinal haemorrhage and perforation [6], which have high morbidity and mortality rates [7]. Thus,the search of the newnontoxic medications is very importanttoday. So the aim of the work was to investigate the preventive effect of low molecular weight organic compound (LMOC) on erosive and ulcerative lesions in the gastric mucosa undere-thanol-induced gastric lesions in rats.

**Methods.**The animals used in the study were bred and kepton a standard diet in terms of accredited vivarium of Educational and Scientific Center "Institute of Biology" Taras Shevchenko National University of Kyiv in accordance with the "standard rules on arrangement, furnishing and maintenance of experimental biological clinics (vivarium)".

The studywas carried out on 30 white laboratory Wistar rats. The research was conducted in accordancewith the Law of Ukraine dated 21.02.2006 № 3447-IV "On protection of animals from abuse" and the ethical standards and rules of working with laboratory animals (Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington DC, 1996) [8]. All animals selected for the experiment were subjected to veterinary examination, were acclimated for five days, and then randomly divided into groups, numbered and marked appropriately.

For examine of the preventive actionof LMOC (sodium 2-(2-hydroxyphenoxy) acetyl)-L-prolinate)rats were divided into 3 groups of 10 animal seach: 1$^{st}$group was intact rats, 2$^{nd}$ and 3$^{rd}$ – rats, which had ethanol-induced ulcer at ion of the gastric mucosa (GM). Rats of 2$^{nd}$ group were injected with normal saline at a volume 2 ml/kg 30 minutes before ulcerogenic factor action, they were the control for the 3$^{rd}$ group. Rats of the 3$^{rd}$ group were treated with LMOC which was injected at thedoseof 1 mg/kg(2 ml/kg of saline solution) 30 minutes before ethanol action (compound was syn the size dat Lomonosov Moscow State University).

Erosive and ulcerative lesions of GM of rats in the 2$^{nd}$ and 3$^{rd}$ groups were caused by intra gastric ethanol infusion. After 1 hour from ethanol exposure the rats were sacrificed.To assess the state of GM of rats after ulcerogenic factor action stomach was removed, cutalong the lesser curvature, turn mucous out and thoroughly was hed with saline. The area and number of ulcers was measure dusing experimental gastroscope.

In the homogenateof GM of rats the content of lipid peroxidation(LP) products(the concentration of hydrogen peroxide, dien conjugates, thiobarbituric acid (TBA)- active products and Shiff's bases) wasmeasured by standard biochemical methods [9-12]. Antioxidant protection of the GM under condition of ethanol administration was assessed by the superoxide dismutase, catalase and glutathione system activity [13-15]. To study the influence of NMOS on glutathione system we examined the content of reduced (GSH) and oxidized glutathione (GSSG), glu-