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INFLUENCE OF THE REACTIVE OXYGEN SPECIES AND IONIZING RADIATION ON YEAST CELLS *SACCHAROMYCES CEREVISIAE* AND *SACCHAROMYCES CARLSBERGENSIS* UNDER ILLUMINATION BY VISIBLE LIGHT

Research article

Abstract

In this present work the yeast *Saccharomyces cerevisiae* and *Saccharomyces Carlsbergensis* against oxidizing stress as a result of exposure includes growing of yeast culture under standard conditions till the end of the logarithmic or the start of the stationary phase of growth, incubation with a protective agent. The protective agent is represented by inhibitor - naphthalene C10H8 in concentration of 5,10-4 M. Naphthalene is a part of heavy oil fractions and is released into the environment as a result of accidents, during the combustion of various fuels, it is found in the exhaust gases of cars and is classified as a carcinogen and mutagen. Dose-effect curves were obtained when exposed to ionizing radiation on yeast *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* cells, the effect of additives (hydrogen peroxide, ethanol, naphthalene) on the growth and viability of yeast in the post-radiation period was evaluated, and the effect of visible light on the growth and viability of cells after irradiation was investigated.

Keywords: yeast, X-ray, hydrogen peroxide, ethanol, naphalene.

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ВЛИЯНИЕ АКТИВНЫХ ФОРМ КИСЛОРОДА И ИОНИЗИРУЮЩЕГО ИЗЛУЧЕНИЯ НА ДРОЖЖЕВЫЕ КЛЕТКИ *SACCHAROMYCES CEREVISIAE* и *SACCHAROMYCES CARLSBERGENSIS* ПРИ ОСВЕЩЕНИИ ВИДИМЫМ СВЕТОМ

Научная статья

Аннотация

В настоящей работе защита дрожжей *Saccharomyces cerevisiae* и *Saccharomyces Carlsbergensis* от окислительного стресса в результате воздействия включает выращивание дрожжевой культуры в стандартных условиях до конца логарифмической или начала стационарной фазы роста, инкубацию с защитным агентом. Защитное средство представлено ингибитором - нафталином C10H8 в концентрации 5,10-4 М. Нафталин входит в состав тяжелых нефтяных фракций и выделяется в окружающую среду в результате аварий, при сгорании различных видов топлива, он содержится в выхлопных газах автомобилей и классифицируется как канцероген и мутаген. Были получены кривые доза-эффект при воздействии ионизирующего излучения на клетки дрожжей *Saccharomyces cerevisiae* и *Saccharomyces carlsbergensis*, оценено влияние добавок (перекись водорода, этанол, нафталин) на рост и жизнеспособность дрожжей в пострадиационный период, а также влияние видимого света на рост и жизнеспособность дрожжей. исследовали клетки после облучения.

Ключевые слова: дрожжи, рентген, перекись водорода, этанол, нафталин.

1. Introduction

It is known [1] that reactive oxygen species (ROS), which include H_2O_2, O_3, O, $^{\bullet}OH$, $^1O_2$, $O_2^{\bullet-}$, $HO_2^{\bullet}$, HO_2^-, NO, some organic free radicals: alkyl R•, alkyl peroxide RO_2•, alkoxyl RO•, anion radicals of a reducing nature D•– and some others are

formed as a result of reactions of catalytic initiation, dissolution of active gases from the atmosphere, radiation, cavitation exposure[2], photoradiation, biological emissionIn the work of Ananyeva E.P. and others [3], the effect of exposure to low-power density of microwave radiation on yeast Saccharomyces cerevisiae and Rhodotorula rubra was investigated. It has been shown that microwave exposure to each yeast reduces the concentration of cells and stimulates their growth at certain parameters. It has been established that ionizing radiation has a significant detrimental effect on living objects, but also at certain doses to show a positive effect (the effect of small doses) [4]. Radiation initiation in the absence of radiation pollution of the aquatic environment is carried out under the influence of a natural radioactive background and occurs mainly as a result of radiolysis of water [5]. Under normal conditions, the contribution of this component to the overall rate of radical formation is insignificant, but increases sharply with radiation contamination. Solar UV radiation initiates the formation of free radicals.

Humic acids, chlorophyll, riboflavin, coumarins [6] of plants and other natural compounds can act as photosensitizers that accelerate the transformation of substances under the influence of solar radiation [7]. Naphthols, polycyclic aromatic hydrocarbons, as well as many synthetic and natural pigments have photosensitizing properties among organic xenobiotics. In the cells of a living organism, free radicals are released during the natural process of metabolism and redox reactions [8].

Free radicals [9] as unstable molecules damage cell components in particular DNA, and induce stress response and repair systems, including antioxidants, enzymes, and mechanisms for changing cellular permeability [10], [11]. When the balance between reactions involving ROS and stress response systems is disturbed, a state of oxidative stress develops, which is one of the most physiologically significant, mediating other types of stress [12]. In this state, there is a sharp increase in oxidative processes, reactions of peroxidation and radical oxidation are intensified, the content of substances with pro-oxidant activity increases with insufficient antioxidant protection system [13], neutralization of the formed ROS, metabolism is rebuilt to the synthesis of proteins necessary for survival. The reproduction of microorganisms in a state of oxidative stress slows down and stops. An increase in the diffusion of oxygen into the cell or a violation of the functioning of the respiratory chain leads to an increase in the intracellular concentration of ROS by 100–1000 times, up to 10^{-5} M or more. All this adversely affects cells and cell populations.

Along with direct chemical and photochemical effects of ROS, primarily H_2O_2, are involved in intracellular processes and the transmission of regulatory signals in prokaryotic and eukaryotic cells [14], changed the redox state of the environment in cellular organelles, participated in hormonal regulation and other metabolic rearrangements.

Usually there are joint effects of stressors and antistressors of different nature, and in relation to the effects of ROS the effects of prooxidants and antioxidants [13], [16], [17], which may be the same compounds. In natural conditions, such a situation is typical on the surfaces of water, soil, and plants illuminated by sunlight where are formed as a result of photochemical exposure to ultraviolet light, H_2O_2 and other reactive oxygen species, radical particles, local zones with elevated temperatures, inducing responses to oxidative stress and heat shock in various organisms. At the same time, the cells are affected by near ultraviolet (UVA and UVB bands) and visible light. The latter can participate in neutralizing negative changes through the mechanisms of photoreparation and cross-reactions.

The purpose of this work is to obtain dose-effect curves when exposed to ionizing radiation [15] on yeast *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* cells [18] and to study the effect of visible light on the growth and viability of cells after irradiation, as well as the effect of additives (H_2O_2, ethanol, naphthalene) on the growth and viability of yeast cells in the post-radiation period was evaluated, and the effect of visible light on the growth and viability of cells after irradiation was investigated.

2. Research methods

In this work, hydrogen peroxide, naphthalene from Ruschem, medical ethanol, 96%, yeast *S. cerevisiae* strain T-985 race Mayen and *S. carlsbergensis* were used. The work used freshly prepared an aqueous solution of naphthalene with a concentration of $5 \cdot 10^{-4}$ M. In the course of replanting, a part of the suspension (5–10% by volume) was selected from the flasks, which was sown on a nutrient medium with subsequent determination of growth indicators in lighting conditions.

The cultivation of *S. cerevisiae* T-985 yeast [19], [20] was carried out under aerobic conditions with stirring of the medium in flasks on a thermostatically controlled rocking chair at 28°C, 150 rpm. The volume of the flasks was 200 ml, the volume of the sterilized medium in the flasks was 50 ml. The seed material was depending on the conditions of the experiment, – from 2 to 10% volume. For comparative evaluation, cultivation variants with yeast consistently adapted to the introduction of H_2O_2 with a light illumination level of 750 Lux on the surface of the medium were used. Composition of the medium for cultivating yeast *S. cerevisiae*, g/l: $(NH_4)_2SO_4$ – 5,0; KH_2PO_4 – 1,0; KCl – 0,15; $MgSO_4 \cdot 7H_2O$ – 0,2; yeast extract – 0,5; sucrose – 30; tap water, pH 5,8. Yeast adaptation to the introduction of hydrogen peroxide was used as a standard procedure for obtaining microorganisms to be sensitive to visible light in a state of oxidative stress [19], [20].

In the course of passivation and adaptation to hydrogen peroxide, 2 yeast lines were conducted by 2 flasks in each line. Line 1 – control, without the introduction of H_2O_2 and line 2 – with the introduction of H_2O_2 for 18–36 hours of cultivation in an amount of 0,6 g/l on all passages. A total of 10 passages were conducted. At the end of cultivation, at the stage of all passages, aliquots of yeast suspension were taken from the flasks, transferred to glass tubes with a volume of 2 ml and after 1 hour were irradiated on the Model-KALAN 4 X-ray machine at the Institute of Materials of Modern Energy and Nanotechnology, MUCTR at the absorbed dose rate of 3 Gy/s according to the Fricke dosimeter [21], [22]. After irradiation, part of the test tubes was kept in the light, and the other part in the dark.

Also, the research tubes were supplemented with solutions (hydrogen peroxide, ethanol, naphthalene) in a volume of 50 ml.

3. Results and discussion

Figure 1 illustrates the dependence of the optical density of the solution on the passage of the effect of the dose of X-ray irradiation of a suspension of yeast cells and the illumination factor on the survival of cells as they pass to oxidative stress by the introduction of hydrogen peroxide.

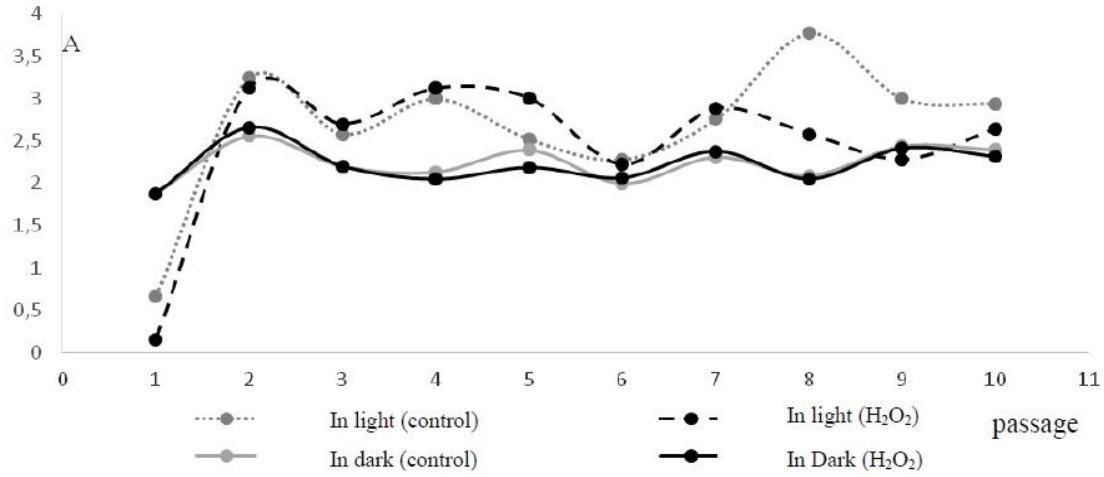

Fig. 1 – The optical density of the solution from passage by strain T 985 under aerobic conditions as the number of passages increasing with the introduction of H_2O_2

It has been recorded that delays the growth of yeast when storing a suspension of yeast cells in the dark, and then the dependence of optical density on passivation practically does not change after the introduction of hydrogen peroxide, an increase in optical density was established in the case of control after the 7th passage under visible light, yeast growth increases, and after the introduction of peroxide at the 7th stage of passivation, a decrease in the growth of yeast cells and the optical density value is almost the same as for the suspension of yeast cells that were stored in the dark. The simultaneous action of visible light creates favorable conditions for stimulating yeast growth. In this case, visible light against the background of X-ray action can act as a factor controlling cell growth.

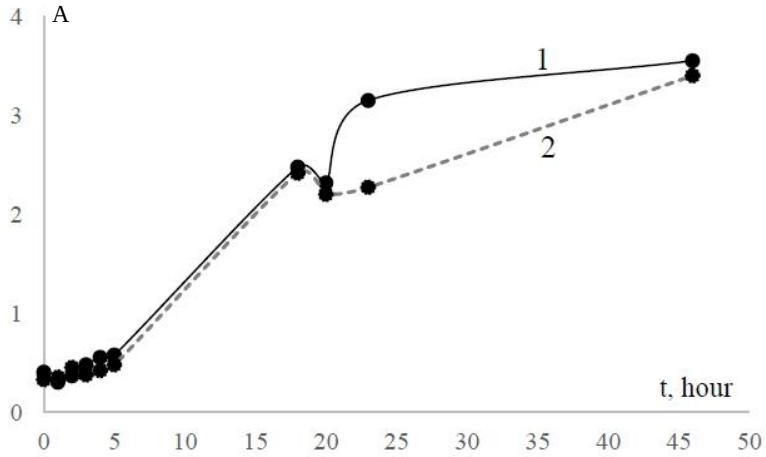

Fig. 2 – Yeast growth of Strain T 985 in the light after freezing-thawing of the inoculum:
1 – S. cerevisiae; *2* – the present of H_2O_2

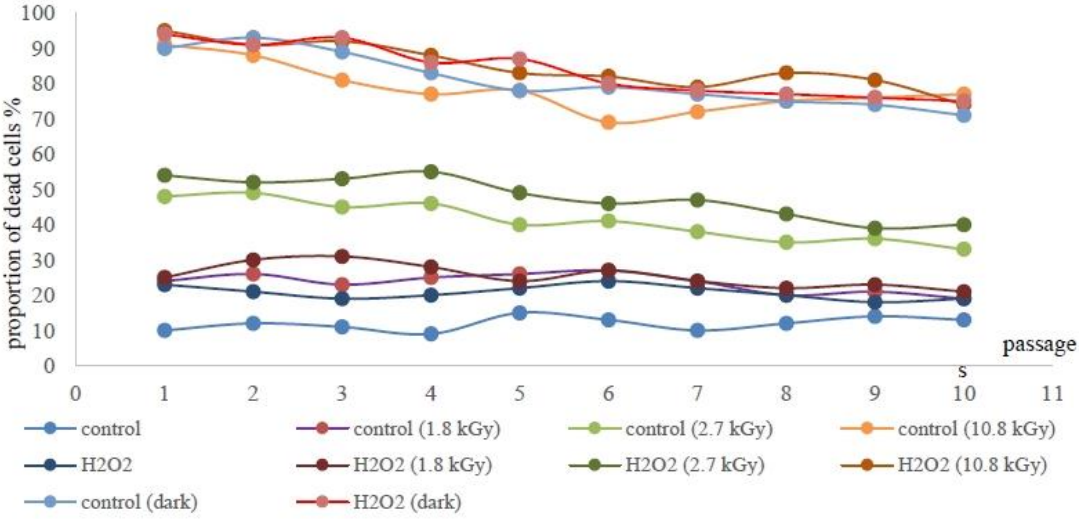

Fig. 3 – Changes in the proportion of dead cells in passages of strain N 985
as they adapt to optimal doses of hydrogen peroxide at different doses of X-ray irradiation
during subsequent exposure of the yeast suspension in daylight and in the dark

The proportion of dead was measured 3 hours after exposure to peroxide. Cultivation in flasks under aerobic conditions, the initial concentration of the substrate is 30 g/ l. As follows from the data shown in Fig. 3, the effect of X-rays is similar to the effect of hydrogen peroxide, while the lighting factor is crucial for adaptation. The proportion of dead cells exposed to the same dose of peroxide or X-ray, with subsequent exposure to darkness, is about 90% and 85%, respectively for the 1st passage. Without lighting, the remains high by the 10th passage: approximately 77% when exposed to peroxide and 74% when exposed to X-ray irradiation. When the environment is illuminated, the proportion of dead cells drops to the level in the control by the 6th passage. At the same time, H_2O_2, control (dark) and control (10.8 kGy) does not significantly affect the survival rate and the rate of adaptation of cells. Thus, X-ray irradiation with dose of 2,7 kGy is sufficient to restore cell viability. Light in this case acts as an antistressor, helping yeast cells to overcome the effects of oxidative stress, probably through the mechanism of photoreparation.

The paper considers the introduction of an aqueous solution of naphthalene into a solution of a suspension of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* cells from the time of cultivation (Fig. 4).

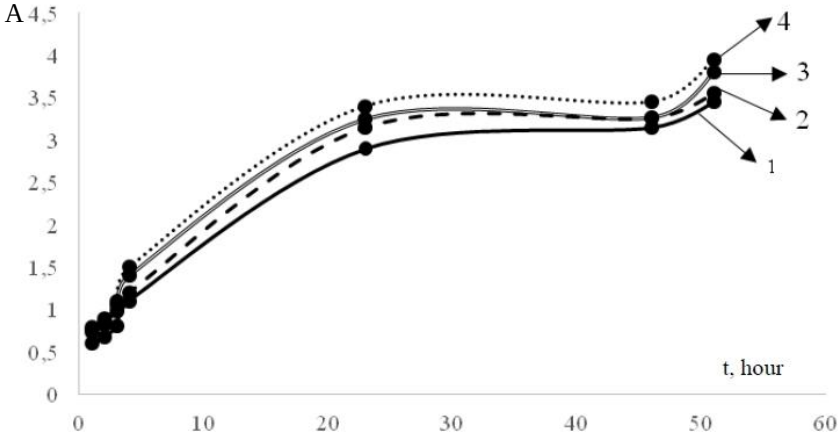

Fig. 4 – Growth dynamics of yeast *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* from the time of cultivation:
1 – S. carlsbergensis (control); *2 – S. cerevisiae* (control); *3 – S. carlsbergensis* ($C_{10}H_8$); *4 – S. cerevisiae* ($C_{10}H_8$)

Protection of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* from oxidative stress as a result of exposure includes growing a yeast culture under standard conditions until the end of the logarithmic or the beginning of the stationary growth phase, incubation with a protective agent. The protective agent is represented by an inhibitor – naphthalene in a concentration of $5 \cdot 10^{-4}$ M. The incubation time with the protective agent is 24–48 hours (Fig. 4). Yeast growth increases when naphthalene is introduced into the cultured medium (Fig. 3, curve 3 and curve 4).

It is known that hydroxylation products of naphthalene in the case of the naphthalene radiolysis are formed, which may manifest itself in an increase in protective properties with respect to yeast cells. The constant of interaction of naphthalene with OH is ($k_1 = 5 \times 10^9$ l/Ms) [23].

$$H_2O \qquad \text{X-ray} \qquad e^{-}, OH^{\cdot}, H^{\cdot}, H_2O_2, O_2^{\cdot -}$$

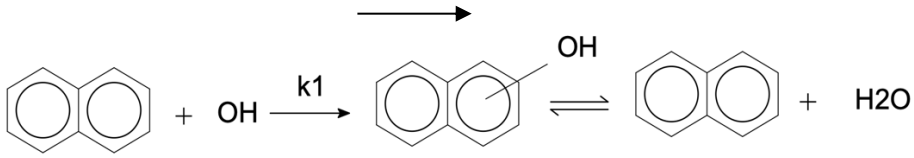

Fig. 5 – The constant of interaction of naphthalene with OH is ($k_1=5\times10^9$ l/Ms)

The number of living cells of *S. carlsbergensis* and *S. cerevisiae* practically does not change after further irradiation up to 800 Gy. It is shown that the change in the proportion of dead cells decreases by 7–9% at a dose of 400 Gy and by 3–8% at 800 Gy in the presence of naphthalene compared with the control (Fig. 6, curve 2). The product of radiolysis – the hydroxy derivative of naphthalene acts as a protective agent of yeast cells from radiation.

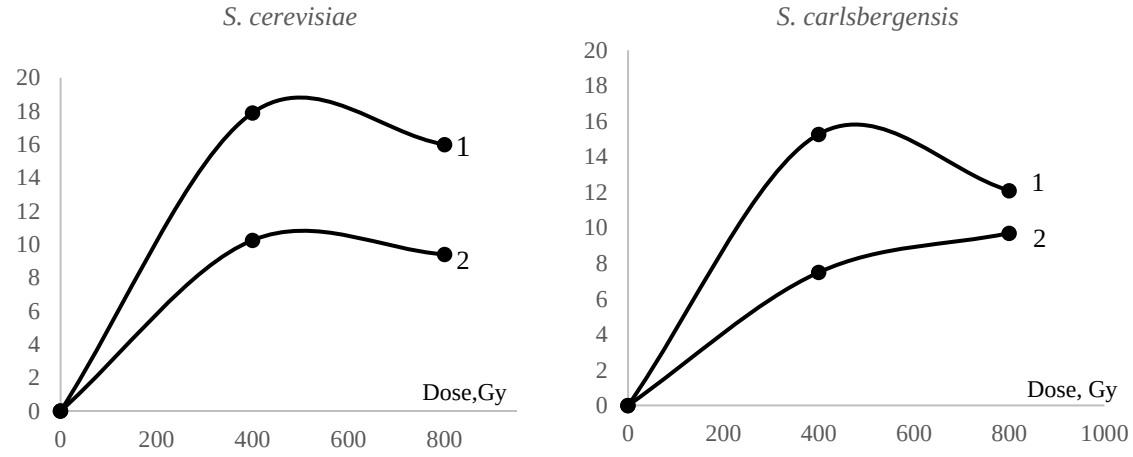

Fig. 6 – The change in the proportion of dead cells of *S. cerevisiae* and *S. carlsbergensis* from the radiation dose during subsequent exposure to yeast suspension:
1 – control; *2* – introduction of $C_{10}H_8$

It has been shown that yeast cells are sensitive to the effects of radiation and the number of dead cells increases from the dose. Figure 6 shows the change in the proportion of dead cells *S. cerevisiae* and *S. carlsbergensis* after exposure to irradiation with an absorbed dose of 400 Gy and 800 Gy in the presence and absence of naphthalene.

Consequently, exposure to ionizing radiation up to a dose of 150 Gy does not affect the viability of yeast cells under our experimental conditions. At doses above 150 Gy, yeast cells were inactivated with a gradual increase in the proportion of dead cells with further exposure to ionizing radiation on the systems. Adding ethanol to culture *S. cerevisiae* strain T-985 in an amount of 15 g /L in the exponential growth phase, followed by the selection of aliquots of yeast suspension at the end of growth into glass tubes and their irradiation 30 minutes after selection at doses of 400 Gy, 800 Gy, 1200 Gy, 1600 Gy and exposure to irradiated suspension in the light led to an increase in the proportion of dead cells by 1,5–2 times (Fig. 7) as the radiation dose increases. The proportions of dead cells in the post-radiation period (when irradiated from 400 Gy to 800 Gy) differed little (within a few %) from the proportion of cells measured immediately both for the ethanol-injected and non-ethanol-injected variants after irradiation.

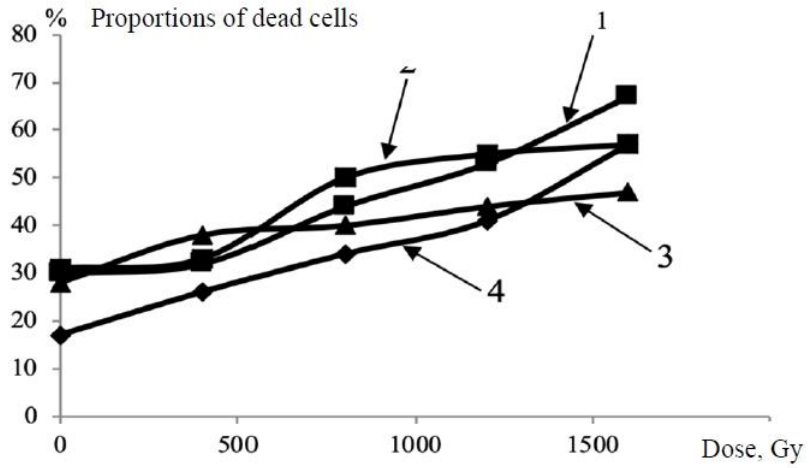

Fig. 7 – Changes in the proportion of dead yeast cells depending on the dose and time after irradiation (measurements immediately after irradiation and after a day):
1 – control; *2* – with ethanol; *3* – control 24 hours after irradiation; *4* – with ethanol 24 hours after irradiation

3. Conclusion

An important conclusion follows from the data presented in Fig.1–3: in order for heterotrophic microorganisms (yeasts) to become sensitive to visible light, including low dose of irradiation, they must be subjected to stressful influences or be in a state of stress. At the same time, the stress state does not necessarily have to be caused by to X-ray radiation, but, for example, by factors such as hydrogen peroxide, mechanical action and, possibly, heat shock, ultrasound, etc.

The results in Fig. 7 indicate that:

– yeast cells with the addition of ethanol in the concentrations used are less resistant to radiation compared to the control;

– there are no significant changes in the proportion of living cells observed in the post-radiation period compared to the measurement carried out immediately after irradiation, i.e., the measurement time factor elapsed after irradiation is less significant compared to other factors.

Cells that have survived irradiation under experimental conditions (with visible light illuminating the medium with yeast cells) remain viable for a sufficiently long period.

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Финансирование

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Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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