

PECULIARITIES OF GROWTH PARAMETERS OF *SACCHAROMYCES CEREVISIAE* UNDER DIFFERENT CONDITIONSA. H. SHIRVANYAN^{1*}, S. N. MIRZOYAN^{1,2,3**}, K. A. TRCHOUNIAN^{1,2,3***}¹ Chair of Biochemistry, Microbiology and Biotechnology, YSU, Armenia² Research Institute of Biology, YSU, Armenia³ Microbial Biotechnologies and Biofuel Innovation Center, YSU, Armenia

Saccharomyces cerevisiae is an essential component of human civilization because of its extensive use in food industry. At the point of industrial usage it is very important to get large amount of biomass but many factors causes yeast's viability loss and death. The aim of this work was to study the peculiarities of growth parameters – specific growth rate (SGR, μ , growth yield) of *S. cerevisiae* wine and beer strains under different external conditions, as well as pH and oxidation-reduction potential (ORP) changes during growth. It was shown that under aerobic cultivation, the conditions 30°C and pH 6.5 are more favorable for the growth of 2 strains (ATCC 9804, $\mu = 0.398$; ATCC 13007, $\mu = 0.407$), where they can enhance the biomass, and under microaerobic conditions the pH 5.0 and 37°C are more favorable (ATCC 9804, $\mu = 0.35$; ATCC 13007, $\mu = 0.436$). During aerobic conditions, lower ORP values are established which did not depend on growth temperature and external pH. Under microaerobic conditions until the establishment of the stationary phase (24 h) pH dependent changes of ORP were established. Obtained results will help to clarify the biochemical, biophysical and bioenergetic phenomena of yeast survival that can enhance the biomass growth and yield and thus applied industrial scale.

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Introduction. Yeasts are the main source of different compounds, such as proteins, lipids, nucleic acids etc. *S. cerevisiae* yeasts are widely used in the making of bread, wine, beer and cider; distilled beverages, such as rum, vodka, whisky, brandy, and sake since ancient times [1, 2]. They are also convenient organisms for using in genetic engineering, and thus we can obtain different valuable compounds, such as nutritional supplements, medicines, enzymes etc. These yeasts can be also used in bioremediation processes [3]. Advantages of *S. cerevisiae* is that they can be easily grown in cheap medias and genetically manipulated [4].

The preservation of biotechnological interest yeasts is a relevant topic both from a scientific and economic point of view. *S. cerevisiae* is an essential component

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of human civilization, because of its extensive use in food and beverage fermentation in which it has a high commercial significance. In the European yeast industry, a 1 million tons is produced annually, and around 30% of which is exported globally. The global market's annual growth rate was 8.8% from 2013 to 2018 [2].

At the point of industrial usage it is very important to get large amount of biomass. Hydric, thermal, nutritional and oxidative fluctuations are among the main causes of the yeasts' viability loss. Moreover, many factors, such as different stressful factors (for example high concentration of by products) can damage cell, therefore lead to cell death, and thus to the reduction of biomass producing. Despite this, new food applications require non-genetically modified strains, free of chemical additives and cells capable of resisting processing [5].

Thus, the fundamental question is to understand the biochemical, biophysical and bioenergetic phenomena of yeast survival that can enhance its preservation and thus control them in industrial scale.

Oxidation-reduction potential (ORP) reflects valuable metabolic information with the cultivation of aerobic and microaerophilic microorganisms [6]. ORP of fermentation systems is a combination indicator for pH, dissolved oxygen and metabolism with a large number of redox pairs, and the intracellular redox balance and physiology of cells at multiple metabolic levels can be partially determined by extracellular ORP. Regulation of extracellular ORP has been shown to enhance stress tolerance of yeast to high-concentration ethanol. At the same time, chip analysis for gene expression also showed that the changes of ORP correlated with the expression of many stress response pathways such as the heat shock protein (HSP) family [6].

Considering the importance of the above problem the aim of this work was to study the peculiarities of growth parameters (SGR, growth yield) of *S. cerevisiae* wine and beer strains under different external conditions, as well as pH and ORP changes during aerobic or microaerobic growth.

Materials and Methods.

Yeast Strains and Growth. Two strains of yeasts, used in this research *S. cerevisiae* ATCC 9804 and *S. cerevisiae* ATCC 13007 (American Type Culture Collection, Manassas, USA) were purchased from Microbial Depository Center of Scientific and Production Center «Armbiotechnology» NAS RA. Yeast were grown in YPD medium (1% yeast extract, 2% peptone, 2% dextrose) overnight, then 3% inoculum were transferred in sterile YPD medium with different pH, which was adjusted to required values with K_2HPO_4 to pH 6.5 or to pH 5.0 by 0.1 normal HCl [7–9]. Yeasts were grown 48 h aerobically (200 mL media in 500 mL conical flasks, 130 rpm rotary shaking) and microaerobically (250 mL media in 250 mL conical flasks, without shaking). The shaking speed was selected based on a previous study that found that optimum agitation speed is 130 rpm [10].

The yeast biomass growth was studied with double beam UV-VIS spectrophotometer (Cary 60, “Agilent Technologies”, Germany) following the optical density (OD) readings of culture absorbance under the wavelength of 600 nm against the medium as blank at half hour intervals [11–13].

The yeast SGR calculated using the following equation, μ :

$$\mu = \frac{\ln(OD_2) - \ln(OD_1)}{t_2 - t_1},$$

where OD_2 and OD_1 are the ODs of the microbial culture at t_2 and t_1 hours, respectively, moreover in (t_2-t_1) hour $OD_2:OD_1=2$ [14]. Growth kinetics of *S. cerevisiae* ATCC 9804 and *S. cerevisiae* ATCC 13007 strains was presented in Fig. 1, which was used for determination of culture doubling time. For other conditions the same principle was applied [15].

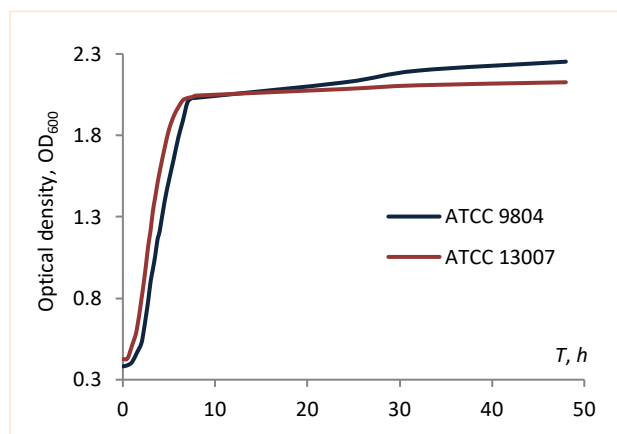


Fig. 1. Growth kinetics of *S. cerevisiae* cells in batch culture in YPD at 30°C and pH 6.5. Determination of ORP and external pH.

Growth yield was stated as the difference of maximum and initially biomass (mg) by using biomass – OD standard curve of each strain [16]. The collected biomass was centrifuged at $6000\text{ G}/min$ at 4°C and washed two times with distilled water. The resulting precipitate was dried at 105°C for 4 h and the yeast biomass was measured with an analytical balance [17].

During yeast growth pH, ORP changes were measured. External pH was measured by Milwaukee MW151 MAX pH/ORP/Temp Logging Bench Meter equipped with MA917 pH electrode. ORP measurement were done by Ionometer I-160.1MP (Republic of Belarus, Gomel Plant of Measuring Devices) by using titanium-silicate (Ti-Si) and platinum (Pt) electrodes at $25\pm 1^\circ\text{C}$. ORP electrode was calibrated by $16.13\text{ g}\cdot\text{L}^{-1}\text{ K}_3[\text{Fe}(\text{CN})_6]$ and $21.12\text{ g}\cdot\text{L}^{-1}\text{ K}_4[\text{Fe}(\text{CN})_6]$ (pH 6.86) reference solution, where the ORP was $254\pm 10\text{ mV}$ at 25°C [14, 18].

Chemicals and Data Processing. Glucose, peptone, yeast extract (“Sigma Aldrich”, Germany), and the other reagents of analytical grade were used.

Sample averages were compared to measure significance of the difference using Student’s t test. Each experiment was repeated three times.

Results and Discussion. Environmental conditions affect almost all physical and biological processes. Ensuring robustness in performance during varying environmental conditions can be an important requirement in these processes [19]. In addition, the conditions during growth also influence subsequent survival [20]. For this reason, to achieve desired performance of a synthetic circuit outside laboratory-controlled environment, it is important to design temperature robustness. Failing to respond to non-optimal temperature and pH can cause cell death as well as alter functional properties of biomolecular systems. Industrial process is largely affected by growth rate of the organism such as yeasts. Thus, microbial growth rate

is an important parameter and it is necessary to understand how this parameter depends on temperature [19, 21]. This can provide us important insight regarding role of temperature in these processes as well as can help design techniques to enhance robustness to temperature variations. While the dependence of yeast growth on different temperatures has been well studied for many species, prediction of yeast growth rate for dynamic temperature changes is relatively unclear [19]. In this work we studied the effect of temperature changes (25–37°C) and pH (5.0 and 6.5) of media in the SGR of *S. cerevisiae* yeasts (Fig. 2).

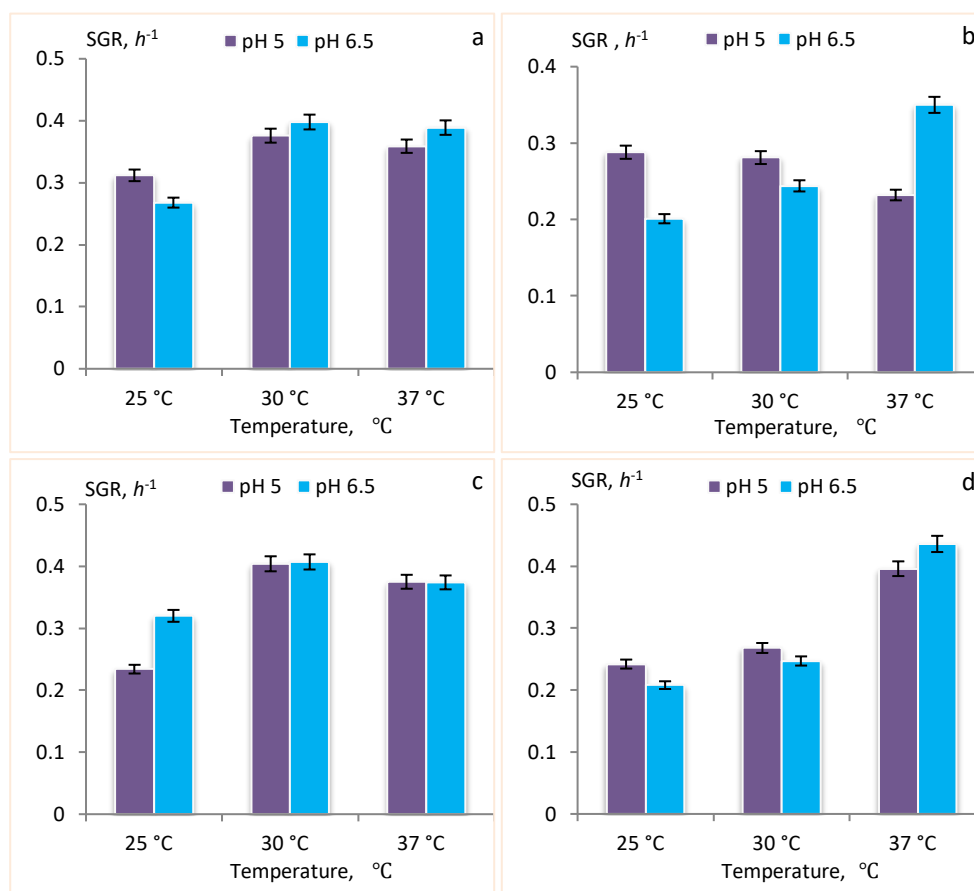


Fig. 2. SGR of *S. cerevisiae* strains (a, b – ATCC 9804; c, d – ATCC 13007) grown under different conditions (a, c – aerobic growth condition; b, d – microaerobic growth conditions) depending on temperature.

In aerobic conditions at 30°C SGR of *S. cerevisiae* ATCC 9804 1.33 times exceeds the same parameter under microaerobic condition at pH 5.0 and 1.63 times at pH 6.5, which may be due to greater tolerance to low oxygen content. However, obtained results show that in the case of *S. cerevisiae* ATCC 9804, aerobic conditions are more conducive to rapid reproduction, which may be associated with the presence of oxygen, which leads to the formation of a large amount of ATP, which can be used for growth and accumulation of substances.

On the hand for *S. cerevisiae* ATCC 13007 in aerobic conditions at 30°C, SGR 1.51 times exceeds the same parameter under microaerobic condition at pH 5.0, which may be related to more oxygen needs for growth. In the same conditions for this strain at pH 6.5 SGR reduction (1.65 times) is similar as in the case of *S. cerevisiae* ATCC 9804, which means that at pH 6.5 adaptive processes to oxygen limitation are not strain – depended and there universal mechanism for adapting to oxygen limitation conditions and to grow in microaerobic conditions. However, SGR of *S. cerevisiae* ATCC 13007 in microaerobic conditions at 37°C SGR at aerobic condition 1.1 and 1.2 times at pH 5.0 and pH 6.5, respectively, whereas for *S. cerevisiae* ATCC 9804 aerobic condition is still more favorable for growing even at high temperatures. Thus, based on the SGR data, we can say that *S. cerevisiae* ATCC 13007 is a more stress-resistant (particularly against high-temperature and oxygen-limiting) strain than ATCC 9804, which is important feature for using them in industry.

Based on the SGR data, it can be suggested that in the case of aerobic cultivation, the conditions 30°C and pH 6.5 are more favorable for the growth of both strains (ATCC 9804, $\mu = 0.398$; ATCC 13007, $\mu = 0.407$) and under microaerobic conditions the pH 5.0 and 37°C are more favorable (ATCC 9804, $\mu = 0.35$; ATCC 13007, $\mu = 0.436$), which is probably due to the fact that at higher temperatures fermentation enzymes are more active, which, in turn, activates the overall fermentation process, giving cells additional energy to ensure adequate growth. These data are in good conformity with the existing data from different groups [21–23]. The ideal temperature range for fermentation and bioethanol production is between 30°C and 38°C (strain-dependent) [21]. Differences in SGR under microaerobic cultivation conditions are due to the nature of the strains: *S. cerevisiae* ATCC 9804 strain was isolated from palm wine [24] and able to produce wine, in fermentation process of which is released also acetic acid, which results in pH decrease (Fig. 3, b, d).

Acetic acid biosynthesis appears to be a mechanism that yeast use to balance NAD^+ (nicotinamide adenine dinucleotide) formed in response to hyperosmotic stress and glycerol overproduction. This may occur through the action of cytosolic, NAD^+ – dependent aldehyde dehydrogenases, which oxidize acetaldehyde to acetic acid while reducing NAD^+ to NADH [25]. Unlike *S. cerevisiae* ATCC 9804, another strain, ATCC 13007, is brewing yeast [26], which synthesizes fewer acids than the latter.

It is also interesting to understand the growth yield of tested yeast strains grown under different external conditions, because due to the protein content and probiotic properties of yeasts of the species *S. cerevisiae*, yeast biomass is an option to consider for animal feed [17]. Thus, dry weigh of yeast has been determined and summarized in Table.

Growth yield differences of *S. cerevisiae* under different conditions

Growth yield, mg						
<i>S. cerevisiae</i> ATCC 9804						
	aerobic conditions			microaerobic conditions		
	25°C	30°C	37°C	25°C	30°C	37°C
pH 6.5	34.7347	34.7013	33.5862	27.6345	27.3692	27.3859
pH 5.0	35.9333	36.0315	36.2598	30.8943	32.564	29.6642
<i>S. cerevisiae</i> ATCC 13007						
pH 6.5	20.177	19.1646	21.0451	16.1522	16.7361	18.3022
pH 5.0	21.7756	21.5366	23.1657	17.7746	18.4273	18.4024

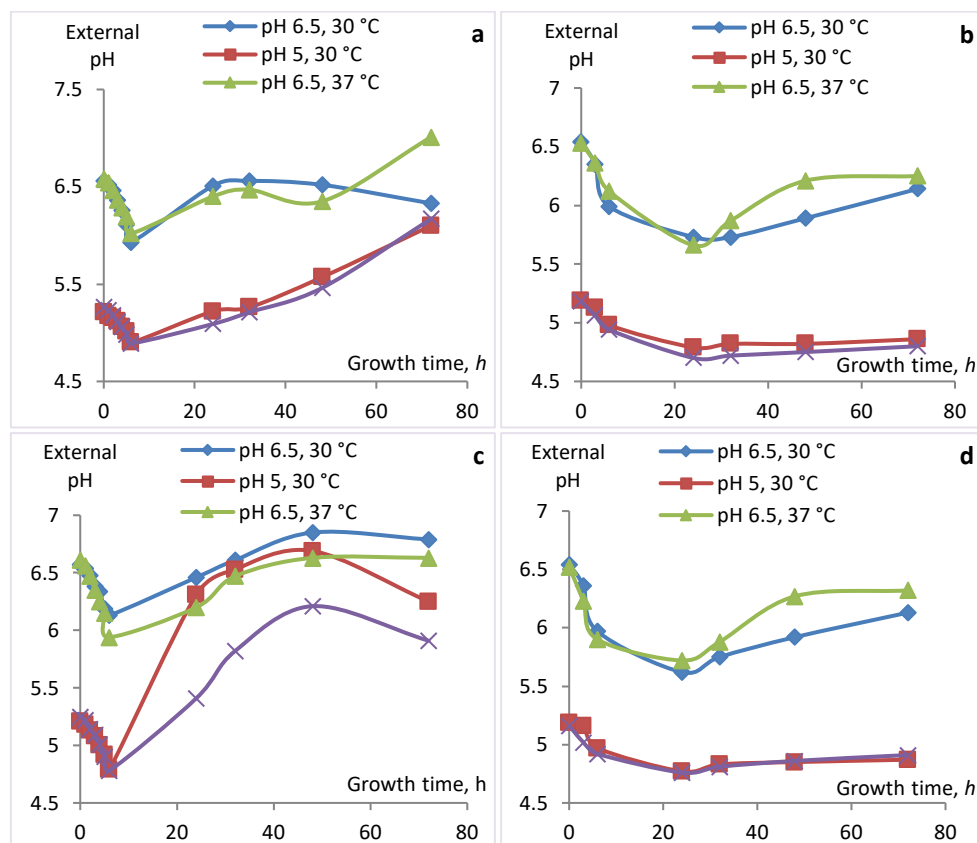


Fig. 3. External pH changes during growth (strains: a, b – ATCC 9804; c, d – ATCC 13007) under different conditions (a, c – aerobic growth condition; b, d – microaerobic growth conditions) depending on temperature.

As could be expected from the data on the SGR, it can also be seen from the growth yield data, that yeast cultivated under aerobic conditions produces more biomass than under microaerobic conditions, which is probably due to the fact that under aerobic conditions they are active as both catabolic and anabolic metabolic pathways unlike microaerobic conditions, activates mainly energy exchange due to lack of oxygen. Under microaerobic conditions growth yield differences and lower biomass production by *S. cerevisiae* ATCC 13007 may be conditioned by active leaking carbon dioxide during fermentation. These results correlated with growth kinetics (Fig. 1): although *S. cerevisiae* ATCC 13007 growing rapidly, in final it produces less biomass than another strain. *S. cerevisiae* strains are well adapted to large amounts of inhibitory compounds. Native oxidoreductases reduce furan aldehydes to less toxic alcohols, which require NADPH (nicotinamide adenine dinucleotide phosphate) as cofactors. The yeast compensates for the increase in cofactor demand by decreasing glycerol production and increasing acetate production to balance the redox potential. Acids interfere with cell membrane permeability and fluidity, affecting membrane protein function and cellular processes including nutrient transport and energy transduction, and once inside the cell, the intracellular pH decreases. Yeast cells counteract proton accumulation by

translocation via the plasma membrane H^+ -ATPase, mediated by ATP hydrolysis. This decreases intracellular ATP levels with a concomitant reduction in biomass yield [27]. *S. cerevisiae* yeast cells may generate energy both by fermentation and aerobic respiration, which are dependent on the type and availability of carbon sources. Cells adapt to changes in nutrient availability, which entails the specific costs and benefits of different types of metabolism but also may cause alteration in redox homeostasis, both by changes in reactive oxygen species (ROS) and in cellular reductant molecules contents [28].

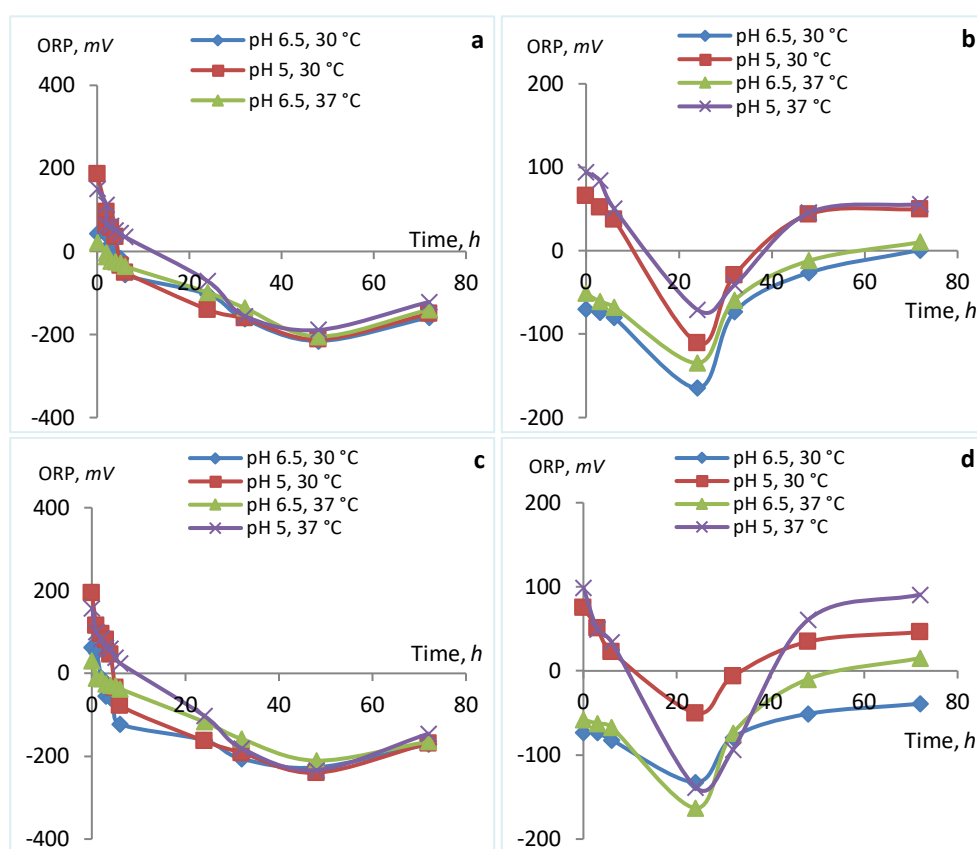


Fig. 4. Monitoring of ORP changes during growth (strains: a, b – ATCC 9804; c, d – ATCC 13007) under different conditions (a, c – aerobic growth condition; b, d – microaerobic growth conditions) depending on temperature.

So for that redox potential during yeast growth was monitored. As it was shown in Fig. 4, the kinetics of ORP are very different in aerobic to microaerobic cultivation conditions. The changes of readings of ORP Pt electrode from positive ($+180 \pm 10$ mV) to negative (-240 ± 10 mV) values were observed upon the growth of *S. cerevisiae* ATCC 13007 (Fig. 4, c; pH = 5.0, 30°C). In the case of *S. cerevisiae* ATCC 9804 the maximum range of ORP was from $+180 \pm 10$ mV to -215 ± 10 mV, which shows that *S. cerevisiae* ATCC 9804 has more oxidizing ability than *S. cerevisiae* ATCC 13007. As it was proposed under aerobic conditions, lower ORP values are established, which indicates the presence of more reductive state, and this

nature of kinetics is almost does not depend on temperature and pH. The latter only affect the growth of yeast, leading to a more intense or passive expression of ORP, but in general, the dynamics of change in ORP is maintained in all samples.

In contrast to aerobic conditions, under microaerobic conditions until the establishment of the stationary phase (24 h) pH dependent changes of ORP were established. In literature it was shown that the behavior of the reduction potential during the fermentation of a Sauvignon blanc, showing the minimum in the redox potential curve was lower at higher temperatures from 0 mV at 15°C to -200 mV at 24°C [29]. The pH dependence of the kinetics of ORP differs between both strains, particularly in the conditions of pH 5.0 and 37°C (Fig. 4, b, d). Changes in the redox conditions in the fermentation medium, and especially in the finished beer, significantly affect the processes of transformation of colloidal substances and the formation of turbidity [30]. As it was shown, the highest ethanol concentration of 131.0 ± 1.8 g/L was obtained for ethanol fermentation of flocculating yeast when ORP was controlled at -150 mV, because the lower ORP level with more reducing power available for maintaining metabolism of yeast cells improved their viability more significantly, which is affected by osmotic pressure and ethanol inhibition, enhancing ethanol productivity and yield. On the other hand, more biomass was accumulated when ORP was controlled at -100 mV [31]. Based on the data on the above data, we can assume that the maximum fermentation activity of *S. cerevisiae* ATCC 13007 and ethanol production was detected at 24 h of growth in conditions of 37°C, pH 5.0 and pH 6.5, where ORP was -163.2 ± 10 mV and -138.5 ± 10 mV, respectively (Fig. 4, d).

It would be of further separate interest to study the products that released during this growth and the final explanation of the metabolic pathways will explain these features.

Conclusion. Here we show that many factors such as temperature, external pH, ORP can affect yeast growth and lead to loss of viability of yeast cell or biomass production decrease. It was shown that in the case of aerobic cultivation, the conditions 30°C and pH 6.5 are more favorable for the growth of 2 strains, in which they can rapidly reproduce, and produce more biomass and under microaerobic conditions the pH 5.0 and 37°C are more favorable. Under aerobic conditions, lower ORP values are established and this nature of kinetics is almost does not depend on temperature and pH. Under microaerobic conditions until the establishment of the stationary phase (24 h) pH dependent changes of ORP were established.

The results obtained will help to better understand the biochemical and bioenergetic basis of yeast growth and stress tolerance mechanisms and to optimize production conditions, ensuring stable biomass and high production yield under changing environmental conditions.

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**SACCHAROMYCES CEREVISIAE ԽՄՈՐԱՍՆԿԵՐԻ ԱՃԻ
ՊԱՐԱՄԵՏՐԵՐԻ ԱՌԱՆՁՆԱՀԱՏԿՈՒԹՅՈՒՆՆԵՐՆ
ԱՃՄԱՆ ՏԱՐԲԵՐ ՊԱՅՄԱՆՆԵՐՈՒՄ**

Saccharomyces cerevisiae-ն մարդկային քաղաքակրթության կարևոր քաղաքիչներից է, քանի որ այն լայնորեն օգտագործվում է սննդի արդյունաբերության մեջ: Արդյունաբերական տեսանկյունից շատ կարևոր է մեծ քանակությամբ կենսազանգվածի ստացումը, սակայն բազմաթիվ գործոններ

առաջացնում են կենսունակության կորուստ և խմորասնկերի մահ: Այս աշխատանքի նպատակն էր ուսումնասիրել տարբեր պայմաններում *S. cerevisiae*-ի գինու և գարեջրի շտամերի աճի պարամետրերի՝ աճի տեսակարար արագության (μ), աճի արտադրողականության, ինչպես նաև այդ աճի ընթացքում pH-ի և օքսիդացման-վերականգնման պոտենցիալի (ՕՎՊ) փոփոխությունները: Ցույց է տրվել, որ աերոբ աճեցման դեպքում 30°C և pH 6,5 պայմաններն առավել արդյունավետ են եղել 2 շտամերի աճեցման համար (ATCC 9804, $\mu = 0,398$; ATCC 13007, $\mu = 0,407$), որտեղ նրանք կարող են արագ բազմանալ և տալ մեծ քանակությամբ կենսազանգված, իսկ միկրոաերոբ աճեցման դեպքում առավել արդյունավետ է pH 5,0 և 37°C (ATCC 9804, $\mu = 0,35$; ATCC 13007, $\mu = 0,436$) պայմանները: Աերոբ պայմաններում է ՕՎՊ-ի ավելի ցածր արժեքներ, և սա կախված չէ աճեցման ջերմաստիճանից և pH-ից: Միկրոաերոբ պայմաններում մինչև ստացիոնար փուլի հաստատումը (24 ժ) նկատվում է ՕՎՊ-ի անկում: Արդյունքները կօգնեն հասկանալ խմորասնկերի գոյատևման կենսաքիմիական, կենսաֆիզիկական և կենսաէներգետիկ հիմունքները, որոնք կարող են ուժեղացնել դրանց գոյատևումը և դրանով իսկ վերահսկել արդյունաբերական մասշտաբով:

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ХАРАКТЕРИСТИКИ РОСТА *SACCHAROMYCES CEREVISIAE* В РАЗНЫХ УСЛОВИЯХ РОСТА

Saccharomyces cerevisiae – важный компонент человеческой цивилизации, поскольку они широко используются в пищевой промышленности. При промышленном использовании очень важно получить большое количество биомассы, но многие факторы вызывают потерю жизнеспособности и гибель дрожжей. Целью данной работы было изучение особенностей параметров роста (удельной скорости роста (μ), урожайности) винных и пивных штаммов *S. cerevisiae* в различных условиях, а также изменений pH и окислительно-восстановительного потенциала (ОВП) во время этого роста. Было показано, что при аэробном культивировании условия 30°C и pH 6,5 более благоприятны для роста двух штаммов *S. cerevisiae* (ATCC 9804, $\mu = 0,398$; ATCC 13007, $\mu = 0,407$), они могут быстро воспроизводиться и производить больше биомассы. А в микроаэробных условиях более благоприятны pH 5,0 и 37°C (ATCC 9804, $\mu = 0,35$; ATCC 13007, $\mu = 0,436$). В аэробных условиях устанавливаются более низкие значения ОВП, и это не зависит от температуры и pH. В микроаэробных условиях до установления стационарной фазы (24 ч) происходит снижение ОВП. Полученные результаты помогут понять биохимические, биофизические и биоэнергетические явления выживания дрожжей, которые могут повысить их сохранность и, таким образом, контролировать их в промышленных масштабах.