

EVALUATION OF ETHANOL AND BIOMASS PRODUCTION RATE  
BY DIFFERENT *SACCHAROMYCES CEREVISIAE* STRAINS DEPENDING  
ON EXTERNAL pH AND TEMPERATUREA. H. SHIRVANYAN<sup>1,2\*</sup><sup>1</sup> Chair of Biochemistry, Microbiology and Biotechnology, YSU, Armenia<sup>2</sup> Research Institute of Biology, Laboratory of Microbiology, Bioenergetics  
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Bioethanol production is in high demand due to its potential to replace transportation fuel and its ability to be renewable and long-lasting. Therefore, the bioconversion of fermentable sugars to ethanol is of central importance. The work aimed to explore yeast properties changes during fermentation at different pHs and temperature conditions. Biomass production, specific growth rate (SGR), fermentation products, and metabolite composition and quantity changes were studied at 25°C, 30°C, and 37°C as well as pH 5 and pH 6.5 conditions. SGR data shows that at 25°C and 30°C pH 5 is optimal for yeast biomass production. At pH 5.0–6.5, the biomass production of the ATCC 9804 strain almost 2 times exceeds the same parameter for another strain. The highest biomass production was detected at 30°C and pH 5. Ethanol production by yeast increases as the growth temperature decreases at pH 5 in contrast to pH 6.5, where the temperature changes within 30–37°C range have no significant effect on it in both strains. At 30°C ethanol concentration in the extracellular medium reached ~117 mM in the case of ATCC 13007 strain, whereas for ATCC 9804 the same parameter was 1.4-fold lower. Both strains had the same substrate assimilation rate. Glycerol production reduces with increasing growth temperature and pH; the highest glycerol concentration (6.1 mM) was observed within 32 h growth of ATCC 9804 strain at 25°C, pH 5 and reaches 5.5 mM within 24 h growth of ATCC 13007 strain under the same conditions. At pH 5 and 6.5, the free energy for glycerol production was 2.3-fold and 4-fold higher compared to that of ethanol and acetate production processes in both strains. Gibbs free energy of ethanol production reaches the lowest value compared to the same parameter of acetate and glycerol production at pH 5, suggesting a preference for the alcoholic fermentation metabolic pathway under these conditions. The lowest acetate production was observed after 24 hours of growth of the ATCC 13007 strain at 37°C and pH 5, with  $\Delta G = 173.9 \text{ kJ/mol}$ . Obtained data highlights temperature stress mechanisms regulation of yeast and can be used for improving ethanol production processes and yeast genetic modification tools.

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**Keywords:** *Saccharomyces cerevisiae*, ethanol production, biomass production, HPLC, fermentation balance, free energy.

**Introduction.** The continued growth of energy consumption and the accumulation of atmospheric greenhouse gases, along with their effects on climate

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change, have made renewable energies, including biofuel production, one of the main challenges of the 21st century. Biofuels are liquid or gas fuels produced from biomass, such as organic waste materials. These fuels are in high demand due to their significant reduction in ecological impact on global warming compared to fossil fuels [1, 2], as well as their renewable and sustainable characteristics. [3]. This technology's key component is the bioconversion of fermentable carbohydrates to ethanol [4]. According to [5], the annual global production of ethanol reached 29000 million gallons in 2019.

*Saccharomyces cerevisiae*, *Zymomonas mobilis*, and *Escherichia coli* have been extensively studied and developed for the generation of ethanol. *S. cerevisiae* is a preferred workhorse for the corn and sugarcane ethanol production industry [4]. A single batch of ethanol produced by some yeast strains has been reported to contain up to 18% (v/v) ethanol [6].

*S. cerevisiae* is the main player in the commercial manufacture of ethanol among the numerous yeasts that produce ethanol through sugar fermentation. *S. cerevisiae* uses glycolysis to catabolize carbohydrates under anaerobic conditions, getting two molecules of pyruvic acid. It then undergoes pyruvate decarboxylase conversion to carbon dioxide and acetaldehyde, which is then reduced to ethanol by alcohol dehydrogenase while simultaneously releasing  $\text{NAD}^+$ . As a result, the terminal step reactions are crucial and serve as the basis for important fermentation industries [7].

Key enzymes in the pyruvate-to-ethanol pathway in yeast cells include pyruvate decarboxylase 1 (Pdc1) and alcohol dehydrogenases 1 and 2 (Adh1 and Adh2). The tetrameric enzyme Adh1 (EC 1.1.1.1) from the yeast *S. cerevisiae* has four equal subunits, each of which has a zinc ion-binding active site. Three distinct ligands, Cys43, His66, and Cys153, let the  $\text{Zn}^{2+}$  ion maintain its tetrahedral geometry while leaving the fourth position open for catalysis. When the cofactor nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) binds to the enzyme at the active site, a complex known as E- $\text{NAD}^+$  is formed that binds the alcohol substrate and subsequently allows a hydride transfer. As a result, a novel complex E-NADH aldehyde is formed. The aldehyde product is finally released, allowing NADH to separate from the enzyme. During catalysis, the oxygen atom of the alcohol substrate displaces a water molecule linked to  $\text{Zn}^{2+}$ . In this manner, zinc maintains the tetrahedral coordination of the intermediate alkoxide [8]. Adh1 (fermentation) is more activated in *S. cerevisiae*, when there is a high concentration of sugar present than Adh2, which uses ethanol as a substrate for respiration and biomass production [9] (see Fig. 1).

It is important to understand the metabolism regulation system's responses under different conditions. The dynamic changes during fermentation under different conditions, especially under stressful environmental conditions, have not been well characterized [10]. Yeast cells frequently experience unpleasant conditions when producing particular products. For instance, during the processing of lignocellulosic biomass, hazardous concentrations of inhibitors may be released. Acetic acid, formic acid, furfural and other acids are some of these inhibitors. Additionally, slightly high temperatures (35–39°C) are desired to perform simultaneous saccharification and fermentation to relieve inhibition of enzyme activity [10]. Additionally, during the production of ethanol, the temperature within a bioreactor may increase from 30°C

to roughly 40°C. High temperatures disable cell growth and the metabolic activity of yeast cells, which lowers ethanol productivity and yield. Therefore, the use of thermotolerant microbes is promising to solve the problem of ethanol production at high temperatures [11]. It is crucial to investigate the relationship between metabolism regulation systems and energy balance with different operating conditions improving the ethanol production efficiency of microbial cell factories.

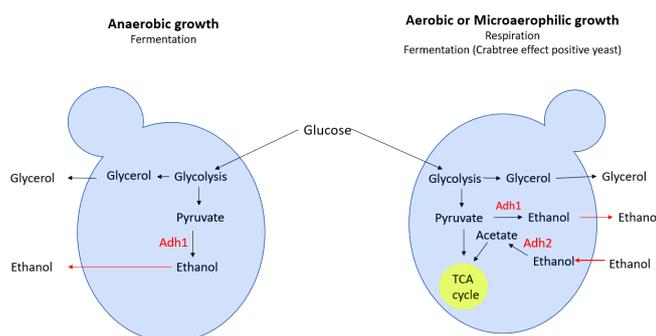


Fig. 1. Alcohol dehydrogenases isozymes function during fermentation and respiration.

This work aimed to study the effect of growth temperature on ethanol production rate and metabolites profile of *S. cerevisiae* wine and beer strains.

### Materials and Methods.

**Growing Media and Cell Culture Preparation.** Both, *S. cerevisiae* ATCC 9804 and ATCC 13007 strains (American Type Culture Collection, USA) used in this study have been purchased from Microbial Depository Center of Scientific and Production Center “Armbiotechnology” NAS RA. The *S. cerevisiae* ATCC 9804 strain was isolated from palm wine [12], whereas the *S. cerevisiae* ATCC 13007 strain is a lager brewing strain [13, 14]. Strain selection was based on their biotechnological potential. The YPD medium (1% yeast extract, 2% peptone, and 2% dextrose) was used to grow the yeasts, and agar (2% w/v) was added for solid cultures. Single colonies were used to inoculate liquid cultures (5–15 mL), which were then incubated at 30°C overnight. After that, 3% v/v inoculum was added to a sterile YPD medium [15] and incubated at 25–37°C in a microaerophilic (250 mL medium in a 250 mL conical flask, without shaking) environment.  $K_2HPO_4$  or 0.1 N HCl was used to change the pH of the medium [16–18].

Following the optical density (OD) readings of culture absorbance under the wavelength of 600 nm against the medium as a blank, the yeast biomass growth was examined using a double beam UV-VIS spectrophotometer (Cary 60, “Agilent Technologies”, Germany) [19–21]. When the yeast growth curve was linear, the specific growth rate (SGR) was estimated as the ratio of the logarithmic difference between a doubled optical reading and doubling time [22].

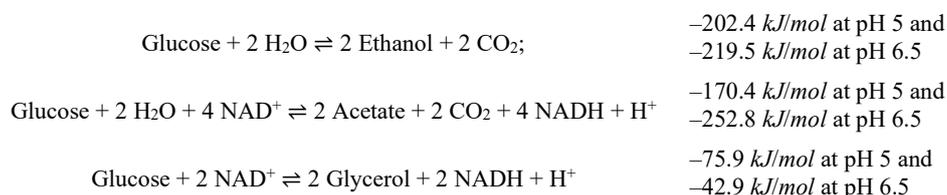
**Organic Acids, Alcohols, and Sugar Determination.** Organic acids (acetate, succinate), alcohols (ethanol, glycerol), and sugars were identified with high-performance liquid chromatography (HPLC, Agilent 1260 Infinity II LC Bioinert) with a refractive index detector (Agilent RID, G1362A, set on positive polarity and optical unit temperature of 55°C) [23]. Data were processed with the Agilent

OpenLAB CDS system. The column (Macherey-Nagel EC 250/4.6 NUCLEOSIL 120–5 C18 column (250 × 4.6 mm, MN720041.46, Düren, Germany)) previously was purged with acetonitrile/water (1:1) at 60°C and then in 10 μL of each sample was injected. The separation of organic compounds was carried out with mobile phase (5 mM sulfuric acid in ddH<sub>2</sub>O) in 42 min analysis time with 0.4 mL/min flow speed [24]. The concentrations of dissolved chemicals were calculated using the standard curves.

The data obtained by HPLC are used to describe several metabolic processes. Substrate utilization and metabolites production rate was calculated as the difference in concentration (mM) for the given growth period. Carbon conversion efficiency (CCE) was determined by following equation:

$$\text{CCE} = \frac{\Delta C_2}{\Delta C_1} \cdot 100\%,$$

where  $\Delta C_1$  is the difference in substrate carbon concentrations during a given growth period and  $\Delta C_2$  is the difference in fermentation product carbon concentrations during the same growth period, mM [24, 25]. Fermentation balance was calculated by the difference in carbon concentration at the stationary and lag growth phase expressed in %. Gibbs free energy was calculated by the following the Josiah Willard Gibbs equation (1870):  $\Delta G = \Delta G^0 + RT \ln Q$ , taking into account the following  $\Delta G^0$  values [26]:



**Chemicals and Data Processing.** Glucose, peptone, agar, yeast extract, (“Carl Roth GmbH”, Germany), Succinate (“Sigma Aldrich”, Germany), and the other reagents and chemicals of analytical grade were used.

Each data point represented was averaged from independent triplicate cultures: the standard deviation was not more than 3%. Statistical analysis was performed by using Student’s *t*-test [27, 28]. Graph presentations were carried out using the GraphPad Prism 8.0.2.263 software (San Diego, CA, USA) and MS Excel [29].

**Results and Discussion.** Optimization of industrial processes requires as high as possible specific growth rate and biomass yield. Many industrial related factors such as high sulfite dosage, high sugar concentration related osmotic stress, high organic acids and alcohols (ethanol and glycerol) can affect on the yeast leading to loss of vitality. Inability to adapt to unfavorable temperature and pH can result in cell death and modify the manner, in which that biomolecular systems work. We were interested in determining, which temperature *S. cerevisiae* exhibits the highest metabolic rate while performing aerobic respiration. Literature lists a wide range of optimal growth temperatures for yeast, including 25°C to 30°C [30], 30°C to 33°C [31], as well as 25°C to 35°C [32]. Our study aimed to determine, which temperature

was closest to the optimal temperature for the metabolism, biomass and ethanol production in *S. cerevisiae*. Previously we have shown that *S. cerevisiae* ATCC 9804 is more stable against to temperature and pH changes than *S. cerevisiae* ATCC 13007 during microaerophilic growth [33]. Growth temperature rise stimulates higher SGR value (Fig. 1), which may be conditioned with the increased fermentation enzymes activities. The only exception is growth pH 5 for ATCC 9804 strain, which optimally growth at 25°C in these conditions. At 25°C and 30°C SGR of yeast is higher at pH 5 than at pH 6.5. SGR of *S. cerevisiae* ATCC 9804 at 37°C, pH 6.5 exceeds the same parameter at pH 5 by 1.51 times, whereas for *S. cerevisiae* ATCC 13007 it was 1.1 times. These results are in good conformity with [34], who showed that rise in temperature (35–45°C) and ethanol concentration challenges the yeasts growth parameters during sugar fermentation: as the temperature rises, yeast's growth rate and metabolism accelerate until they reach the optimal level.

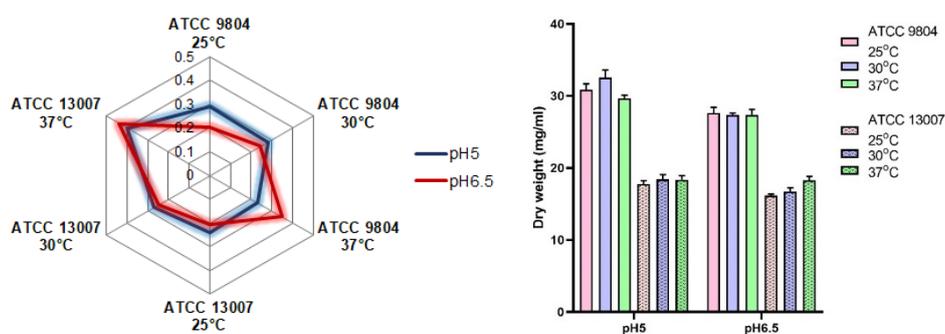


Fig. 2. Specific growth rate and dry weight of *S. cerevisiae* different strains depending on growth temperature (25–37°C) and pH (5 and 6.5)

Growth yield analysis showed that in both pH 5 and pH 6.5 the biomass production activity of the ATCC 9804 strain almost 2 times exceed the same parameter for another strain. Biomass production reduction may be replaced with a high carbon dioxide production rate with is typical for beer strains such as ATCC 13007 [35]. The highest biomass production was detected at 30°C and pH 5 growth conditions (Fig. 2). pH and temperature affect the yeast growth lag and exponential phase, but not the stationary phase, which results in almost the same biomass production rate at different conditions. In comparison with SGR results growth yield studies showed that the latter is not pH and temperature dependent, which may be conditioned by the adaptive ability of yeast to those conditions.

The ethanol concentration was strongly affected by both cell concentration and pH. Previously shown that high temperatures inhibit cell growth and the metabolic activity of yeast cells, resulting in a reduction in ethanol yield and productivity [36]. Results show that at pH 5 ethanol production ability of yeast increase as the growth temperature decreases, while at pH 6.5 the temperature changes at the 30–37 range have no significant effect on ethanol production in both strains (Fig. 3). Ethanol production rate reaches the same values for both strains at pH 6.5. The ethanol production capacity of ATCC 13007 is slightly higher than ATCC 9804. Thus, at 30°C ethanol concentration in the extracellular medium reached 117.25 mM in the case of ATCC 13007 strain, whereas for ATCC 9804 the

same parameter was 1.4-fold lower. The maximal ethanol yield was obtained during glucose fermentation at 25–30°C, pH 5 by *S. cerevisiae* ATCC 13007. Other works have shown that maximum ethanol concentration (73 mM) was achieved at pH 5.5 after 15 h of fermentation [36], as opposed to which these strains demonstrate maximal ethanol production at 24–32 h of growth.

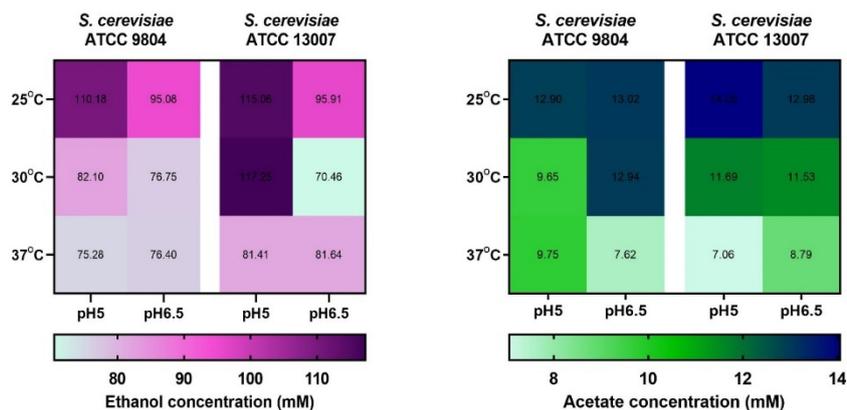


Fig. 3. Heat maps of maximal ethanol and acetate production by different strains of *S. cerevisiae*.

Increased ethanol concentration during fermentation can impede the development and viability of microorganisms [34]. In the presence of excess ethanol yeast can exhibit reduced cell viability and growth, such as a decrease in cell volume. There can also be effects on yeast metabolism (e.g., stress-response proteins, lowered protein levels, and denaturation), cell structure, and membrane function (e.g., inhibition of endocytosis, loss of electrochemical gradients). Ethanol toxicity to yeast is primarily due to cell membrane damage. However, maintaining an ion balance (e.g., magnesium and potassium) can provide the membrane with protective effects from ethanol toxicity and temperature changes [2].

By comparing the results of ethanol and acetate production, we can notice that temperature and pH decrease affects similar in both products accumulation. Acetate concentration reaches its highest rate (14 mM) during 24 h of growth ATCC 13007 at pH 5 and 25°C, and the lowest production was observed when the same strain was grown at 37°C and pH 5 conditions. Acetate accumulation in growth medium decreases pH, which leads to cell viability loss. So lower SGR and biomass yields at 25°C may be conditioned with high fermentation products levels, as well as by-products accumulation.

Metabolites HPLC analysis results are summarized in Tab. 1.

Results show that both strains have the same substrate assimilation rate and 110 mM glucose is completely consumed within 24 h reaching the glucose assimilation rate of 4.6 for both strains. Although strains differ with ethanol production rate; at pH 5 in all growth temperatures ethanol production rate of ATCC 13007 strain, 1.1–1.8 times exceeds the same value for another strain in contrast to pH 6.5, where this value is almost the same for both strains. At higher pH alcohols and acids ratio remains at lower values indicating that at pH 6.5 more acids and low alcohols produced than at pH 5. Results show that at 25°C and 37°C glycerol production has

the same manner for both strains contrary to 30°C, pH 6.5 conditions where ATCC 13007 produces 35.1% more glycerol than ATCC 9804 strain. Glycerol production reduces in correlation with temperature and pH arise; the highest glycerol level (6.1 mM) was observed within 32 h growth of ATCC 9804 strain at 25°C, pH 5 and reaches 5.53 mM within 24 h growth of ATCC 13007 strain at the same conditions. Glycerol formation plays a major role: it regulates intracellular NADH/NAD<sup>+</sup> ratio, acts as an osmoregulator, and prevents cells from freezing by acting as an anti-freezing agent [37, 38].

Table 1

24 h growth analysis of *S. cerevisiae* different strains metabolites depending on growth temperature (25–37°C) and pH (5 and 6.5)

	ATCC 9804					ATCC 13007				
	Gluc AR, mM/h	Et PR, mM/h	(Et+Glyc)/ (Ac+Suc)	CCE, %	FB, %	Gluc AR, mM/h	Et PR, mM/h	(Et+Glyc)/ (Ac+Suc)	CCE, %	FB, %
25°C										
pH 5	4.6±0.1	3.1±0.09	6.8±0.2	60.2±1.8	62.1±1.9	4.6±0.1	3.6±0.1	7.1±0.2	93.5±2.8	68.5±2.0
pH 6.5	4.6±0.1	3.2±0.1	6.2±0.19	91.8±2.8	60.4±1.8	4.6±0.1	3.3±0.1	6.7±0.2	71.9±2.2	62.5±1.9
30°C										
pH 5	4.6±0.1	2.5±0.08	7.7±0.23	59.7±1.8	47.7±1.4	4.6±0.1	4.4±0.1	8.6±0.3	98.2±2.9	74.2±2.2
pH 6.5	4.6±0.1	2.9±0.09	6.1±0.18	59.7±1.7	50.8±1.5	4.6±0.1	2.3±0.05	5.2±0.2	44.9±1.3	45.1±1.35
37°C										
pH 5	4.6±0.1	2.0±0.06	6.3±0.19	58.6±1.8	44.5±1.3	4.6±0.1	3.3±0.1	11.1±0.3	74.7±2.2	55.4±1.7
pH 6.5	4.6±0.1	2.7±0.08	8.1±0.24	62.6±1.9	48.6±1.5	4.6±0.1	2.8±0.08	8.5±0.2	66.6±2	55.3±1.7

Abbreviation: Gluc – glucose, Glyc – glycerol, Et – ethanol, Ac – acetate, Suc – succinate, AR – assimilation rate, PR – ethanol production rate, FB – fermentation balance.

Trace amounts of succinate are detected by HPLC by both strains at 25°C and pH 5, but not at higher temperatures. Succinate is produced by the metabolic respiration pathway during microaerophilic growth and is constantly involved in successive reactions of the Krebs cycle and did not secrete to the extracellular medium and captures a very small amount in the carbon conversion process. There may be several reasons for this. First of all, succinate is a weak acid, although when secreted into the extracellular environment, it can carry protons, which will lead to an increase in intracellular pH and membrane potential, which is inefficient for the cell. Furthermore, the free energy was almost 2 times higher (–124 kJ/mol) than that of ethanol production (–203.75 kJ/mol) at 25°C, pH 5, which facilitates succinate production in trace amounts (2–3 mM).

Gibbs energy studies (Table 2) show that the free energy for alcoholic fermentation reaches its lowest state at pH 5, compared to the same parameter for acetate and glycerol production. This is why ethanol is the predominant end product in yeast at pH 5.

The temperature does not affect the free energy values of alcoholic fermentation, but it has important role in regulation of acetate production rate in both strains. The higher acetate production rate at 25°C and pH 5 conditions can be attributed to the high fermentation efficiency of yeast, while, for example, at 37°C, the lowest acetate levels were recorded, as indicated above. Besides that, it was

shown that at pH 4.5 the uncharged acetic acid molecules enter cells by facilitated diffusion through the Fps1p aquaglyceroporin channel, encounter a more neutral pH in the cytoplasm and dissociate into acetate and protons. The protons lead to cytoplasmic acidification thereby inhibiting important metabolic processes [39].

Table 2

Gibbs free energy changes ( $\Delta G$ , kJ/mol) of *S. cerevisiae* different strains during different alcohol, acetate and glycerol production processes depending on temperature (25–37°C) and pH (5 and 6.5)

	ATCC 9804			ATCC 13007		
	Et	Ac	Glyc	Et	Ac	Glyc
25°C						
pH 5	-203.75±6.1	-179.86±5.4	-84.14±2.5	-202.78±6.08	-176.79±5.3	-83.69±2.5
pH 6.5	-219.94±6.6	-259.0±7.8	-51.38±1.5	-220.52±6.6	-259.95±7.8	-50.47±1.5
30°C						
pH 5	-203.63±6.1	-179.63±5.4	-84.2±2.5	-202.59±6.1	-177.75±5.3	-83.89±2.5
pH 6.5	-220.85±6.6	-246.05±7.4	-52.86±1.6	-221.54±6.5	-266.89±7.7	-52.0±1.5
37°C						
pH 5	-204.02±6.1	-178.04±5.3	-85.38±2.6	-203.26±6.1	-179.3±5.4	-86.12±2.5
pH 6.5	-220.79±6.3	-262.83±7.8	-51.91±1.5	-220.65±6.6	-262.2±7.9	-51.1±1.5

Weak acids such as the above-mentioned succinate or acetic acid, induce activation of the proton-translocating ATPase Pma1p in yeast plasma membrane, which pumps out the protons generated by weak acid dissociation in the cytosol in an ATP-dependent manner. This ensures the maintenance of the electrochemical potential across the plasma membrane regulating ion and pH balance and providing energy for nutrient uptake [39].

Glycerol production Gibbs free energy was 2.1–2.3-fold and 4-fold higher compared to ethanol and acetate production processes at pH 5 and pH 6.5, respectively. This explains the higher glycerol production rates at lower pH. However, taking into account its roles (regulates intracellular NADH/NAD<sup>+</sup> ratio, osmoregulatory, and antifreezing agent) for yeast metabolism and stress (e.g., acidic stress) resistance mechanisms regulation for improving ethanol production efficiency it is assumed that it would be more reasonable to genetically modify the pathway of acetic acid formation, which would lead the ethanol yield not only at pH 5, but especially at pH 6.5. Nevertheless, further experiments needed to confirm or reject the hypothesis.

**Conclusion.** Increasing growth temperature activates yeast's SGR. At 25–30°C pH 5 was optimal for growth of both strains. SGR of *S. cerevisiae* ATCC 9804 at 37°C, pH 6.5 exceeds the same parameter at pH 5 by 1.5 times, while for *S. cerevisiae* ATCC 13007, it was 1.1 times higher. The biomass production activity of the ATCC 9804 strain is nearly double that of the other strain at both pH 5 and pH 6.5. The highest biomass production was achieved at 30°C and pH 5. At pH 5, ethanol production ability of yeast increases as the growth temperature decreases, whereas at pH 6.5, changes in temperature within the 30–37°C range have no significant effect on ethanol production in both strains. The extracellular medium's ethanol content for the ATCC 13007 strain reached 117.25 mM at 30°C, but it was 1.4-fold higher for the ATCC 9804 strain. The maximal ethanol yield was obtained

during glucose fermentation at 25–30°C and pH 5 by *S. cerevisiae* ATCC 13007. The substrate assimilation rate is equivalent for both strains. At pH 5, in all growth temperatures, ethanol production rate of the ATCC 13007 exceeds that of the other strain by 1.1–1.8 times. At 25°C and 37°C, glycerol production behaves similarly for both strains, contrary to the conditions of 30°C and pH 6.5, where ATCC 13007 produces 35.1% more glycerol than ATCC 9804. Temperature and pH cause a decrease in glycerol synthesis; the highest glycerol level (6.1 mM) was observed within 32 hours of ATCC 9804 strain growth at 25°C and pH 5, whereas it reached 5.5 mM within 24 hours of ATCC 13007 strain growth under the same conditions. Gibbs energy studies show that at pH 5, ethanol fermentation reaches the lowest free energetic state compared to acetate and glycerol production. This is why ethanol is the predominant end product in yeast fermentation at pH 5. Temperature does not impact free energy values but does have an influence on acetate production rates in both strains. Glycerol production Gibbs free energy was 2.1–2.3-fold and 4-fold higher compared to that of ethanol and acetate production processes at pH 5 and pH 6.5, respectively.

Obtained data highlights temperature stress mechanisms regulation of yeast and can be used for improving ethanol production processes and yeast genetic modification tools.

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Ա. Հ. ՇԻՐՎԱՆՅԱՆ

*SACCHAROMYCES CEREVISIAE*-ի ՏԱՐՔԵՐ ՇՏԱՄԵՐԻ ԿՈՂՄԻՑ  
 ԷԹԱՆՈԼԻ ԵՎ ԿԵՆՍԱԶՉԱՆԳՎԱԾԻ ԱՐՏԱՊՐՈԴԱԿՈՒԹՅԱՆ  
 ԱՐԱԳՈՒԹՅԱՆ ԳՆԱՀԱՏՈՒՄԸ ԿԱԽՎԱԾ ՋԵՐՄԱՍՏԻՃԱՆԻՑ  
 ԵՎ ԱՐՏԱԶԻՆ թH-ԻՑ

Կենսաէթանոլի արտադրությունը մեծ պահանջարկ ունի՝ տրանսպորտային վառելիքային միջոցները փոխարինելու ներուժի և վերականգնվող լինելու ունակության շնորհիվ, ուստի խմորվող շաքարների էթանոլի կենսավերափոխումը առանցքային նշանակություն ունի: Աշխատանքի նպատակն է եղել ուսումնասիրել խմորասնկերի հատկությունների փոփոխությունները խմորման ընթացքում տարբեր pH-ների և ջերմաստիճանների պայմաններում: Կենսազանգվածի արտադրության, աճման տեսակարար արագության (SGR), խմորման արգասիքների և միջնորդանյութերի կազմի և քանակի փոփոխություններն ուսումնասիրվել են 25 °C, 30 °C և 37 °C, ինչպես նաև pH 5 և pH 6.5 պայմաններում: SGR-ի տվյալները ցույց են տալիս, որ 25 °C և 30 °C ջերմաստիճանում pH 5-ը օպտիմալ է խմորասնկերի կենսազանգվածի արտադրության համար: pH 5–6.5 արժեքներում ATCC 9804 շտամի կենսազանգվածի արտադրության ակտիվությունը գրեթե 2 անգամ գերազանցում է մյուս շտամին: Կենսազանգվածի ամենաբարձր արտադրությունը հայտնաբերվել է 30°C և pH 5 պայմաններում: pH 5-ում էթանոլի արտադրությունը խմորասնկերի կողմից աճում է ջերմաստիճանի նվազման հետ, մինչդեռ pH 6.5-ում ջերմաստիճանի փոփոխությունը 30–37°C միջակայքում էական ազդեցություն չունի էթանոլի արտադրության վրա երկու շտամներում էլ: 30°C ջերմաստիճանում էթանոլի կոնցենտրացիան արտաբջջային միջավայրում հասել է 117,25 մՄ-ի ATCC 13007 շտամի դեպքում, մինչդեռ ATCC 9804-ի դեպքում նույն պարամետրը 1.4 անգամ ցածր է եղել: Երկու շտամներն ունեն սուբստրատի յուրացման նույն արագությունը: Գլիցերոլի արտադրությունը նվազում է ջերմաստիճանի և pH-ի աճին զուգահեռ. գլիցերոլի ամենաբարձր մակարդակը (6,1 մՄ) դիտվել է ATCC 9804 շտամի 32 ժ աճի ընթացքում 25°C-ում, pH 5 և հասնում է 5.53 մՄ-ի ATCC 13007 շտամի 24 ժ ընթացքում նույն պայմաններում: Գլիցերոլի արտադրության ազատ էներգիան 2.1–2.3 անգամ և 4 անգամ ավելի բարձր է եղել էթանոլի և քացախաթթվի արտադրության գործընթացների ազատ էներգիայի համեմատ pH 5 և pH 6.5 պայմաններում համապատասխանաբար: Էթանոլի խմորման համար Գիբսի ազատ էներգիան հասնում է նվազագույն արժեքի՝ համեմատած ացետատի և գլիցերինի արտադրության հետ pH 5-ում, հետևաբար ածխածնի փոխակերպումը ընթանում է հիմնականում ալկոհոլի խմորման ճանապարհով: Ամենացածր ացետատի արտադրությունը գրանցվել է ATCC 13007 շտամի աճի 24 ժ-ի ընթացքում 37 °C և pH 5 պայմաններում,  $\Delta G=173.9$  կՋ/մոլ: Ստացված տվյալները ընդգծում են խմորասնկերի ջերմաստիճանի նկատմամբ սթրեսի մեխանիզմների կարգավորումը և կարող են օգտագործվել էթանոլի արտադրության գործընթացները և խմորասնկերի զենետիկական ճարտարագիտության գործիքները բարելավելու համար:

А. А. ШИРВАНЯН

ОЦЕНКА СКОРОСТИ ПРОДУКЦИИ ЭТАНОЛА И БИОМАССЫ  
РАЗЛИЧНЫМИ ШТАММАМИ *SACCHAROMYCES CEREVISIAE*  
В ЗАВИСИМОСТИ ОТ ТЕМПЕРАТУРЫ И ВНЕШНЕГО pH

Производство биоэтанола находится в высоком спросе из-за его потенциала заменить транспортное топливо и способности быть возобновляемым и долговечным. Поэтому биоконверсия ферментируемых сахаров в этанол имеет центральное значение. Работа направлена на изучение изменений свойств дрожжей во время ферментации при различных значениях pH и температурных условиях. Производство биомассы, удельная скорость роста (УСР), продукция ферментации и изменения состава и количества метаболитов изучались при температурах 25 °С, 30 °С и 37 °С, а также при условиях pH 5 и pH 6.5. Данные по УСР показывают, что при 25 °С и 30 °С оптимальным для производства биомассы дрожжей является pH 5. При значениях pH от 5 до 6.5 производство биомассы штамма ATCC 9804 почти в 2 раза превышает такой же параметр для другого штамма. Максимальное производство биомассы было обнаружено при 30 °С и pH 5. Производство этанола дрожжами увеличивается при снижении температуры роста при pH 5, в отличие от pH 6.5, где изменения температуры в диапазоне от 30 °С до 37 °С не оказывают значительного влияния на него в обоих штаммах. При 30 °С концентрация этанола во внеклеточной среде достигла ~117 мМ в случае штамма ATCC 13007, в то время как для штамма ATCC 9804 этот же параметр был в 1.4 раза ниже. Оба штамма имели одинаковую скорость ассимиляции субстрата. Производство глицерина уменьшается с увеличением температуры роста и значения pH; максимальная концентрация глицерина (6.1 мМ) наблюдалась при 32-часовом росте штамма ATCC 9804 при 25 °С и pH 5 и достигала 5.5 мМ при 24-часовом росте штамма ATCC 13007 при тех же условиях. При значениях pH 5 и 6.5 свободная энергия производства глицерина была в 2.3 раза и 4 раза выше по сравнению с таким же параметром производства этанола и ацетата в обоих штаммах. Свободная энергия Гиббса производства этанола достигает наименьшего значения по сравнению с таким же параметром производства ацетата и глицерина при значениях pH 5, что указывает на предпочтение алкогольного метаболического пути в этих условиях. Наименьшее производство ацетата наблюдалось после 24 часов роста штамма ATCC 13007 при 37 °С и pH 5, с  $\Delta G = 173.9$  кДж/моль. Полученные данные подчеркивают механизмы регуляции стресса от температуры у дрожжей и могут использоваться для улучшения процессов производства этанола и инструментов генетической модификации дрожжей.