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Research article

Effect of ultraviolet radiation on physiological and biochemical properties of yeast *Saccharomyces cerevisiae* during fermentation of ultradispersed starch raw material

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ABSTRACT

Background: Study of correlation between pretreatment of yeast with ultraviolet radiation and efficiency of further fermentation of wort made of ultrafine grain particles to ethanol.
Results: We investigated three races of industrial yeast *Saccharomyces cerevisiae* (native and irradiated by ultraviolet). Physiological properties during fermentation of starchy wort were tested in all variants. It was shown that activation of the yeast by ultraviolet radiation allows to further increase the ethanol yield by 25% on average compared with the native yeast races when using thin (up to micro- and nano-sized particles) or standard grain grinding.
Conclusions: Using mechanical two-stage grinding of starchy raw materials and ultraviolet pretreatment of yeast, the efficiency of saccharification of starch and fermentation of wort to ethanol was increased.

1. Introduction

Important properties in the ethanol industry are intensification of wort fermentation process and to save energy, raw materials, and time. The ethanol yield depends on a number of factors (pH, temperature, etc.) and the physiological state of industrial yeast culture.

In ethanol production from dry milled grain, enzymatic hydrolysis of starch is carried out in two steps: gelatinization with the addition of thermostable amylase resulting in dextrin formation (liquefaction) and subsequent incubation with glucoamylase at lower temperatures to convert dextrins into glucose (saccharification). In industrial settings, the combination of pretreatment time and temperature varies from 165 °C (3–5 min) to 90–105 °C (1–3 h).

In wet milling technologies, granular starch is obtained and then hydrolyzed without heat cooking at 30–32 °C using special enzyme preparations (the so-called granular starch hydrolyzing (GSH) enzymes) [1]. Approaches to GSH enzyme technology application for dry milling have also just begun to develop.

Another problem of the alcohol industry is industrial yeast culture. The general direction of yeast new strain selection is to obtain races that provide the required fermentation rate and alcohol yield at wort fermentation for 3 d or less and resistance to stress factors such as ethanol and dry matter concentration, temperature, and presence of inhibitors [2]. There are a number of basic methods to obtain new races. Classical methods of selection include hybridization, polyploidy, mutagenesis, and genetic engineering [3,4,5,6]. One such mutagenic factor is UV radiation with a wavelength of 254 nm [7,8,9,10]. Depending on the radiation intensity, both activation of yeast (as a protective mechanism against stress) and mutation can occur. Some authors have shown that mutation of various genes occurs under UV exposure [11,12]. It was found that inactivation of genes responsible for radiosensitivity changes the frequency of mutations induced by different mutagenic factors. Therefore, rad2 mutation increases the frequency of UV-induced mutations of resistance to serine and respiratory failure mutations. Moreover, xrs2 and xrs4 mutations reduce the frequency of UV-induced mutations of resistance to serine and reversions to adenine independence by ADE2 gene nonsense mutation. The xrs2 mutation increases the frequency of cytoplasmic mutations, suggesting that the repair system in yeast acts not only in the nucleus but also in the cytoplasm [11,13]. Transcriptome analysis of mutant and wild-type alcohol yeast showed that the ethanol tolerance is caused by increased levels of oxidative processes in the mitochondria, which also stimulates glycolysis [14]. It was also found that several genes were highly expressed only in the ethanol-tolerant

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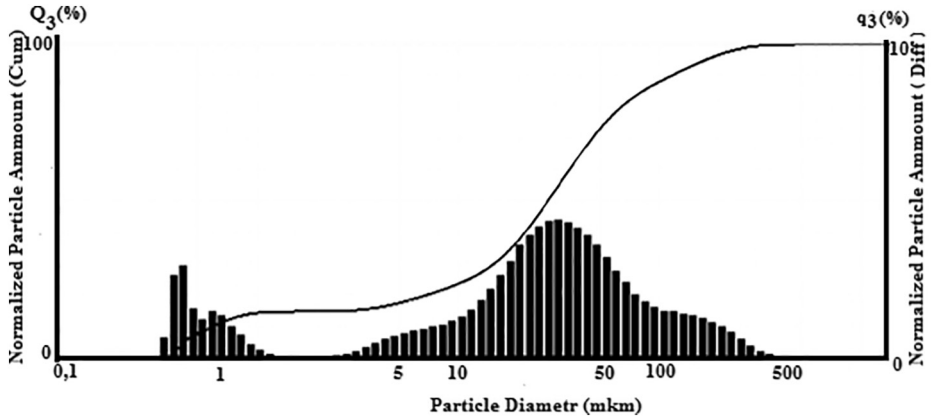

Fig. 1. Size distribution of the obtained ultradispersed raw material particles.

mutant but not in the parent strain. These genes were known to be induced in cells that were exposed to various stresses, such as ethanol, heat, and high osmolarity stress, or at the stationary phase but not log phase. In the ethanol-tolerant mutant, the expression level of these stress-responsive genes was further increased after exposure to ethanol. It was found that substances such as catalase, glycerol, and trehalose, which may have protective roles under stressful conditions, were accumulated in high amounts in the ethanol-tolerant mutant. The ethanol-tolerant mutant also exhibited resistance to other stresses including heat, high osmolarity, and oxidative stress in addition to ethanol tolerance [15,16,17].

In this article, a highly efficient yeast strain for producing ethanol from starch raw materials was obtained by UV radiation. Efficient ethanol producers were selected depending on sugar tolerance, ethanol tolerance, and fermentation activity.

2. Experimental

2.1. Yeast strains

Nine strains of *Saccharomyces cerevisiae* (Safdistil C-70 (Fermentis, France) and two mutant variants, Angel (Angel yeast, China) and two mutant variants, and Oeonoferm (Erbsloh, Germany) and two mutant variants) were used in this study. Mutant variants were selected after UV treatment from among 10 strains of each race of yeast with relatively high ethanol production and high tolerance to ethanol and sugar.

2.2. UV treatment conditions

Yeast culture was inoculated in 10 ml of YPD medium (2% glucose, 1% peptone, and 2% yeast extract) and incubated overnight at 30 °C until cell density reached 2×10^8 cells/ml. The cells were diluted to obtain a final density of 10–100 cells/ml. Then 100 μl of this suspension was taken and inoculated on Saburo agar plate. Exposure was conducted at UV irradiation intensity (wavelength 254 nm) of 200 mW/cm^2 for 5, 10, and 15 min. To stop photoreactions, plates were kept in the dark for 24 h. The colonies were incubated for 3 d at 30 °C.

2.3. Raw materials

We used wheat grain with the following characteristics as raw material: trash admixture 0.22%, humidity 10%, and conventional starch content 59%.

2.4. Preparation of raw materials for fermentation

For wort preparation, grains were ground in two stages [18,19]. In the first stage, grains were ground to a size of approximately 1 mm in a laboratory grain mill LZM-1 (Russia). Further grinding of the grain raw materials was performed in the planetary ball mill Retsch PM 100 (Retsch GmbH, Germany) at 18 g for 30 min. Particle size was determined by the device Laska 1K (Lumex, Russia). Measurement of the actual size of the ultradispersed grain mixture particles showed that the two-stage grinding results in nanometer- (about 10%) and micron-sized (the lion's share from 10 to 200–300 μm) samples (Fig. 1).

Mashes with grain to water ratio of 1:3.5 were prepared using ultradispersed raw materials. For enzymatic hydrolysis, the following commercial enzyme preparations were used: Mezomey-2500 and Glucomey-8000 (Beijing Shifa Multi-Business Agency, China) and Laminex BG2 and Maxazyme NNP DS+ (Genencor International Oy, Finland). The dosage of enzyme preparations was chosen according to the manufacturer recommendation. Pretreatment temperature was 60 °C. Hydrolysis was carried out according to the following scheme: addition of the enzyme preparations Mezomey-2500, Laminex BG2, and Maxazyme NNP DS+ and incubation at 60 °C for 30 min, acidification of the mixture to pH 4.2 with 1 N sulfuric acid, and addition of Glucomey-8000. Saccharification was carried out for 1 h. Soluble material, carbohydrate, and cationic composition and viscosity of the wort were controlled during the experiment.

2.5. Modeling of fermentation conditions

The experiment consisted of two stages: activation of freeze-dried yeast and wort fermentation. Yeast activation was conducted for 24 h at a constant agitation of 150 rpm at 30 °C using wort prepared according to the method described above. Dry yeast was introduced at a concentration of 0.1 g/l.

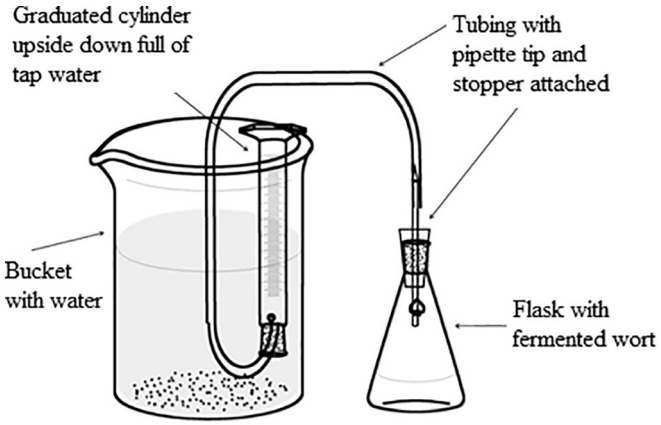

Fig. 2. Device for measurement of fermentation activity.