Enzyme complex influence on physiological properties of alcoholic yeast during cultivation

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Abstract. The effect of enzyme preparations of neutral Prolife BS Liquid protease and Kingfos phytase on the physiological and technological properties of Saccharomyces cerevisiae yeast of race XII in alcohol production was studied. The maximum accumulation of yeast cells occurs when the enzyme preparation of neutral Prolive BS Liquid protease was added to the wort at a dosage of 0.2 units PS/g of starch and Kingfos phytase with a dosage of 0.5 units FS/g of starch. The content of yeast cells in ripe yeast reached 170 million cells/cm^3 after 16-18 hours of cultivation. Such yeast has a high fermentation activity.

1 Introduction

The effectiveness of the alcoholic fermentation process depends mainly on the physiological and technological properties of the yeast used for this purpose. In the production of ethyl alcohol, unicellular microorganisms (yeast Saccharomyces cerevisiae) are used. Yeast Saccharomyces cerevisiae are facultative anaerobes; by the type of nutrition, they are chemoorganoheterotrophs; in relation to the active acidity of the medium, they are acidophiles.

The main requirements for the yeast used in the production of ethyl alcohol from starch-containing raw materials are as follows. The alcoholic yeast used in the processing of starchy raw materials must have high fermentation activity, full fermentability of sugars, resistance to metabolic products (especially alcohol), resistance to the development of extraneous microflora.

For the normal metabolism of yeast cells, it is necessary to contain in the culture medium all substances necessary for the normal functioning of cells, as well as conditions excluding stress effects caused by temperatures, high concentrations of carbohydrates and ethanol.

The set of characteristics reflecting the needs of yeast organisms in certain physical and chemical conditions of the environment is referred to as physiological properties. One of the main physiological properties is the ability to ferment sugars to ethanol and carbon dioxide. The ability of yeast to ferment a certain amount of sugar per unit of time is called fermentation activity, which is determined by the amount of carbon dioxide released per unit of time. At the same time, the process of yeast reproduction and the accumulation of glycogen in cells are determined.

An increase in the fermentation activity of yeast can be achieved by using a balanced composition of the nutrient medium.

2 Materials and methods

The proteolytic enzyme preparation Prolive BS Liquid was used as a source of protease. The enzyme preparation of the neutral protease Prolive BS Liquid is obtained by deep cultivation of the bacterial strain Bac. subtilis. It is a syrupy liquid of light brown color with a specific density of 1.25 g/cm^3 and high proteolytic activity. Prolife BS Liquid contains proteases that hydrolyze peptide bonds in proteins to form soluble peptides and amino acids, which contributes to the fortification of the medium with additional nitrogenous nutrition intended for yeast. The activity of the enzyme preparation is 600 - 750 units PS/cm^3. Optimal conditions of the drug are pH 5.5 – 7.5; the temperature is 50 – 55 ºC.

The enzyme Kingfos preparation was used as a source of phytase. The enzyme Kingfos preparation contains phytase (Mioinositollhexaphosphatephosphohydrolase ECNo.: 3.1.3.8) with an activity of at least 10000 units/g (10%). The producer is Pichiapastoris. The enzyme Kingfos preparation is a white or yellowish powder. Optimal conditions of the action of the drug are pH 5.5 – 6.5; the temperature is 40 – 45 ºC.

A Goryaev cell was used to count yeast cells.

To determine the percentage of viable cells on a slide, a drop of Saccharomyces cerevisiae yeast was mixed with a drop of methylene blue. The prepared preparation was viewed in 10 fields of view. The percentage of non-viable cells to the total number of cells observed in the field of view was determined. Good seeded yeast contains no more than 1% of dead cells.

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The number of budding cells characterizes the ability of yeast to reproduce: active yeast contains 50-70% of cells with buds. The preparation "crushed drop" is prepared from a yeast suspension and the percentage of budding cells is determined in relation to the total amount of yeast with and without buds.

3 Results and discussion

The effect of enzyme preparations of the neutral Prolive BS Liquid protease and Kingfos phytase on the fermentation activity of alcoholic Saccharomyces cerevisiae yeast of race XII was studied. When preparing yeast wort, the enzyme Prolive BS Liquid preparation was added to it in a dosage of 0.3 units PS/g of starch and the enzyme preparation Kingfos phytase in a dosage of 0.5 units FS/g of starch. No additional enzyme preparations were added to the control sample during the preparation of yeast wort. Yeast was introduced into the wort at a temperature of 30 ° C in an amount of 10% of the wort volume. The yeast generation process was carried out for 18-20 hours at a temperature of 28-30 °C in flasks of 750 cm^3. The amount of carbon dioxide released was determined hourly by the weight method (Figure 1).

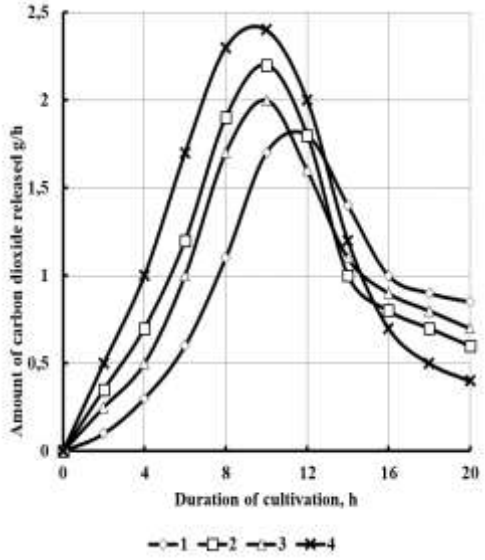

Figure 1. The effect of enzyme preparations on the fermentation activity of alcoholic yeast: 1 – control; 2 – protease of 0.2 units PS/g of starch; 3 - phytase of 0.5 units FS/g of starch; 4 - protease of 0.2 units PS/g of starch, phytase of 0.5 units FS/g of starch.

It can be seen from Figure 1 that the yeast cultivated on the wort obtained using protease and phytase has the maximum fermentation activity. The maximum fermentation activity of alcoholic yeast in the control is observed after 12 hours of growth and after 10 hours of growth in experimental samples. Direct assimilation of amino acids provides not only the intensification of protein synthesis, but also activates the enzymes contained in the yeast cell as indicated by high fermentation activity. Trace elements, such as calcium, zinc, magnesium, obtained by hydrolysis of phytin, also promote an increase in the fermentation activity of yeast.

Figure 2 shows that the yeast cell has 4 phases of vital activity, which are characterized by different growth of yeast cells.

1. Lag phases are characterized by a very complex enzymatic process; they are extremely sensitive to the composition of the medium, its temperature, and active acidity. In this phase, the volume of yeast cells increases slightly, nucleic acids are synthesized, and energy is accumulated for further active accumulation of biomass. The duration of the lag phase for yeast in the experiment is 1-2 hours and 2 – 3 hours in the control.

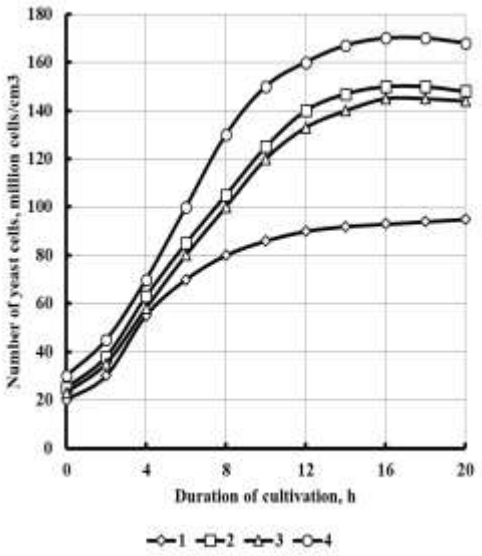

Figure 2. The effect of enzyme preparations on the dynamics of yeast biomass accumulation: 1 – control; 2 – protease of 0.2 units PS/g of starch; 3 - phytase of 0.5 units FS/g of starch; 4 - protease of 0.2 units PS/g of starch, phytase of 0.5 units FS/g of starch.

2. Logarithmic phases or exponential growth phases are characterized by high activity of cell reproduction. The number of budding cells increases rapidly, reaching 45-50%. At the same time, the biomass of yeast gradually increases due to the formation of new daughter cells, which, growing to the mother cell, begin to bud, forming a new generation. In this phase, the maximum growth rate of yeast cells is observed. The duration of the phase in the experiment is 14-16 hours and 18-20 hours in the control. The exponential phase is described by the Mono equation:

$$M_t = M_0 \cdot e^{kt},$$

where Mt is the amount of yeast during time t, kg;
M_o is the initial, seeded amount of yeast, kg;
e is the base of the natural logarithm;
k is the specific growth rate or the coefficient of biomass formation (depends on the yeast race, the concentration of the nutrient medium and its composition, aeration and other factors);

t is yeast reproduction time.

3. A stationary phase is characterized by a gradual slowdown in the growth rate. The formation of new cells practically stops, their budding ends, because the nutrient medium is depleted, metabolic products are formed that inhibit the growth and reproduction of cells. To maintain the vital activity of the cells, the nutrients remaining in the culture medium are used. The cells increase in size, their mass also increases. The increase in biomass in this phase can be 5-10% of the mass of yeast accumulated in the logarithmic phase. During this period, intracellular biosynthetic processes occur; they form the maturity of the yeast cell. The enzyme systems of the yeast cell are rebuilt from the active synthesis of biomass to the metabolic processes that support their vital activity.

The proportion of dying cells increases and the reproduction of new ones slows down. A phase of decline is coming.

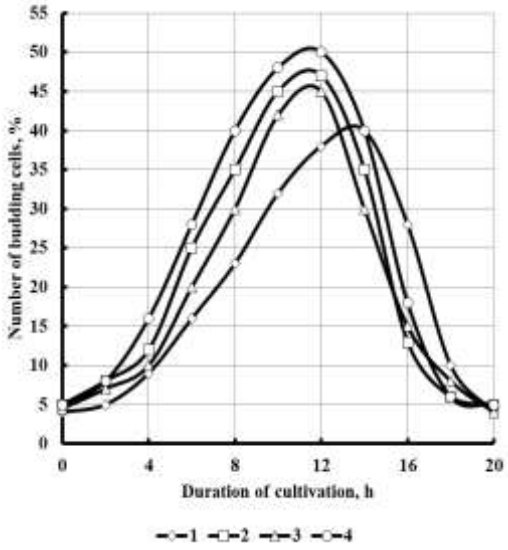

Figure 3. The effect of enzyme preparations on the dynamics of yeast reproduction: 1 – control; 2 – protease of 0.2 units PS/g of starch; 3 - phytase of 0.5 units FS/g of starch; 4 - protease of 0.2 units PS/g of starch, phytase of 0.5 units FS/g of starch

4. The phase of attenuation means dying off. Metabolic products accumulate in a significant amount in the medium. The phase is characterized by the absence of cell growth and reproduction. The mass of cells decreases, because all the nutrients of the culture medium were used in the previous phase. There is a gradual death of cells. To maintain vital activity, cells begin to use their own reserves. Autolysis of cells occurs.

The yeast in the experiment, compared with the control, multiplies more intensively, after 14-16 hours of growth it accumulates about 170 million yeast cells in the culture medium, and the yeast in the control accumulates about 95 million yeast cells after 18-20 hours of growth. Therefore, the process of yeast cultivation with the use of additional enzyme preparations can be limited to 14-16 hours of growth, and at the same time yeast cells accumulate almost twice as much as in the control. All this makes it possible to reduce the amount of seeded yeast introduced into the fermentation apparatus, to reduce the loss of fermented carbohydrates to the accumulation of yeast biomass and to increase the yield of alcohol.

A higher level of the budding of yeast cells (Figure 3) in the experiment was reached after 12 hours and after 14 hours in the control, which corresponds to about the middle of the logarithmic phase and amounts to 50.0% in the experiment and 40.0% in the control.

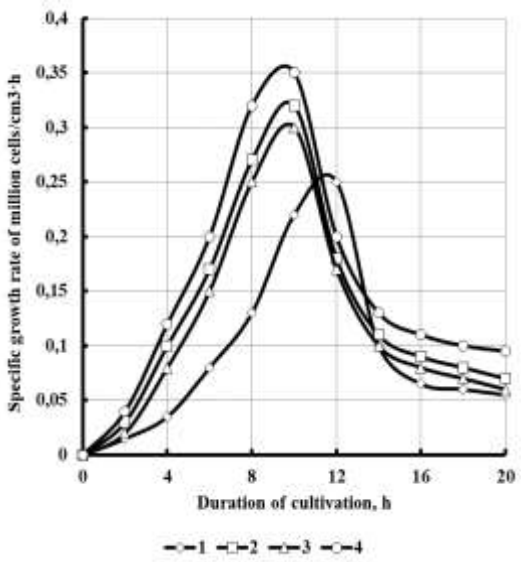

Figure 4. The effect of the yeast reproduction rate of enzyme preparations: 1 – control; 2 – protease of 0.2 units PS/g of starch; 3 - phytase of 0.5 units FS/g of starch; 4 - protease of 0.2 units PS/g of starch, phytase of 0.5 units. FS/g of starch.

The rapidly occurring dynamic fermentation inhibits the respiration process and consequently suspends budding and cell reproduction, as well as the rapid depletion of the substrate with nutrients, which in turn creates unfavorable conditions for budding. At the end of cultivation, yeast cells consumed their reserve substances, such as glycogen, and the number of budding cells reaches 4-5%.

A very characteristic indicator is the growth rate of yeast culture. We used the specific growth rate, which was determined by the increase in the number of cells over a certain period of time. The specific growth rate was maximal in the logarithmic phase (Figure 4), when fermentation was weak and amounted to 0.35 million cells/cm^3·h in the experimental samples and 0.25 million cells/ cm^3·h in the control.

4 Conclusion

The influence of various sources of supplementary nutrition and enzyme preparations of protease and phytase on the physiological and technological properties of yeast in alcohol production has been

studied. The proteolytic enzyme Prolive BS Liquid preparation was used as a source of protease. The enzyme Kingfos preparation was used as a source of phytase. It was found that the maximum accumulation of yeast cells was observed when an enzyme preparation of neutral Prolive BS Liquid protease was added to the wort with a dosage of 0.2 units PS/g of starch and enzyme Kingfos phytase with a dosage of 0.5 units FS/g of starch. The content of yeast cells in mature yeast reaches 170 million cells/cm^3 after 16-18 hours of cultivation; the yeast has a high fermentation activity.

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