Culture Conditions and Optimization of Glutathione-producing Yeast Fermentation Tank

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Abstract. In this paper, yeast was used as the starting strain, and the obtained strain was inoculated in 5L fermentor by plate, slope and seed culture. The fermentation temperature, fermentation time, rotation speed and ventilation condition were changed by single factor experiment. The content of glutathione was determined by high performance liquid chromatography (HPLC). Finally, the optimum amount of biomass accumulation and target product accumulation were obtained. condition.

1. Introduction

Since the role of glutathione has been discovered more and more, and Chinese research is still not perfect. In recent years, China has begun to pay attention to the production, research and application of glutathione. Research in this area by other developed countries such as Japan is currently superior to China, so that glutathione is mainly imported from Japan.\cite{1-3} In order to get rid of this situation, China's research on the production and application of glutathione should be accelerated. In summary, this experiment mainly studies the expansion of yeast strains in 5L fermenter, measures the yield of glutathione, and obtains the optimal fermentation tank culture conditions, which provides a theoretical basis for industrial production.

2. Training method

a). The yeast was inoculated on a plate medium and cultured in an inverted state at 30 °C for 48 hours.

b). The strain grown on the plate was inoculated to a slant medium and cultured at 30 °C for 48 h.

c). In the ultra-clean workbench, use 5ml pipette to suck the sterile water into the inclined surface, and then inoculate the seed culture medium with the large-mouth 5ml pipette to absorb the strain on the inclined surface. The culture temperature is 30 °C, and the 250mL shake flask bottle volume 50 mL, incubated at 250 rpm shaker speed for 16 h.
d). The seed solution on the shaker was combined and inoculated into a 5 L fermenter for cultivation, and the inoculum amount was 10% (v/v), and glutathione was fermented under a certain temperature, time, rotation speed and aeration condition.

3. Determination of the standard curve

Take 6.8 g of potassium dihydrogen phosphate, 2.2 g of sodium heptan sulfonate, dilute to 1 L, and then adjust the pH to 3.0 with phosphoric acid. A C18 column was used with a wavelength of 210 nm and a flow rate of 1 ml/min. A 0.1% phosphate buffer solution was extracted. The prepared standard solution was analyzed, the injection amount was 20 μL, the concentration was plotted on the abscissa, and the average peak area was plotted on the ordinate. A standard working curve was drawn, and each concentration was repeated three times or more.

HPLC instrument operation steps:

a). Methanol over organic film; water first passed through 0.45μm organic film, then passed through 0.22μm organic film; phosphate

The buffer solution was passed through a 0.45 μm aqueous membrane, passed through a 0.22 μm aqueous membrane, and all reagents were sonicated for 20 min.

b). Install the column, tighten the knob, open the liquid meter and wash with methanol and water for 20 min.

c). Stop the cleaning, change the water to a buffer solution, and after running for 10 minutes, turn on the UV lamp.

d). Take the baseline for about 20 minutes, and after the baseline is flat, start the injection. (Injection 20μL)

e). After the injection is finished, clean the instrument and turn it off.

4. Results and analysis of the effect of temperature on glutathione production

Temperature is one of the important factors in the fermentation of fermenter to produce glutathione, which has a significant impact on cell growth and GSH production. In this part of the experiment, the effect of temperature on the fermentation results was mainly investigated. Under the same conditions, the fermentation temperatures were 28 °C, 29 °C, 30 °C, 31 °C, 32 °C, and 33 °C, respectively. The experimental results are shown in Table 1.

| T(℃) | 28  | 29  | 30  | 31  | 32  | 33  |
|------|-----|-----|-----|-----|-----|-----|
| Biomass (g/L) | 16.5 | 17.2 | 18.3 | 18.6 | 19.1 | 18.5 |
| Yield (mg/L)  | 253.6 | 261.4 | 270.4 | 290.1 | 281.0 | 273.7 |

According to the chart in Table 1, the optimal temperature conditions are obtained more intuitively, as shown in Figure 1.
Figure 1. Effect of temperature on cell biomass and GSH production

The temperature mainly affects the growth of the early strain and the synthesis of glutathione in the middle and late stages, because the fermentation of yeast to produce glutathione is closely related to the activity of the enzyme in the bacteria. If the temperature is too low, the activity of the enzyme becomes low, resulting in a slow growth rate of the strain and a low product yield. If the temperature is too high, the activity of the enzyme will be reduced or even inactivated, the growth of the cells will be slow, and the production of glutathione will be reduced. It can be seen from Figure 1 that as the fermentation temperature increases, both yeast cell biomass and glutathione production increase. At a temperature of 31 °C, glutathione production reached a maximum. At a temperature of 32 °C, the bacterial biomass accumulated the most. Therefore, the optimum temperature at which the target product has the largest accumulation of glutathione is 31 °C.

5. Results and analysis of the effects of fermentation time on glutathione production

The length of fermentation affects the growth of the strain and the synthesis of intracellular glutathione. In the experiment, the biomass and glutathione production were measured every 5 hours after fermentation for 35 hours under the conditions of other factors, as shown in Table 2.

Table 2. Effect of fermentation time on cell biomass and GSH production

| Time (h) | 35  | 40  | 45  | 50  | 55  | 60  |
|----------|-----|-----|-----|-----|-----|-----|
| Biomass (g/L) | 13.2 | 15.3 | 16.1 | 17.6 | 17.8 | 17.3 |
| Yield (mg/L)  | 220.1 | 232.6 | 264.7 | 283.2 | 279.5 | 273 |

According to Table 2, it is more intuitive to see the effect of fermentation time on the fermentation results, as shown in Figure 2.
Figure 2. Effect of fermentation time on cell biomass and GSH production

As the fermentation time increases, the biomass of the cells and the accumulation of the target product become more, but the time is too long, and the biomass and product accumulation of the cells become less. It may be because the production of by-product ethanol affects the growth of bacteria and the synthesis of glutathione, and for a long time, the synthesized glutathione is also hydrolyzed by the enzyme in the bacteria. Therefore, too long fermentation time will reduce the yeast cell biomass and the accumulation of glutathione. It can be seen from Fig. 2 that the biomass and glutathione production in the pre-fermentation period increased rapidly. At 50 h, the glutathione production reached its maximum value, and at 55 h, the bacterial biomass reached its maximum value. After that, the bacterial biomass and glutathione production no longer increased or even decreased. In addition to the above factors, it is also related to the consumption of nutrients such as carbon sources and nitrogen sources in the fermentation broth. Therefore, it can be determined that the fermentation time is between 50h and 55h, which is the optimal time range for bacterial biomass and glutathione accumulation.

6. Results and analysis of the effect of rotational speed on glutathione production

The rotation speed of the fermenter mainly affects the contact between the strain and the fermentation medium and the dissolved oxygen in the fermentation liquid, which is closely related to the carbon source, nitrogen source and inorganic salt in the medium. In this experiment, the fermenter rotation speed was adjusted to 100 rpm, 150 rpm, 200 rpm, 250 rpm, 300 rpm, respectively. After the other factors were unchanged, the biomass and glutathione production were measured after 55 h of fermentation. Speed culture conditions.

Table 3. Effect of rotational speed on cell biomass and GSH production

| Rotating speed (rpm) | 100 | 150 | 200 | 250 | 300 |
|----------------------|-----|-----|-----|-----|-----|
| Biomass (g/L)        | 15.5| 17.2| 17.6| 18.6| 17.8|
| Yield (mg/L)         | 212.5| 221.7| 267.7| 286.5| 289.3|

According to the drawing in Table 3, the effect of the fermenter rotation speed on the fermentation result is more intuitively obtained, as shown in Fig. 3.
Figure 3. Effect of rotational speed on cell biomass and GSH production

The fermenter rotation speed is too low, the oxygen content in the fermentation liquid is low, the growth of the bacteria body is slow, the fermentation time is prolonged, and the product accumulation is reduced. If the fermenter speed is too high, the oxygen content in the fermentation liquid will increase, the growth rate of the bacteria will be too fast, and the water in the fermentation liquid will be lost, resulting in a decrease in the biomass of the bacteria. As can be seen from Fig. 3, when the fermenter rotation speed is 250 rpm, the accumulation of bacteria reaches a peak and then decreases. Glutathione production increased with the speed of the fermenter, and the yield increased, most at 300 rpm. Therefore, the rotation speed at 300 rpm is most advantageous for the accumulation of the target product.

7. Results and analysis of effects of ventilation on glutathione production

Ventilation is mainly manifested by the effect of dissolved oxygen in the fermentation broth on cell growth and glutathione accumulation. In this experiment, the fermenter aeration was adjusted to 0.1 VVM, 0.3 VVM, 0.5 VVM, the fermenter rotation speed was set to 300 rpm, the fermentation temperature was 31 °C, and the bacterial biomass and glutathione production were measured after 55 h of fermentation.

Table 4. Effect of ventilation on cell biomass and GSH production

| Ventilation (VVM) | 0.1 | 0.3 | 0.5 |
|-------------------|-----|-----|-----|
| Biomass (g/L)     | 16.5| 18.3| 19.1|
| Yield (mg/L)      | 189.3| 259.3| 281.4|

According to the drawing in Table 4, the influence of the aeration amount on the fermentation result was visually obtained, as shown in Fig. 4.
Figure 4. Effects of aeration on cell biomass and GSH production

The aeration rate of the fermenter increases, and the oxygen content in the fermentation broth increases, which is beneficial to the growth of the cells and the synthesis of the product. When the oxygen content is increased, the metabolic process of the bacteria is accelerated, and the target product is easier to synthesize. As can be seen from Fig. 4, as the aeration increased, the accumulation of bacteria and the production of glutathione gradually increased. Under other conditions, when the ventilation is 0.5VVM, the utilization of the substrate in the fermentation broth is more favorable. When the strain is in full contact with the fermentation broth, the amount of bacterial accumulation is more, and the amount of glutathione synthesis is also significantly increased. Therefore, the ventilation of 0.5VVM is more suitable for the accumulation of bacteria and products.

8. Conclusion
In this paper, the single factor test method was used to change the temperature, time, aeration and rotational speed conditions, and the biomass and glutathione production were measured. The following conclusions were obtained: the temperature was 31 °C, the fermentation time was 50 h, and the fermentation tank speed was 300 rpm. When the aeration rate is 0.5VVM, the highest yield of glutathione is the optimum fermentation condition.

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