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Application of foam separation in production of β-glucanase in Pichia

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β-glucanase is widely used in many fields and has great economic value and development space, but it faces the difficulties of separation and nutrient destruction in the process of industrial production. Foam separation is a simple, mild and efficient adsorption separation technique that enables efficient separation and extraction of β-glucanase. In this study, five single factors(loading volume, pH, separation gas velocity, fermentation loading concentration, surfactant concentration) of foam separation and harvest of β-glucanase produced by Pichia pastoris were studied. The best univariate condition was: 600 mL/min separation gas velocity, loading volume of 200 mL, initial enzyme concentration of 100 g/mL, surfactant concentration of 0.3 mg/mL and pH of 5. Based on the best univariate condition, the optimal separation conditions of β-glucanase were further explored, and the five-factor four-level orthogonal test was designed. From the experimental results, the best separation condition was: 600 mL/min, loading volume of 200 mL, initial enzyme concentration of 100 μg/mL, surfactant concentration of 0.5 mg/mL and pH of 5. Under this separation condition, the enrichment ratio (E) was 0.56 and the recovery rate (R) was 96.01%.

Keywords: Foam separation; β-glucanase; recovery rate; enrichment ratio.

INTRODUCTION

β-glucanases can catalyze the breakdown of β-glucans, being present as structural elements in the cell walls of yeast, fungi, and grains[1]. β-glucanases can be divided into four categories, among which the most noteworthy study is-1,3-1,4-β-glucanase[2, 3]. It has a strict substrate specificity[4–7], so it is widely used in food[8], detergent[9], animal feed[10–11] and other industries.

The source of β-glucanase mainly includes plant extraction and microbial fermentation: β-glucanase is widely found in the cell wall of higher plants, especially barley, oats and other grains are rich in β-glucanase, so it can be extracted from plants[12]. Microbial fermentation is the main source of industrial production of β-glucanase, which is found in bacteria, clostridium, fiber bacteria and fungi. M. Sait Ekinci et al.[13] isolated β-glucanase from Streptococcus bovis; Alice Grassick et al.[14] isolated the first β-glucanase with thermal stability from chloromycetes. After holding at 100 °C for 15 min, it can still retain 15% of its original activity; Chen Zixian et al.[15] isolated β-glucanase from Aspergillus fumigatus by fermentation.

The methods of purifying β-glucanase from the fermentation liquid are double water phase extraction and hydrophobic chromatography[16, 17], gel filtration[18], ion-exchange chromatography[19], ammonium sulfate precipitation[20], membrane separation, etc. Ma Li et al.[17] separated and purified β-glucanase by hydrophobic chromatography, and the recovery rate was 25%. The purity of β-glucanase extracted by Dingyemei et al.[21] through gel filtration was 2.48 times higher than that of crude enzyme solution. Zhang Zhiqiang et al.[19] isolated and purified β-glucanase from Aspergillus niger fermentation powder by ion-exchange chromatography; Li Weifen et al.[20] purified β-glucanase by ammonium sulfate precipitation method.

However, these methods have their own disadvantages. For example, the recovery rate of hydrophobic chromatography is only 25%[17]; gel filtration, ion-exchange chromatography require a large amount of water washing[21]; sodium sulfate precipitation method pollutes the environment[20]; the price of membrane in membrane separation method is high, and the membrane is easy to be damaged.

With the application field of β-glucanase gradually expanding and its application value gradually discovered, its market value and demand are further increasing. Therefore, seeking an efficient, low-cost, simple operation and high enzyme activity has become an urgent problem in the application of β-glucanase.

Foam separation, also known as gas-floating separation or bubble adsorption separation technology, with air bubbles as the separation carrier, using the different surface activities of different substances to achieve the purpose of separation. In this century, it was gradually applied in the field of biological engineering, mainly for the extraction of surficial active biological macromolecules[22]. With the characteristics of a simple process, high safety, low industrial cost, high production efficiency and green and environmental protection, it can be used as a new method to extract β-glucanase.

Based on the above description, this study investigated the effects of five single factors (loading volume, pH, separation gas velocity, fermentation loading concentration, surfactant concentration) on the production of β-glucanase from Pichia pastoris. The effects of five single factors on the enrichment ratio and the recovery rate of enzyme activity were analyzed. Later, we designed orthogonal experiments with five factors and four levels based on one-factor conditions to explore and optimize the conditions of foam separation of β-glucanase, and provide basic experimental conditions for the application of foam separation in the production of β-glucanase in the next stage.

EXPERIMENTAL SECTION

Instruments and reagents

SQP Electronic Balance (Sartorius), ST 5000 Laboratory pH meter (Auhaus Instruments), SKY200B (Sukun Industrial), Minifors 2 desktop standard fermor (Everson Biotechnology, Ltd), NCB-1210A low-temperature sink (EYELA), LZB-3WB rotor flowmeter (Changzhou Double huan Thermal Instrument Co., Ltd), WH-861

oscillator (Taicang Hualida Experimental Equipment Limited), UV-1780 Spectrophotometer (Yanjin Instrument Co., Ltd.), TGL-16G high-speed centrifuge (Shanghai Anting Scientific Instrument Factory), DK-8D constant temperature sink (Shanghai Yiheng Scientific Instrument Co., Ltd.).

Trypsin (AR Oxoid), glucose (AR Beijing SoleTechnology), yeast extract (AR Oxoid), glycerol (AR, Ltd), methanol (AR, Ltd), sodium dodecyl sulfate (AR), Coomassie Blue (AR Solarbio), DNS (AR Solarbio), β-glucan (AR Solarbio).

Experimental installation

The device is mainly composed of three parts: foaming device, separation device and collection device, as shown in Figure 1.

Fobbing device includes air cylinder, pressure reduction valve, regulator valve, stop valve, rotor flowmeter (model) LZB-3WB, with a measured flow range of 100–1000 mL/min).

Separation device includes: gas distributor, air hose (120 cm length, inner diameter of 30 mm glass pipe)

Collection devices include a foam collection beaker.

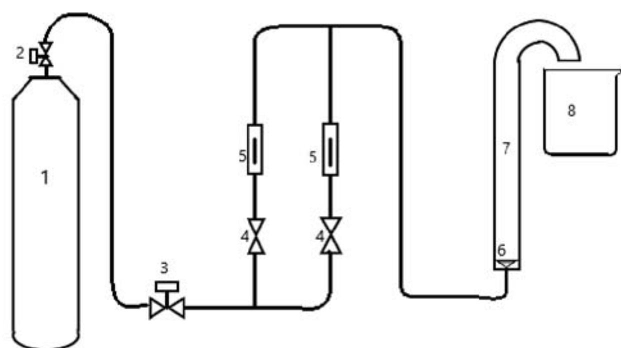

Figure 1. Schematic diagram of foam separation devices

1 – Air cylinder; 2 – pressure relief valve; 3 – regulating valve; 4 – stop valve; 5 – rotor flow meter; 6 – gas distributor; 7 – air float pipe; 8 – Foam collection beaker

Analytic procedure

Determination of β-glucanase concentration

β-glucanase concentration was determined by Coomassie blue staining: the extracted sample solution was centrifuged for 10 min and 800 μL of sterile distilled water and 200 μL supernatant were transferred to the tube. Add 5 mL of Coomassie blue stain and stand for 5 min. Absorbance was measured at 595 nm as a blank control by adding 1 mL of sterile distilled water, and the β-glucanase concentration was detected from the standard curve.

Determination of β-glucanase enzyme activity

In this experiment, the enzyme activity was determined by the color development reaction between β-glucanase hydrolysis products and DNS reagent: 1 mL of bacterial solution was centrifuged at 10,000 rpm for 5 min, and the supernatant was poured into a new 1.5 mL centrifuge tube and precipitated for cell lysis. Samples of the enzyme to be tested were obtained after the lysis. 1 mL substrate solution was taken in four tubes, three as test tubes and one as blank control and insulated at 50 ± 0.2 °C for 2–3 min. After the insulation, 1 mL of enzyme fluid was added to the three test tubes and 1 mL of distilled water to the blank tubes for control. The four tubes were then heated simultaneously in a 50 °C water bath for 10 min, removed with 2 mL DNS of reagent per tube, and heated with boiling water for 5 min. After cooling, add 10 mL of unionized water to each tube. Finally, zero was adjusted with a blank tube and the absorbance was measured at 540 nm. The measured parallel absorbance was averaged and the β-glucanase activity was calculated following this formula:

$$\frac{(bx+a)\times n\times F\times 1000}{10\times W}$$ Formula 1

Formula x: the average sample OD;
The b and a: the maltose concentration and the corresponding OD values were obtained by the regression equation;
n: Dilutions of the enzyme solution;
10: Time of the enzymatic reaction;
F: Substrate correction factor (1.047);
W: Weight of the enzyme sample (1 mL);

Evaluation of the separation effect

The effect of foam separation was evaluated by the enrichment ratio (E) and the enzyme active recovery rate (R):

$$E=\frac{C_1}{C_0}$$ Formula 2

$$R=\frac{A_1V_1}{A_0V_0}$$ Formula 3

C in Formula 2: β-glucanase protein concentration in the foam liquid, mg/mL;
C_0: Initial β-glucanase protein concentration of the sample solution, mg/mL;
V_1: Foam loading volume after separation, mL;
V_0: Initial volume of the sample fluid, mL;
A_1: Foam liquid enzyme was active after separation, U/mL;
A_0: The sample fluid was initially enzymatic, U/mL.

Empirical method

Fermentator fermentation produces β-glucanase

The fluids stored with glycerol were activated and after 48 h of culture picked a ring was seeded in a shake bottle loaded with 25 mL BMGY medium, with conditions set to 30 °C and 250 rpm. After 12 h, 2 mL of culture medium was transferred to four bottles of 50 mL BMGY medium for 18 h to obtain the seed solution, which was set to 30 °C and 250 rpm.

The equipment was adjusted before the start of the test to confirm whether the pH electrode oxygen-soluble electrode, refrigeration system, peristaltic pump, filling pipe and so on of the fermententer are normal. After the inspection, the autoclaved BSM medium will be connected to the fermentation tank. After the culture medium is cooled, open the pH electrode regulating medium from pH to 5.0 and pass into the air conditioning gas intake for 4 L · min^{-1}, open the dissolved oxygen electrode, cooling circulating water, and mixing motor. When the seed solution was accessed, the mixing speed was set at 800 rpm, a fermentation temperature of 30 °C, pH of

5.0, and fermentation growth for approximately 16–18 h. About 16 h the dissolved oxygen value starts to drop slowly, and by about 25 h it suddenly rises and remains above 80%, at this time, at 3.6 mL · h^{-1} · L^{-1}. The rate flow plus a fermentation solution volume of 5% glycerol was added to the fermententer. After completing the glycerol addition, waiting for 3 h, the sudden rise in dissolved oxygen indicates that the glycerol has been completely exhausted when methanol induction begins. The fermentation liquid was adjusted pH to 6.0 and the speed was 800 rpm, and the fermentation loading volume was 0.5% methanol every 12 h flow. During the dissolved oxygen was regulated at the flow acceleration of methanol, and the dissolved oxygen was maintained at about 35%. The fermentation process was completed 9 times for about 108 h. End the fermentation, transfer the fermentation liquid into the storage container, and clean the fermented tank.

Foam separation experimental operation

First, the air tightness and connection stability of the separation device are checked. According to the experimental design, the sample solution with the corresponding concentration, pH, liquid volume, etc. is configured to be used. Open the pressure reduction valve and the gas cylinder valve, and adjust the rotor flow meter according to the experimental design, so that the air flow speed meets the experimental design conditions. The sample solution was added to the separation column, foam was collected at the vent outlet, the timing was started while the sample was added, and collection was stopped after 10 min. Seal the beaker mouth containing the foam liquid with plastic wrap, and place it in a 4 °C refrigerator for static antifoam. After foam elimination, the remaining loading volume was measured, and the separation liquid-dextrase concentration and activity were measured according to the analysis method. The enrichment ratio and recovery rate of the separation test were analyzed to determine the optimal separation conditions and evaluate the separation effect.

Gextrase univariate experiment

Univariate experiments were designed to examine the effects of different separation gas velocity, sample liquid concentration, pH, loading volume, and amount of surfactant on foam separation harvesting β-glucanase when other conditions were unchanged. The concentration and activity of the original sample solution were determined before separation. After adding the separation column, the control separation conditions of the dextrase concentration and activity of the isolate were collected, and the recovery rate and enrichment ratio of the foam separation were calculated to analyze the optimal separation univariate condition.

Orthogonal test of dextrase harvested by foam isolation

According to the optimum gas separation velocity, sample solution concentration, pH, loading volume and surfactant amount obtained from the single factor experiment, the orthogonal test of five factors and four levels is designed. According to the analysis of the orthogonal test results, the recovery rate is the main factor to determine the best combination of separation conditions and combined with the influence of various factors on the separation effect of foam, the best separation conditions and scheme are finally determined.

RESULTS AND DISCUSSION

The isolation conditions of dextrase

Effects of ventilation speed

Five sets of foam separation experiments were designed with ventilation velocity change gradient of 200 mL/min. 200 ml/min, 400 ml/min, 600 ml/min, 800 ml/min and 1000 ml/min were taken respectively. Other conditions were determined: loading volume of 100 mL, initial enzyme concentration of 100 g/mL, surfactant concentration of 0.4 mg/mL, solution pH of 6 and collection time of 10 min. The results are shown in Figures 2.

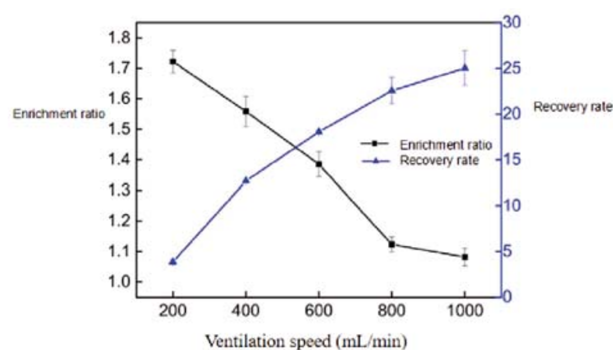

Figure 2. The effect of intake speed on the separation of β-glucanase

Available from experimental results, β-glucanase enrichment ratio E decreased with increasing ventilation rate, with a maximum of 1.69 than E at a ventilation rate of 200 mL/min.

However, the enzyme active recovery rate R increased with the ventilation rate, with a maximum of 25.09% at a ventilation rate of 1000 mL/min. Combined with the foam separation principle of the analysis, when the ventilation speed is low, the bubbles are less and unstable, easy to rupture, the adsorption effect of β-glucanase is weak, so with the increase of ventilation speed, foam becomes stable, β-glucanase and liquid contact times more, adsorption effect becomes stronger, so the separated enzyme recovery rate gradually increased. On the other hand, as the speed of ventilation increases, the large number of bubbles also brought more water, resulting in a large increase in the content of water in the separation collection and a gradual decrease in the enrichment ratio of separation. Therefore, the comprehensive analysis considered the effect of enrichment ratio E and enzyme active recovery R, which concluded with the optimal separation gas rate of 600 mL/min.

Effects of the loading volume

Five sets of foam separation experiments were designed, with loading volume of 100 mL, 150 mL, 200 mL, 250 mL, and 300 mL, respectively. Other conditions were determined as ventilation speed of 400 mL/min, initial enzyme concentration of 100 μg/mL, surfactant concentration of 0.4 mg/mL, solution pH of 6, and collection time of 10 min. The results are shown in Figures 3.

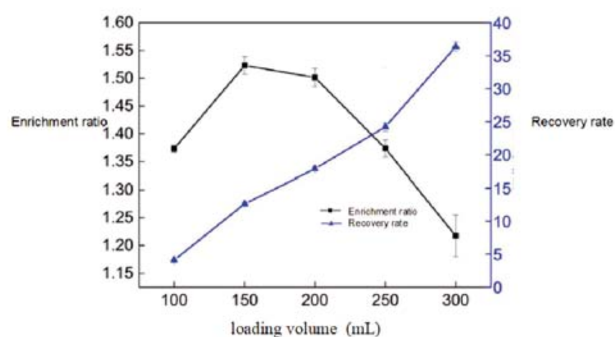

Figure 3. The effect of loading volume on the separation of β-glucanase

From the experimental results, the enzymatic active recovery R of β -glucanase increased with the loading volume, with a maximum of 35.76% at the loading volume of 300 mL. The β-glucanase enrichment ratio E tended to increase first and then decrease with the loading volume, with a maximum of 1.51 at the loading volume of 200 mL. The experimental results can be analyzed combined with the principle of foam separation. When the loading volume is low, the loading surface is low and the short binding area of the sample liquid and bubble leads to the short binding time and insufficient binding. When the loading volume increases, the β-glucanase and bubble binding effect become better, so the enzyme active recovery rate increases. On the other hand, with the increase of loading volume, the rise of liquid surface, while better bringing out

β-glucanase, but also bring out a large amount of water. After reaching a certain limit, with the increase of loading volume will gradually decrease the separation enrichment ratio E. Therefore, the comprehensive analysis considered the effect of the enrichment ratio E and the enzyme active recovery rate R, finally selecting the optimal loading volume of 200 mL under univariate conditions.

Effects of the initial enzyme concentration

Five sets of foam separation experiments were designed with an initial enzyme concentration change gradient of 25 μg/mL. 50 μg/mL, 75 μg/mL, 100 μg/mL, 125 μg/mL and 150 μg/mL were taken respectively, other conditions determined as ventilation speed of 400 mL/min, loading volume of 100 mL, surfactant concentration of 0.4 mg/mL, solution pH of 6, and collection time of 10 min. The results are shown in Figures 4.

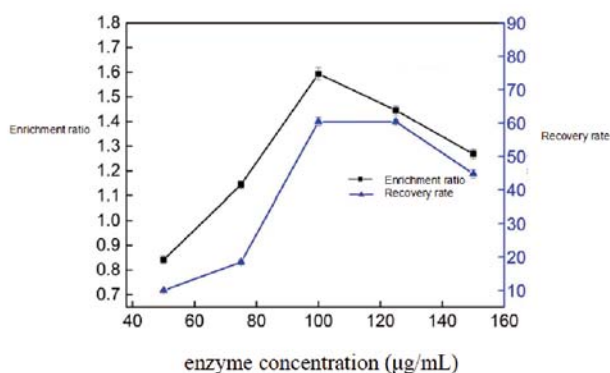

Figure 4. The effect of initial enzyme concentration on the separation of β-glucanase

Available from the results, the enzyme active recovery R of β-glucanase showed a trend of increasing first and then decreasing with the initial enzyme concentration, with a maximum of 61.03% at an initial enzyme concentration of 100 μg/mL. However, the β-glucanase enrichment ratio E also showed a trend to decrease before increasing with the initial enzyme concentration, with a maximum of 1.59 at the initial enzyme concentration of 1000 μg/mL. Analysis of the experimental results combined with the foam separation principle is available, when the initial enzyme concentration is low, the amount of foam is less, so the enzyme active recovery rate and enrichment ratio are low. When the initial enzyme concentration gradually rises, the amount of foam produced increases and the foam is also in a more stable state. When its concentration increases to a certain critical cut-off value, the viscosity of the sample liquid is too large, and the conclusion phenomenon between the enzyme and the enzyme will occur, leading to bubble instability, and the enzyme active recovery rate and enrichment ratio will gradually decrease. Therefore, the comprehensive analysis considered the effect of the enrichment ratio E and the enzyme active recovery rate R, finally selecting the optimal initial enzyme concentration of 100 g/mL under univariate conditions.

Effects of surfactant concentration

Five groups of foam separation experiments were designed, with surfactant (SDS) concentration change gradient of 0.1 mg/mL. 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL and 0.5 mg/mL were taken respectively. Other conditions were determined: ventilation speed of 400 mL/min, loading volume of 100 mL, initial enzyme concentration of 100 μg/mL, solution pH of 6 and collection time of 10 min. The results are shown in Figures 5.

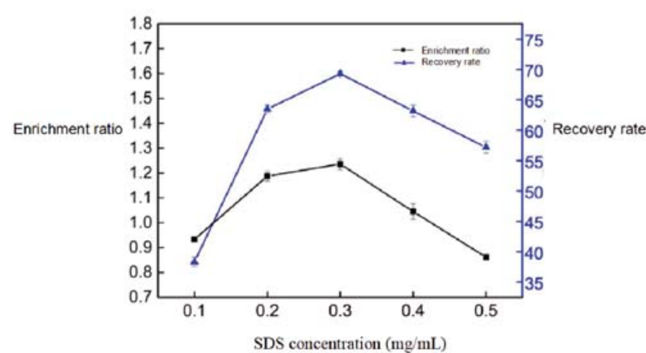

Figure 5. The effect of SDS concentration on the separation of β-glucanase

According to the experimental results, both the enzymatic active recovery rate R and enrichment ratio E showed a trend of first increasing and then decreasing with the increasing SDS concentration. The enzyme active recovery rate R was 69.26% at SDS concentration of 0.3 mg/mL, and the enrichment ratio E was also 1.25 at SDS concentration of 0.3 mg/mL. To analyze the experimental results combined with the principle of foam separation, when the SDS concentration is low, less foam is produced, as its concentration increases, the adsorption force to the β-glucanase is enhanced, so the enrichment ratio and recovery rate increase. When the SDS concentration increases to a certain boundary

value, the SDS concentration is too high due to the association between its molecules, the adsorption force to β-glucanase gradually decreases, and the enrichment ratio also gradually decreases due to excessive bubbles bringing out a large amount of water. Therefore, the comprehensive analysis considered the effect of enrichment ratio E and enzymatic active recovery R, finally with an optimal surface SDS concentration of 0.3 mg/mL in selected univariate conditions.

Effects of the sample liquid pH

Five groups of foam separation experiments were designed with a pH gradient of 1, taking 4, 5, 6, 7 and 8, and other conditions: ventilation speed of 400 mL/min, loading volume of 100 mL, initial enzyme concentration of 100 g/mL, surfactant concentration of 0.4 mg/mL, and collection time of 10 min. The results are shown in Figures 6.

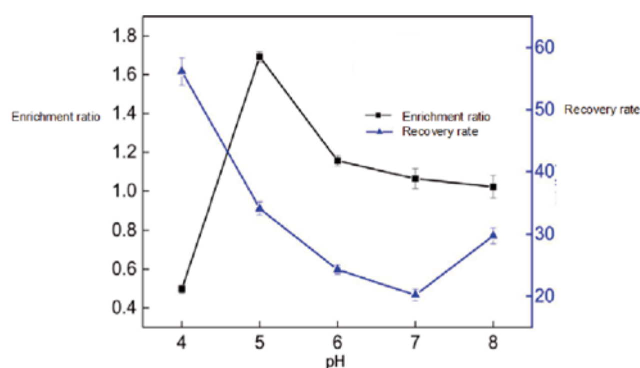

Figure 6. The effect of pH on the separation of β-glucanase

Available from the experimental results, the enzymatic active recovery rate R of β-glucanase showed a trend to decrease first and then slightly increase with increasing pH, with a maximum of 54.25% at 4. However, the β-glucanase enrichment ratio E showed a trend of increasing first and then decreasing with pH, with a maximum of 1.67 at a pH of 5. Analysis on the results of the foam separation principle can be obtained. β-glucanase is stable in weak acidic solution, and the best pH is 4~5. In this range, the most stable and easily adsorbed, so the enzyme active recovery and enrichment ratio is the highest, and the sample liquid pH increases to alkaline. The comprehensive analysis considered the effect of enrichment ratio E and enzyme live recovery rate R, and the optimal sample liquid pH was 5 was finally selected under univariate conditions.

Orthogonal test to investigate the separation conditions of foam separation and harvesting-dextrase

According to 3.1, the best univariate separation conditions for foam separation and harvesting-dextrase were: ventilation speed of 600 mL/min, loading volume of 200 mL, initial enzyme concentration of 100 g/mL, surfactant concentration of 0.3 mg/mL, and pH of 5. Five factors four-level orthogonal tests were involved according to the best univariate conditions and actual conditions, as shown in Table 1.

A total of 16 orthogonal tests of five factors and four levels were designed according to Table 1, and foam separation harvesting-dextrase experiments were performed

Table 1. Orthogonal experiment factors and level table of β-glucanase separation

level	factor				
	Ventilation speed (mL/min)	SDS concentration (mg/mL)	Initial enzyme concentration (μg /mL)	Install loading volume (mL)	pH
1	200	0.2	50	100	5
2	400	0.3	75	150	6
3	600	0.4	100	200	7
4	800	0.5	125	250	8

according to the experimental method. The experimental design and results are shown in Table 2.

According to the orthogonal test results, the $\overline{K}$ value and R value distribution of the enrichment ratio E of foam separation and harvesting of β-glucanase were analyzed, as shown in Figures 7 and 8. The $\overline{K}$' and R' value distribution of R of foam separation and harvesting of β-glucanase as shown in Figures 9 and 10.

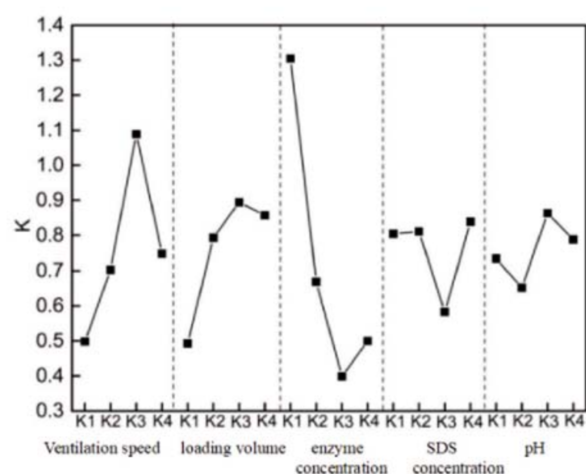

Figure 7. Mean distribution of enrichment ratio

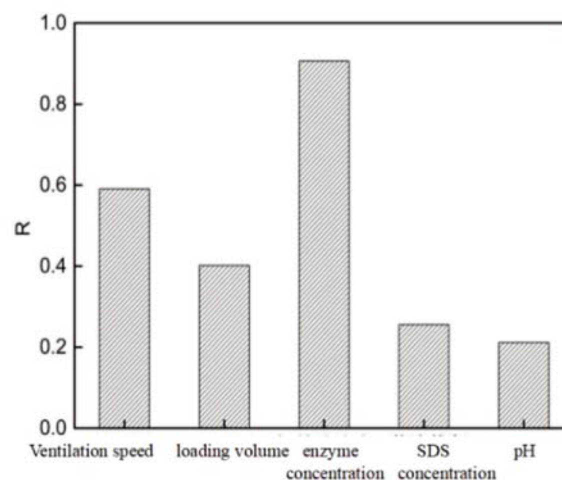

Figure 8. Range distribution of enrichment ratio

By analyzing the distribution of the above values, using recovery R as the main measure of foam separation-glucextrase effect, the optimal separation condition was 200 mL, surfactant concentration was 0.5 mg/mL, initial enzyme concentration of 100 g/mL, ventilation speed 600 mL/min and pH was 5. Under this separation condition, enrichment ratio E was 0.56 and recovery R was 96.01%. The effect on foam separation and harvesting of β-glucanase recovery rate R ranged from large to small

Table 2. β-glucanase orthogonal test protocol and results Orthogonal experiment design and results analysis of β-glucanase

number	Ventilation speed (mL/min)	SDS concentration (mg/mL)	Initial enzyme concentration (μg/mL)	Install loading volume (mL)	pH	concentration ratio E	percent recovery R(%)
experiment 1	200	0.2	50	100	5	0.80	20.11
experiment 2	200	0.3	75	150	6	0.38	52.70
experiment 3	200	0.4	100	200	7	0.37	79.78
experiment 4	200	0.5	125	250	8	0.44	81.87
experiment 5	200	0.2	75	200	8	0.20	87.89
experiment 6	200	0.3	50	250	7	1.47	81.44
experiment 7	200	0.4	125	100	6	0.52	87.98
experiment 8	400	0.5	100	150	5	0.63	91.69
experiment 9	600	0.2	100	250	6	0.60	93.94
experiment 10	600	0.3	125	200	5	0.66	95.74
experiment 11	600	0.4	50	150	8	1.85	90.66
experiment 12	600	0.5	75	100	7	1.25	95.10
experiment 13	800	0.2	125	150	7	0.38	85.18
experiment 14	800	0.3	100	100	8	0.66	87.97
experiment 15	800	0.4	75	250	5	0.85	94.88
experiment 16	800	0.5	50	200	6	1.11	95.01
$\bar{K}_1$	0.50	0.49	1.30	0.80	0.73		
$\bar{K}_2$	0.70	0.79	0.67	0.81	0.65		
$\bar{K}_3$	1.09	0.89	0.40	0.58	0.86		
$\bar{K}_4$	0.75	0.86	0.50	0.84	0.79		
R	0.59	0.40	0.91	0.26	0.21		
$\bar{K}_1'$	58.61	71.80	71.80	66.30	75.60		
$\bar{K}_2'$	80.78	79.46	82.66	80.06	75.91		
$\bar{K}_3'$	93.86	81.83	88.35	89.63	85.38		
$\bar{K}_4'$	90.76	90.92	81.20	88.03	87.12		
R'	35.24	19.11	16.54	23.33	11.52		

K_1, $\bar{K}_2$, $\bar{K}_3$, $\bar{K}_4$ represent the mean of E corresponding to four different levels, and R indicates the extreme difference of each K value; $\bar{K}_1'$, $\bar{K}_2'$, $\bar{K}_3'$, $\bar{K}_4'$ represent the mean of R corresponding to four different levels, the R' indicates the extreme value of $\bar{K}'$.

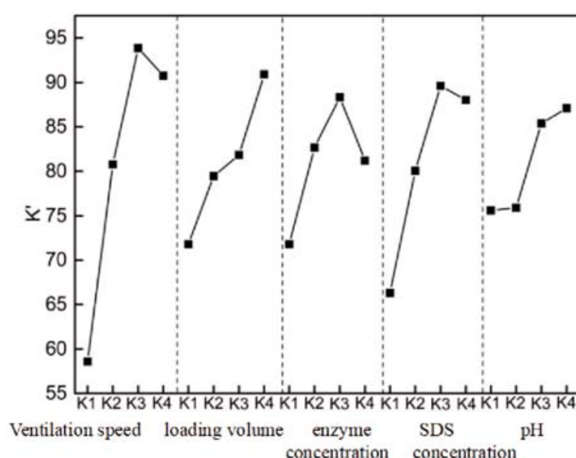

Figure 9. Average distribution of recovery rates

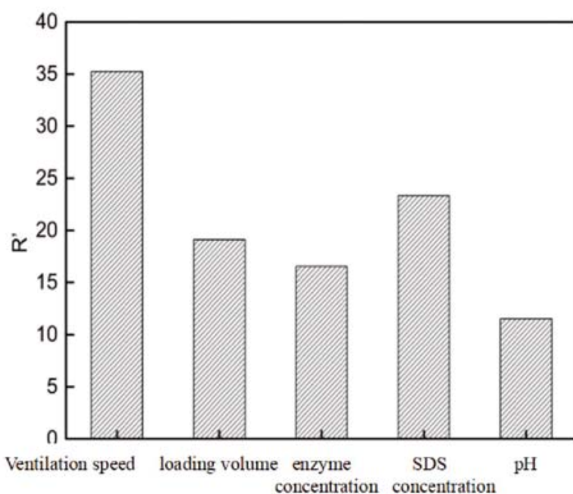

Figure 10. Range distribution of recovery rate

as follows: ventilation velocity, loading volume, surfactant concentration, initial enzyme concentration, and pH.

CONCLUSION

In this work, we achieved the first extraction of β-glucanase by using foam separation technology, which was efficient, simple, mild and low-cost. And in the process of extraction, the use of surfactant can be reduced by using the surface activity of β-glucanase, so as to reduce the pollution to the product. The mild separation conditions in the process of foam separation also reduce the influence of the denaturation of the separated substances to the greatest extent. The experimental data show that the recovery rate of β-glucanase extracted by foam separation technology can reach 96.01%, which is higher than the traditional extraction method. At the same time, the extraction process is more environmentally friendly and efficient than the traditional extraction method. In addition, the foam separation technology requires simpler production equipment and less consumables, which greatly reduces the production cost and threshold, and realizes the efficient, low-cost and green production of β-glucanase.

From five univariate experiments, the optimal univariate condition for foam separation and extraction of β-glucanase was: ventilation speed of 600 mL/min, loading volume of 200 mL, initial enzyme concentration of 100 g/mL, surfactant concentration of 0.3 mg/mL, and pH of 5. Based on the optimal univariate condition, the optimal separation condition of β-glucanase was further explored, and the five-factor and four-level orthogonal test was designed. The optimal separation condition was 600 mL/min, loading volume of 200 mL, initial enzyme

concentration of 100 μg/mL, surfactant concentration of 0.5 mg/mL and pH of 5. The analysis is available from the experimental results, foam separation is a feasible method to achieve simple, mild and effective extraction of β-glucanase.

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