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The Effect of Glucose Stress on the Cell-viability of *Corynebacterium glutamicum*

Zhou Jing-bo[1] and Huang Ji-hong[2]

[1]South China Normal University, Guangzhou - 510631, China.
[2]He·nan University of Technology,Zhengzhou - 450001, China.

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To keep the optimally physiological state of the strain in the process of glutamic acid fermentation, the one-factor-at-a-time method and response surface methodology experiment about the effect of glucose stress on the strains (FM00-187) in the fermentation medium were performed, and the physiological status (cell-viability) of *corynebacterium glutamicum* flora in different concentrations of glucose was detected by Cyto Analysis 2000. With the changes in the glucose(substrate, initial glucose, fed-batch glucose) concentration, the cell-viability of the strains, changed significantly: increasing the concentration of the substrate, the cell-viability tended to increase .When the concentration of substrate glucose reached 8.93 g/L, the initial glucose concentration 39.14 g/L and the fed-batch glucose concentration 634.99 g/L, the cell-viability peaked (7.650μA), if the glucose continues to increase, the cell-viability would decrease, which was the phenomenon of glucose concentration inhibition. The sensitivity of cell-viability to the three factors, substrate glucose, fed-batch glucose ,initial glucose. *Corynebacterium glutamicum* fermentation performance can be displayed through the cell-activity, provides the reference for the actual fermentation production.

Key words: Glucose stress *Corynebacterium glutamicum* Cell-viability.

The component and content of the culture medium of biological processes are complex and time-varying, with gas, solid and liquid state coexisting[1,2] .In addition,the sterile and sealing requirement in training process and the issue of probe sensitivity bring much difficulty to the technological monitoring in situ. Therefore there is great limitation on the process optimization of microbial expression products[2, 3].

Glutamic acid-producing strain has experienced a series of complex biological metabolism and the change in physiological activity determines the quality of fermentation results[4,5]. How to keep the best physical condition of the strain in the fermentation process? When the sole carbon source is glucose, glucose metabolism is mainly regulated by the control of energy charge, namely the energy level of cells[6]. The mainly physiological function of glucose metabolism is supplying energy in the form of ATP.In the process of glucose oxidation, when the intermediate product accumulates or reduces, the change of energy charge will be influenced and the end-product of metabolism APT will be excess or decreased.. The metabolic pathways are regulated by these intermediate products and adenine nucleotides through inhibiting or activating key enzymes in each stage of glucose metabolism [7]. The change of the energy charge, can we named cell-activity. The starting point of this study is that through competition for nutrients i.e. glucose stress in the medium, the positive effect of glucose at different concentrations on metabolic pathways of

* To whom all correspondence should be addressed.
E-mail: 329241034@qq.com; huangjih1216@126.com

corynebacterium glutamicum and physiological state(cell-viability) of the flora ,has been analyzed by the Cyto Analysis.

MATERIALS AND METHODS

Bacterial strains

Corynebacterium glutamicum FM00-187, Provided by henan lotus gourmet powder group.

Primary instrument

DSW-CJ-2F Clean Bench; BSC-130 Biosafety Cabinet, Cyto Analysis 2000, 752 Spectrophotometer, TGL-16 Table-top High Speed Centrifuge, LDS-10 Low Speed Centrifuge, LRH-250A Biochemical Incubator, YQ001 Adjustable Micro Pipette, Emperature Control Fermentation Machine.

Culture medium

Fermentation medium, Initial Glucose, 20-100g/L, KH2PO4, 3 g/L, MgSO4, 1 g/L, FeSO4, 0.02 g/L, MnSO4 0.02 g/L, TN, Soybean hydrolysate, :1.2g/L, Biotin: 0.50 mg / L, VB1:0.20 mg /L

Cultural methods

The concentration of initial glucose, 40-60 g/L; Fermentation temperature, 34-38°C Air volume was controled by ventilation ratio, 1:0.12-0.45;pH,6.7-7.5. The glucose which was added in fed-batch fermentation process was fed-batch glucose, and the time-varying concentration of glucose was the substrate concentration[8].

Determination of cell-viability

The pretreatment of fermentation broth[9]: 0.5mL fermentation broth was taken by the adjustable micropipette into the centrifuge tube of 1.5mL, then stratified by the high speed centrifugal (8000r/min).The supernatant was poured out to determinate the concentration of glutamic acid and glucose, and the sediment was used for cell-viability.

The determination of cell-viability in fermentation liquid: the sample, which was composed of the thallus treated as 1.3.2 and the dedicated diluent of Cyto Analysis 2000, was injected in the sample slot by a micropipette,then the result was displayed automatically[10,11].

The effect of glucose concentration in medium on the cell-viability of glutamic acid-producing strain

In order to investigate the effect of glucose concentration in culture medium on the cell-viability of bacterial strain, orthogonal test as table (1) and uniform design as table (2) were designed ,and combined with each other to avoid the phenomenon of data fault. Three different strains (A: strain S9114; B: strain FM84-415, strain C: FM00-187) were independently tested. The concentration of the glucose (substrate, initial glucose, fed-batch glucose) has a significant effect on cell-viability, so the three factors of substrate, initial glucose, fed-batch glucose need to be investigated in the fermentation process.

Each strain was tested repeatedly for 5 times at the same level,with the cell-viability (E) as the Index and substrate concentration (S1), initial glucose concentration (S2) and fed-batch glucose concentration (S3) as the factors.

Methods of analysis

The results of the experiment about cell-viability (E) were gotten by the general software —Design-Expert.8.05b

RESULTS AND DISCUSSION

The single factor test

The relationship of cell-activity and initial concentration

Corynebacterium glutamicum (FM00-187) had been fermented for 36h and With the cell-viability measured in the initial glucose concentration(20 g/L030 g/L040 g/L050 g/L060 g/L), fed-batch glucose concentration 500 g/L and substrate glucose 8.5 g /L, Emperature 35!, Ventilation rate1:0.25, pH 7.0, As shown in figure 1, When the concentration of the initial glucose concentration 40 g/L, the cell-viability peaked, if the glucose continues to increase, the cell-viability would decrease.

The relationship of cell-activity and fed-batch glucose concentration

Corynebacterium glutamicum (FM00-187) had been fermented for 36h and With the cell-viability measured in the fed-batch glucose concentration (300 g/L0400 g/L0500 g/L0600 g/L0700 g/L), initial glucose concentration 40 g/L and substrate glucose 8.5 g /L, Emperature 35!, Ventilation rate1:0.25, pH 7.0, As shown in figure 2, When the concentration of the fed-batch glucose concentration 600 g/L, the cell-viability peaked, if the fed-batch glucose continues to increase, the cell-viability would decrease.

Table 1. Response surface test factors and levels

Factors	Code	Levels		
	value	-1	0	1
Fed-batch (g/L)	X_1	500	600	700
Substrate (g/L)	X_2	7	8.5	10
lnitial (g/L)	X_3	30	40	50

Results are shown in table 2 by Box-Behnken

Table 2. The result response surface design and experiment

NO.	X_1 /(g/L)	X_2 /(g/L)	X_3 /(g/L)	Cell-activity E (μA)
1	0	0	0	7.57
2	1	0	1	7.5
3	-1	0	-1	6.81
4	0	-1	1	5.65
5	-1	-1	0	5.37
6	1	1	0	7.25
7	0	0	0	7.77
8	-1	1	0	6.37
9	0	0	0	7.5
10	0	1	-1	6.97
11	0	1	1	6.36
12	0	0	0	7.85
13	-1	0	1	5.57
14	1	0	-1	6.87
15	0	-1	-1	6.37
16	0	0	0	7.66
17	1	-1	0	5.72

The relationship of cell-activity and substrate glucose concentration

Corynebacterium glutamicum (FM00-187) had been fermented for 36h and With the cell-viability measured in the substrate glucose concentration(5.5 g/L07.0 g/L08.5 g/L010 g/L011.5 g/L), initial glucose concentration 40 g/L and fed-batch glucose 500 g /L, Emperature 35!, Ventilation rate1:0.25, pH 7.0, As shown in figure 2, When the concentration of the substrate glucose concentration 8.5 g/L, the cell-viability peaked, if the substrate glucose continues to increase, the cell-viability would decrease.

Response surface analysis

Results show that initial0fed-batch and substrate concentration (there are five repeated and each factors three levels) have some influence on the cell-activity by Box-Behnken, table 1

Prediction equations were derived using the Design - Expert. 8.05 b of the data secondary multivariate regression fitting in table 2 about fed-batch (X1), the substrate concentration (X2) and the initial concentration (X3) to cell activity, as follows:

Y=7.67+0.40 X1+0.48X2-0.24X3+0.13 X1 X2+0.47X1X3+0.028 X2 X3-0.57 X12-0.92 X22-0.41 X32

Then we use analysis of variance quadratic model to find the remarkable factors, as shown in table 3.

The model P =0.0003 in reasonable agreement with the adjusted P (Lack of fit) = 0.0706

Table 3. Analysis of variance quadratic model

source	Sum of squares	df	mean square	*F* value	*P* value	significant
model	10.79	9	1.20	20.74	0.0003	*
X_1	1.30	1	1.30	22.43	0.0021	*
X_2	1.84	1	1.84	31.90	0.0008	*
X_3	0.47	1	0.47	8.14	0.0246	*
X_1X_2	0.070	1	0.070	1.22	0.3067	
X_1X_3	0.87	1	0.87	15.13	0.0060	*
X_2X_3	3.025E-003	1	3.025E-003	0.052	0.8256	
X_1^2	1.37	1	1.37	23.78	0.0018	*
X_2^2	3.57	1	3.57	61.85	0.0001	**
X_3^2	0.71	1	0.71	12.32	0.0099	*
residual	0.40	7	0.058			
Lack of fit	0.32	3	0.11	5.29	0.0706	
Pure error	0.081	4	0.020			
cor total	11.17	16				

** P≤0.0001 highly significant* P≤0.05significant.

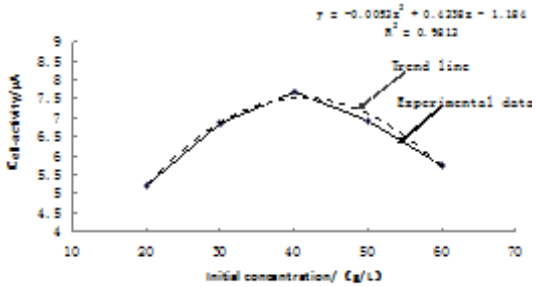

Fig. 1. The relationship of cell-activity and initial concentration

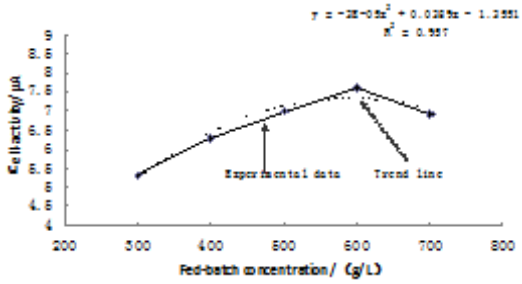

Fig. 2. The relationship of cell-activity and fed-batch concentration

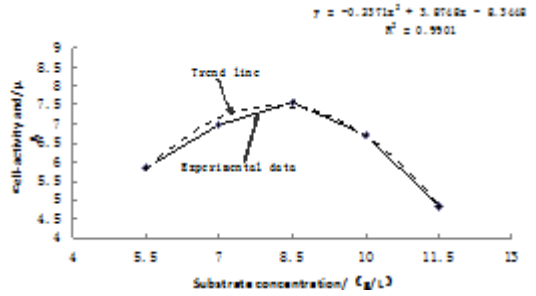

Fig. 3. The relationship of cell-activity and substrate concentration

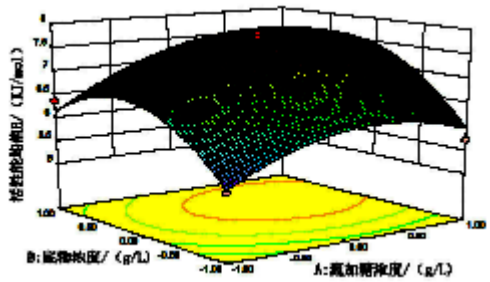

Fig. 4. Response surface and contour of fed-batch, substrate

ÿ 0.05, Adequate precision measures the Cell-activity E of FM00-187, by changes in fed-batch (X1), the substrate concentration (X2) and the initial concentration (X3). One item of the X1, X2, X3 significantly; Quadratic term X2 more significantly, the X1, the X3 is significant, the cubic interaction term X1X3 is significant, that the sensitivity of cell-viability to which appeared in the decreasing order: substrate glucose, fed-batch glucose, initial glucose.

Significant interaction term X1X3 is displayed With the changes in the glucose(fed-batch, substrate)concentration,the cell-viability of the strains, changed significantly: With the increase of fed-batch and substrate, cell-activity E increased , if the glucose continues to increase, the cell-viability would decrease. There is a high cross area the cell-viability peaked in elliptica contour.

The best process parameters by analyzing the Design - Expert and the quadratic regression equation have been predicted: Cell-activity of strain FM00-187 prediction theoretical E value is 7.819μA, when fed-batch 634.99g/ L0substrate 8.93 g/L and initial 39.14 g/L. Through validation test, cell-activity of strain FM00-187 E value (the actual average) was 7.708μA, Actually E value was 7.650μA with the concentration were correct: fed-batch 635.0g/L0substrate9.0 g/L and initial 40.0 g/L. Results show that the model is reasonable and the predicted are more accurate, the actual value is consistent with the theoretical value, for the relative error is 2% [(7.819-7.650) / 7.8^9, The experimental results are in accord with the theoretical ones.

DISCUSSION

Effects had been studied for substrate concentration, initial glucose and fed-batch glucose on the cell-viability of strain FM00-187. It can be seen from the variance analysis and range value of substrate, initial glucose and fed-batch glucose, that the sensitivity of cell-viability to which appeared in the decreasing order: substrate glucose, fed-batch glucose, initial glucose; Through the tracking and detecting of the glutamic acid fermentation process, Cell-activity is a good way to determine glucose concentration of stress on the influence of glutamic acid fermentation,

provides the reference for industrial fermentation production.

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