Optimizing yeast cell (*Saccharomyces cerevisiae*) disruption by sonication treatment

Le Nguyen Han, Le Huynh Hong Van, Tran Van Duc, Dong Thi Anh Dao

Abstract— **Extracellular products are currently easy to obtain but with intracellular products, the disruption of cells is one of the most important steps which need to be carried out. There are many methods used in cell disruption which are based on mechanical techniques, such as high pressure homogenization and bead mills, physical techniques and chemical or enzymatic treatment. In this study, however, we examined the physical disruption of the yeast cell walls *Saccharomyces cerevisiae* by sonication (Sonicator®; Misonix, USA) at various duty cycle rates and sonication time values; then we used an indirect method for the determination of the degree of yeast cell disruption through the soluble protein release. The degree of yeast cell disruption is also evaluated by counting the percentage of disruption cells on hemocytometer. The results showed the release of soluble protein and percentage of disruption cells are in direct relationship with the duty cycle rates and sonication time; at 532.5W-5.226 minutes sonication treatment we got the highest disruption of yeast cells (36.263 ± 0,884 %) and the highest concentration of soluble protein (446.385µg/mL). The data was processed by Minitab and Modde 5.0**

Index Terms— **Optimization, sonication, yeast cell disruption.**

I. Introduction

Yeasts are microscopic, eukaryotic, heterotrophic fungi. The most known and widely applied yeast, *Saccharomyces cerevisiae* is traditionally applied in the production of alcoholic beverages, industrial alcohol and glycerol; it is also used for baking and as addition to animal feed [4]. Yeast cells are formed by high molecular weight components such as protein, glycoprotein, polysaccharide, polyphosphate, lipid, nucleic acid [2]. These components can be varied, depending on culture conditions and physiological state of cells. The yeast cell is protected by a thick and rigid cell wall which makes it difficult extracting the intracellular products [3].

There are many solutions to collect the intracellular products by breaking the cell wall such as chemical treatment. However, the chemical removal in the final product and the purification stages need to be conducted. With enzyme treatment, we should also inactivate the enzymes to reach the desired purity. Therefore, the physical one such as sonication is highly appreciated in cell disruption because of short time treatment and the simple purification stage.

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II. Materials and Method

A. Materials

In this study, we use the spent yeasts which are collected from experimental brewing factory of HCMC University of Food Industry. Spent yeasts are washed with sterile water with ratio 1:3 (w/w), settled in gravity for 1 hour with the above water layer removed afterwards. After that, spent yeasts are washed with sterile salt water 0,9% with ratio 1:3(w/w), settled in gravity for 1 hour and then with the above water layer removed again [5]. Later, yeast cell suspension was centrifuged to recover the cells at 3000rpm and 4^oC for 3 minutes [6]. After that, we remove the liquid phase and add salt water into centrifuge tube, shake well and conduct the centrifugation again. We repeat this centrifugation step until the liquid phase in centrifuge tube becomes purified. This step aims to remove the beer in spent yeast and avoid the falsified measurement results in Lowry method by soluble protein in residual beer. Clean and wet yeast cells are preserved in cool condition (4^oC) and can be used in 2-3 days.

B. Research methodology

Determine the concentration of cells before and after sonication treatment

The concentration of yeast cells is determined by counting in hemocytometer. To determine the proportion of ruptured cells, yeast cells are dyed by methylene blue 0.01% with the ratio 1:1. Undamaged cells display blue because the cytoplasm is still remained inside.

Sonication process

The disruption experiments were performed by using horn tip sonicator, Sonicator®; Misonix, USA. The tip of the sonicator horn (TT13 Titanium tapered tip of 12.7mm diameter) was immersed about h = 10 mm into the solution that processed in a 50mL cylindrical glass vessel with a diameter of d = 50mm. The temperature of the yeast solution was intermittently checked and kept constant 10 ± 1^oC by the use of a cooling bath containing ice-water mixture.

The effects of duty cycle % of a sonicator, time treatment and cell concentration on protein release and proportion of ruptured cells were examined. Yeast mass was randomly diluted at different concentrations and treated with sonication to find out the optimal disruption rate (with fixed capacity of 375W and fixed time of 5 minutes). Based on the found-out optimal cell concentration, we carry out examining the effects of the sonication capacity in range of 150W-600W with the 75W jump (with fixed time of 5 minutes). Finally, the sonication time is examined in range of 1-9 minutes with the 2 minute jump (with the optimal disruption rate and concentration found previously).

Measurement of protein

The cell disruption is evaluated by the proportion of ruptured cells and indirectly by the amount of soluble protein released from cells through sonication. After sonication treatment, soluble protein is separated from cells by the centrifugation process at 10.000 rpm, 10 minute period, 4^0C. The concentration of protein (background protein, total protein and protein released after processing) was determined by the Lowry method using bovine serum albumin as standard. [8]

C. RESULTS AND DISCUSSION

Effect of yeast mass concentration on cell disruption

The effect of yeast mass concentration was investigated in the range of 25-317 million cells per mL yeast, and the results were given in Figure 1. As seen in this chart, the protein release is dependent on cell concentration. The effect of ultrasound evenly distributes in yeast mass solution and disrupt the yeast cell walls. Consequently, the increase of cell concentration means ultrasound is likely to interact with more cells, causing the increase of soluble protein in yeast mass solution.

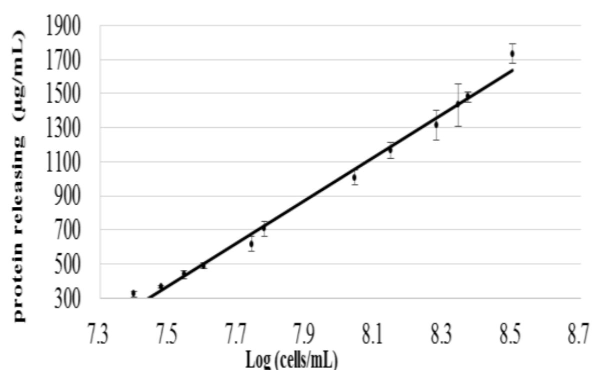

Fig 1. Effect of yeast mass concentration on protein releasing at 50 % duty cycle, 375W acoustic power.

However, the rate of ruptured cells on the condition of increased cell concentration hardly shows any statistical differences ($p_{value} > 0.05$) as seen in Fig. 2. Therefore, yeast cell concentration does not impact the process of cell disruption by sonication method.

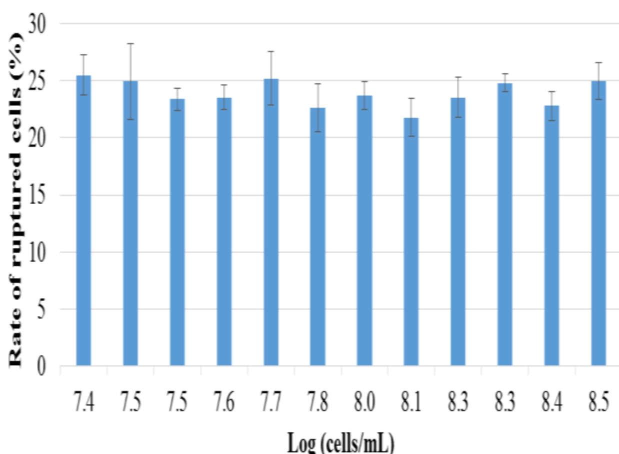

Fig 2. Effect of yeast mass concentration on rate of ruptured cells at 50 % duty cycle, 375W acoustic power

Effect of acoustic power on cell disruption

With acoustic power increasing from 150W-300W, the fact that the amount of protein collected from ruptured cells is quite small, and so is the rate of ruptured cells counted through the microscope suggests that the above applied power is not adequate to interrupt the yeast cell walls to the desired extent. For power ranging from 375-450W, the collected protein rises remarkably. Similarly, the amount of protein measured and the rate of ruptured cells collected are quite high on the condition of 525-600W acoustic power. However, data measured at the points of 525W and 600W respectively do not show statistical difference; consequently, we prefer the case of 525W for its better efficiency.

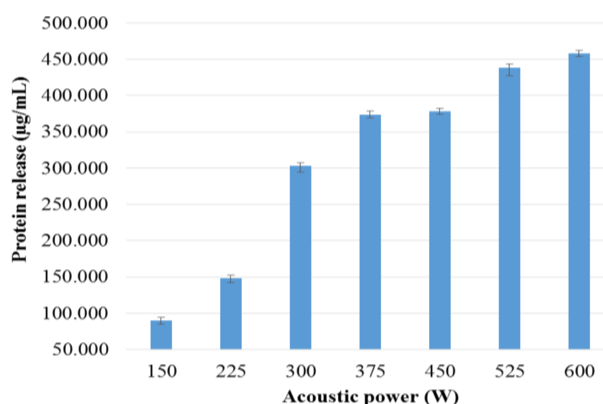

Fig 3. Effect of acoustic power on protein release at 5 minutes and 30 million cells/mL yeast mass concentration

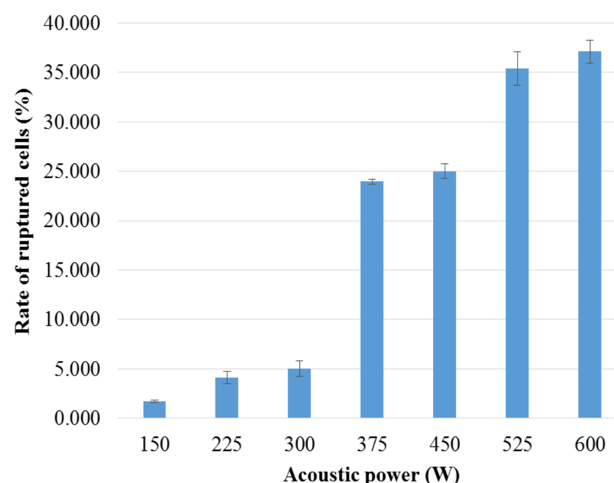

Fig 4. Effect of acoustic power on rate of ruptured cells at 5 minutes and 30 million cells/mL yeast mass concentration

Effect of sonication time on cell disruption

Examining the impact of acoustic power on yeast cell disruption, we find out the optimal power is 575W and use this value for the experiments on sonication time. In range of 1-3 minutes, the amount of protein released and ruptured cell rate is low. At 5 minute period, the collected data increase significantly. However, under 7 and 9 minute treatment, the rate of ruptured cells measured remains stable and is not statistically different from that under 5 minute although the amount of protein varies and shows statistical difference. Therefore, 5 minute treatment is selected for its efficiency.

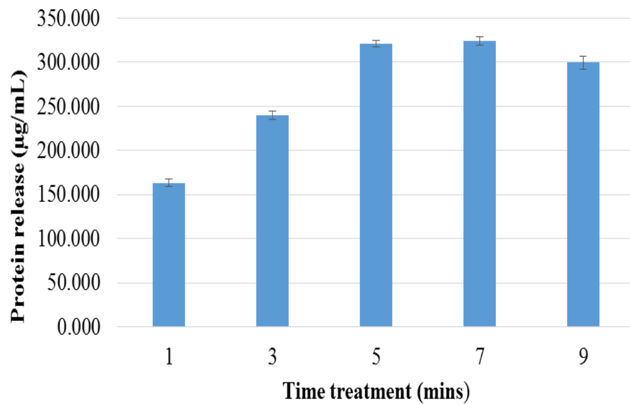

Fig 5. Effect of time treatment on protein release at 575W acoustic power and 30 million cells/mL yeast mass concentration.

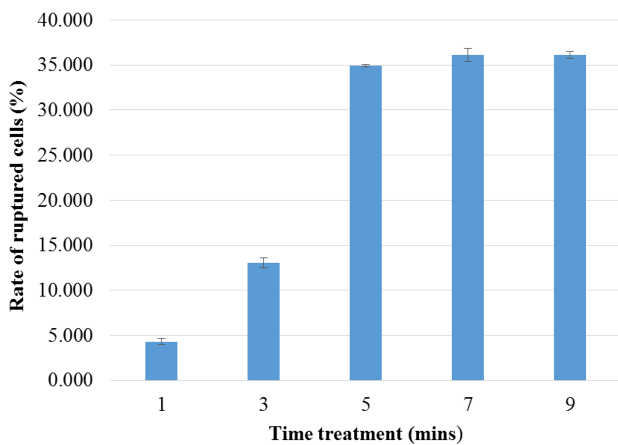

Fig 6. Effect of time treatment on rate of ruptured cells at 575W acoustic power and 30 million cells/mL yeast mass concentration.

Under observation through microscope, the mature and aged yeast cells have thicker walls than the immature ones. However, most of the mature and aged cells are broken during the sonication process while the immature ones are likely to be more stable. It can be explained that the mature and aged yeast cells have gone through several divisions, which left many bud scars on their walls. In fact, those with bud scars are more prone to ultrasound impact than the immature ones which have never divided (with only one birth scar though). At these bud scars, chitin exists in great amount changing the mechanical properties of the cell wall there.

The area around the bud scar is proved unlikely to enlarge on the cell growth in comparison to other spots, leading to a significant decrease in the elasticity of the cell wall there. This change in it turn causes a tension concentration under sonication treatment, making cell broken here. Meanwhile, the birth scar contains little if no chitin so the cell wall there is similar to other spots. The area around the birth scar is also proved to have the same growth rate as others within a cell.

Cell division process is believed to cause holes on β-1,3-glucan layers at the bud scar, making this spot easier to be ruptured.

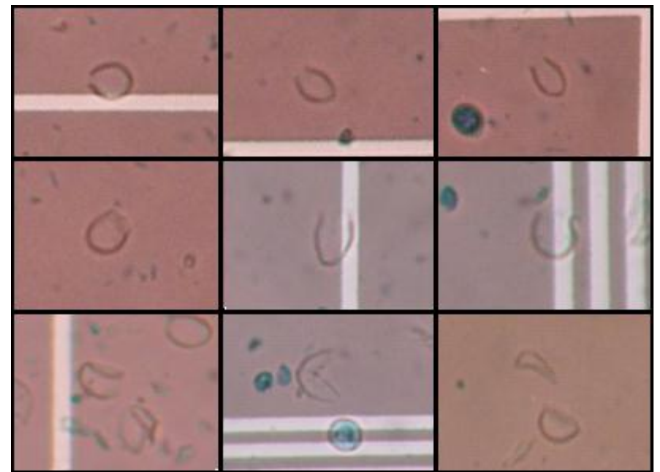

Fig 7. Ruptured cells at different rate

Optimizing the conditions for cell disruption by sonication

For increasing cell disruption rate by sonication, this optimizing process was exercised. Orthogonal Second-order Design (level 2) was carried out. From the above experiments, it can be seen that 2 factors of power and sonication time have the greatest impact on the yeast cell disruption rate, so these factors are chosen whereas the values for other factors are selected at their appropriateness.

We in turn change the values of acoustic power and sonication time, then determine their impact pattern on cell disruption rate and the amount of soluble protein. After that, regression function is built up and optimal parameters are identified.

➢ *Optimizing the objective function as the soluble protein amount*

➢

The experiments are conducted with the following factors:

- Sonication time: $X_1 \in [3;7]$ and basic level: $X_{01} = 5$ minutes
- Acoustic power: $X_2 \in [450; 600]$ and basic level: $X_{02} = 525W$
- The amount of soluble protein: Y_1 (µg/mL)

To seek for the regression function, 13 experiments are conducted including 5 in experimental center and 8 on coordinate axes.

The design of experiment with outcomes of design points is given in Table 1:

Variation levels	*Time treatment (mins) - X_1*	*Acoustic power (W) - X_2*
Basic level	5	525
Variation interval	2	75

	No. trials	X_o	X_1	X_2	X_1X_2	Y
Core point (2^k)	1	+1	-1	-1	+1	319.222
	2	+1	+1	-1	-1	396.733
	3	+1	-1	+1	-1	379.273
	4	+1	+1	+1	+1	396.233
Starlike points: 2k	5	+1	-1	0	0	403.148
	6	+1	+1	0	0	420.664
	7	+1	0	-1	0	375.529
	8	+1	0	1	0	405.649
Null points	9	+1	0	0	0	465.356
	10	+1	0	0	0	465.689
	11	+1	0	0	0	465.707
	12	+1	0	0	0	465.133
	13	+1	0	0	0	465.467

Table 1: Central composite rotatable design of the objective function as the soluble protein amount.

By processing outcomes, we obtain estimates of regression coefficients for the second-order regression model:

$$Y_1 = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2$$

Regression coefficients are calculated by Moddle 5.0 as follows:

Regression coefficients	Value	Standard error	P	Condition P<0,05
b_0	460.446	5.432	$8.37.10^{-12}$	Accept
b_1	13.506	3.776	0.0090	Accept
b_2	10.815	3.776	0.0242	Accept
b_{11}	-19.049	3.936	0.0019	Accept
b_{22}	-27.784	3.936	0.0002	Accept
b_{12}	-7.746	3.270	0.0497	Accept

Table 2: Regression coefficients for second-order regression model Y_1

The regression equation becomes:

$Y_1 = 460.446 + 13.506 X_1 + 10.8146X_2 – 7.74563 X_1X_2- 19.0487 X_1^2 – 27.7841X_2^2$

Coefficient $R^2 = 0.953$, $Q^2 = 0.750$ shows the reliability of the testing model with high compatibility between the experimental and theoretical models. What follows is the dimensional model produced by Modde 5.0 on building regression function on 3-dimensionsl axes:

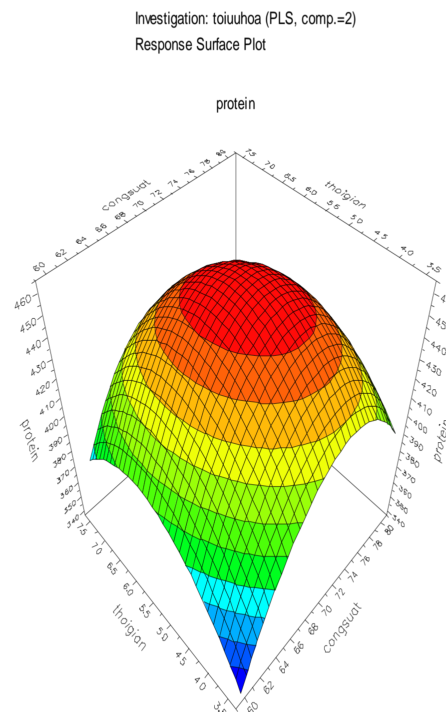

Fig 8. The dimensional model produced by Modde 5.0 on building regression function on 3-dimensionsl axes (Y_1)

On optimizing 2 variable function, the results are as follow: the amount of soluble protein $Y_1 = 463.443$ (μg/mL) on the optimal condition of time $X_1 = 5.4667$ (mins) and acoustic power $X_2 = 532.880$ (W) (equivalent to 71.0507% of the max power).

- *Optimizing the objective function as the cell disruption rate*

The experiments are conducted with the following factors:

- Sonication time: $X'_1 \in [3;7]$ and basic level: $X'_{01} = 5$ minutes
- *Acoustic power:* $X'_2 \in [450; 600]$ and basic level: $X'_{02} = 525$W
- *Cell disruption rate:* Y_2 (%)

Variation levels	*Time treatment (mins) -X'1*	*Acoustic power (W) -X'2*
Basic level	5	525
Variation interval	2	75

To seek for the regression function, 13 experiments are also conducted including 5 in experimental center and 8 on coordinate axes.

The design of experiment with outcomes of design points is given in Table 3:

	No. trials	X'$_o$	X'$_1$	X'$_2$	X'$_1$X'$_2$	Y'
Core point (2^k)	1	+1	-1	-1	+1	23.027
	2	+1	+1	-1	-1	25.233
	3	+1	-1	+1	-1	28.047
	4	+1	+1	+1	+1	27.143
Starlike points: (2k)	5	+1	-1	0	0	30.477
	6	+1	+1	0	0	32.610
	7	+1	0	-1	0	30.057
	8	+1	0	+1	0	32.613
Null points	9	+1	0	0	0	36.797
	10	+1	0	0	0	36.217
	11	+1	0	0	0	36.817
	12	+1	0	0	0	36.140
	13	+1	0	0	0	36.493

Table 3: Central composite rotatable design of the objective function as the cell disruption rate

. By processing outcomes, we obtain estimates of regression coefficients for the second-order regression model:

$$Y_2 = b_o + b_1 X'_1 + b_2 X'_2 + b_{12} X'_1 X'_2 + b_{11} X'^2_1 + b_{22} X'^2_2$$

Regression coefficients are calculated by Moddle 5.0 as follows:

Regression coefficients	Value	Standard error	P	Condition P<0,05
b_0	36.563	0.246	$6.19.10^{-12}$	Accept
b_1	0.447	0.162	0.0329	Accept
b_2	1.168	0.162	0.0004	Accept
b_{11}	-2.874	0.180	$3.79.10^{-6}$	Accept
b_{22}	-2.924	0.180	$3.43.10^{-6}$	Accept
b_{12}	-0.424	0.147	0.0277	Accept

Table 4: Regression coefficients for second-order regression model Y_2

The regression equation becomes:

$$Y_2 = 36{,}563 + 0{,}447X'_1 + 1{,}168X'_2 - 0{,}424X'_1X'_2 - 2{,}874X'^2_1 - 2{,}924X'^2_2$$

Coefficient $R^2 = 0{,}993$, $Q^2 = 0{,}765$ shows the reliability of the testing model with high compatibility between the experimental and theoretical models. What follows is the dimensional model produced by Modde 5.0 on building regression function on 3-dimensionsl axes:

Investigation: toiuuhoa_tylevo (PLS, comp.=2)
Response Surface Plot

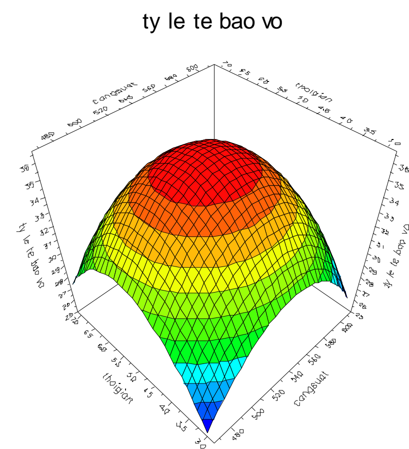

Fig 9. The dimensional model produced by Modde 5.0 on building regression function on 3-dimensionsl axes (Y_2)

On optimizing 2 variable function, the results are as follow: the *cell disruption rate* Y_2 = 36.6914% on the optimal condition of time $X'_1 = 5.0951$ (mins) and acoustic power X'_2 = 536.03 (W) (equivalent to 71.4707% of the max power).

Optimizing the dual objective functions of protein release and cell disruption rate

To obtain the above objective, it is necessary to solve the problem of multi-objective optimization (2 objectives in this study). After optimizing each of the objective functions, the regression function between the independent variables (sonication time (X_1), acoustic power (X_2)) and the objective function (protein release (Y_1), cell disruption rate (Y_2)) is established. Next, the problem of optimizing the dual objective functions (Y_1, Y_2) is solved based on optimal solution for multi-objective function by linear convolution method.

However, it is not practical to find a single solution for both optimal purposes and obtain the max values for the objective function: Y_{1max} and $Y_{2max.}$ Instead, a compromise solution should be identified so that Y_1 and Y_2 are closest to Y_{1max} and $Y_{2max.}$ To seek for the compromise solution, linear convolution is employed:

$Y_L = \alpha_1.Y_1 + \alpha_2.Y_2$

Where:

- α_1: important factor for the objective function 1: protein release (Y_1)
- α_2: important factor for the objective function 2: cell disruption rate (Y_2)

For the purpose of collecting yeast cell walls for further research, we give priority to objective function 2: cell disruption rate (Y_2). With the important factors for Y_1 and Y_2 respectively equal to 0.4 and 0.6 ($\alpha_1 = 0.4$; $\alpha_2 = 0.6$), we get the equation of multiple objective function as follow:

$$Y_L = 0.4Y_1 + 0.6Y_2.$$

Regression coefficients are calculated as follow:

Regression coefficients	Y_1	Y_2	Y_L
b_o	460.446	36.563	206.116
b_1	13.506	0.447	5.671
b_2	10.814	1.168	5.026

Table 5: Regression coefficient for Y_L

Then, we get the following regression equation:

$$Y_L = 206{,}116 + 5{,}671X_1 + 5{,}026X_2 + 28{,}502X_1X_2 + 32{,}160X_1^2 + 25{,}261X_2^2$$

To calculate the step size of the factors:

Variation levels	Independent variables	
	X_1(mins)	X_2 (%)
Basic level	5	70
Variation interval (Δ)	2	10
Factor b_j	5.671	5.026
b_j Δ	11.342	50.262
Step size (δ)	0.226	1

Table 6: The factors' step size

Steepest descent method is designed as follows:

Factor	X_1 (mins)	X_2 (%)	Y_1	Y_2	Y_L
1 (experimental center point)	5	70	454.077	36.768	203.691
2	5.226	71	460.231	36.545	206.019
3	5.451	72	417.154	36.458	188.736

Table 7: Steepest descent method

The above table shows that the second experiment brings the max value for the convolution of 2 functions (Y_{L}). Then, we choose $X_1 = 5.226$ minutes and $X_2 = 71\%$ for the experimental test.

Checking the adequacy of the model

Experiments were conducted to rebuild the closest theoretical factors of optimization for comparing experiment and theory. Experiments were conducted 3 times with the fixed factors as follows:

- Cell concentration: 29 x 10^6 cells/mL
- Acoustic power X_2 = 532.5 (W) (equivalent to 71% of the max power).
- Sonication time: 5 mins 14 seconds

Results are as follows:

- Soluble protein Y_1= 446.385 ± 7.580 (µg/mL) (96.31% compared with theoretical value Y_1= 463.443 (µg/mL);
- Cell disruption rate Y_2= 36.263 ± 0.884 (%), (98.832% compared with theoretical value Y_2 = 36.696%).

III. CONCLUSION

Those significantly impact yeast cells are sonication treatment, time treatment, and acoustic power. When yeast cells are treated at 532.5W (acoustic power) during a period of 5 minutes 14 seconds, cell disruption rate is recorded remarkable (at 36.263 ± 0.884 %) and is so the amount of soluble protein (at 446.385 ± 7.580 µg/mL). The fact that the optimal time treatment and acoustic power in cell disruption by sonication can be identified lays foundation for the successful production of intracellular products without time consumption as in methods using chemicals and enzyme.

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