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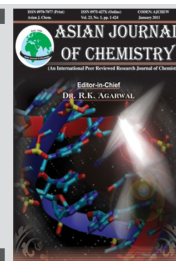

# Citric Acid Induction and Statistical Optimization for Production Enhancement of Catalase by *Serratia marcescens* SYBC08

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The process of enhancing production of catalase by *Serratia marcescens* SYBC08 was carried out in submerged fermentation. Catalase production of critic acid induction was increased by 92 % compared to that of glucose. Statistical experimental designs were used in optimization of catalase production. Temperature, initial pH and corn steep liquor powder concentration were identified as the most significant factors by using Placket-Burman design. The optimum values of three significant factors were determined using central composite design and response surface analysis. While three optimum significant factors were revealed as temperature (32.8 °C), initial pH (5.9) and corn steep liquor powder concentration (33.8 g $L^{-1}$), the maximum predicted catalase production of 11632.88 U $mL^{-1}$ was calculated out using a two second-order polynomial equation. In a validate experiment, maximum catalase production of 12182.74 U $mL^{-1}$ was achieved and had 28.27 % increasement compared to that under unoptimized conditions.

**Key Words: Carbon metabolism, Statistical optimization, Catalase, *Serratia marcescens*, Sludge.**

## INTRODUCTION

Hydrogen peroxide is used as a bleaching or microbicidal agent in the paper, food, textile and semiconductor industries[1]. The excess hydrogen peroxide is toxic to organism and need to be removed after those industrial processes. Reducing agents such as sodium hydrogen sulfite are often used to decompose hydrogen peroxide in industries, but sodium hydrogen sulfite is also toxic to organism. Catalase can efficiently catalyze the decomposition of hydrogen peroxide into water and molecular oxygen and have been widely and safely applied in the industrial section. Commercially available catalase is prepared from bovine livers or microorganisms, an approach limited by high cost due to low yield[2]. Inducers such as $H_2O_2$ and pectin have been explored to enhancing catalase production[3-5]. Catalase systhesis has been related to TCA cycle and can be largely induced by carbon source derived from TCA cycle[6,7]. Therefore those carbon sources not only are fermentation substrate, but also are inducers of catalase.

A classical approach of the one-factor-at-a-time optimization has been applied in improving catalase production[8,9]. Plackett-Burman design combination with response surface methodology (RSM) are very useful tools for enhance production of enzyme, help in understanding the relationship between a set of controllable experimental factors and observed results and the interaction among the possible influencing parameters by less number of experiments[10,11]. However, as per literature survey, there is no report on using Plackett-Burman combined response surface methodology for enhancing production of catalase.

*Serratia marcescens* is a conditional pathogenic bacterium capable of causing disease in diverse organisms, including humans[12], coral[13], insects[14], but it is thought as very important industrial strain which has been applied in fermentation for the production of many enzymes[15-17].

In present study, the authors reported the enhancing catalase production by *Serratia marcescens* SYBC08. Firstly, citric acid was chosen as a suitable inducer for improving catalase production. Secondly, cultural conditions for maximum catalase production were optimized by using Plackett-Burman and central composite design.

## EXPERIMENTAL

**Microorganism and preparation of seed:** The train used in the study was isolated from sludge with hydrogen peroxide in bleaching workshop of textile factory. It was identified as *Serratia marcescens* SYBC08 by 16 S rDNA sequence (Genbank Accession no GU188473) and conserved in China

General Microbiological Culture Collection Center (Preserved No. CGMCC 3449). Prior to use, the strain was recovered from 20 % (v/v) glycerol stocks stored at -70 °C. The microorganism was inoculated into 50 mL seed medium (glucose, 20 g $L^{-1}$; peptone, 10 g $L^{-1}$; beef extract,5 g $L^{-1}$; NaCl, 5 g $L^{-1}$ and pH 7.2) in a 250 mL flask and the flask culture was carried out on rotary shaker with 200 rpm for 12 h at 30 °C for preparing seed. The seed broth was inoculated into fermentation medium with 4 % size (v/v).

**Preparation of crude enzyme:** The broth (15 mL) was centrifugated at 10800 g for 10 min at 4 °C. The stain was collected and suspended in 5 mL of 100 mM $Na_2HPO_4$-$NaH_2PO_4$ buffer (pH 7.0) and then the suspend liquid was disrupted by sonication at 4 °C. Cell debris was removed by centrifugation at 18000 g for 10 min at 4 °C. The crude enzyme was collected and assayed its catalase activity.

**Determination of catalase activity:** Catalase activity was measured spectrophotometrically by monitoring the decrease in absorbance at 240 nm caused by the decomposition of hydrogen peroxide[18]. The reaction mixture in a total volume of 3 mL was composed of 0.1 mL enzyme solution, 50 mM $Na_2HPO_4$-$NaH_2PO_4$ buffer (pH 7.0), 120 mM $H_2O_2$. The molar absorptivity for hydrogen peroxide at 240 nm was assumed to be 43.6 $M^{-1}$ $cm^{-1}$. The linear range of the reaction (30 s) was used to calculate the rate of the reaction and one unit of catalase activity was determined as the amount of enzyme required to transform 1 μmol of hydrogen peroxide to water and oxygen per min[19].

**Evaluation of inducers for the production of catalase:** Submerged fermentation was performed using a fermentation medium which contained (g $L^{-1}$): corn steep liquor powder 36, glucose 22. The initial pH of the medium was adjusted to 6.75. Glucose in fermentation medium was replaced by L-malic acid, citric acid, L-glutaminic acid and succinic acid having equimolar carbon concentration for individual evaluation of catalase production. For fermentation, 50 mL of those mediums were placed in 250 mL flasks. The flasks were incubated at 35 °C on a rotary shaker at 250 rpm for 36 h.

**Experimental design**

Base on the results from single factor experiments, statistical optimization was carried out by using two steps.

**Plackett-Burman design:** For screening significant factors, Plackett-Burman design and experiment was carried out. Plackett-Burman design based on the first-order polynomial model:

$$Y = \beta_0 + \Sigma\beta_i X_i \quad (1)$$

where Y is the response (catalase production), $\beta_0$ is the model intercept, $\beta_i$ is the linear coefficient and $X_i$ is the level of the independent variables. In the experiment, eight factors (temperature, initial pH, rotation speed, inoculum volume, corn steep liquor powder concentration, citric acid concentration, loading liquid volume, fermentation time) were chosen and defined as $X_1$, $X_2$, $X_4$, $X_5$, $X_7$, $X_8$, $X_{10}$, $X_{11}$, respectively. Three dummy variables were defined as $X_3$, $X_6$, $X_9$, respectively. In those factors, high level and low level was encode as "+1" and "-1", respectively. Plackett-Burm design was undertaken by SAS software 8.0 and is shown in Table-1.

**Central composite design:** For maximum catalase production, response surface methodology using central composite design (CCD) was used in optimizing the significant factors from Plackett-Burman design. The three significant factors (temperature, initial pH and corn steep liquor powder concentration) were defined as $X_1$, $X_2$, $X_7$, respectively and studied in different levels (-2, -1, 0, 1 and 2) (Table-2). According to central composite design, the results from a set of 20 experiments were calculated by SAS software 8.0 and showed in Tables 2 and 3.

The second order polynomial equation which relates the response measured to the independent variables was obtained by a multiple regression analysis of data. The equation is:

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i X_i + \sum_{i=1}^{j-1}\sum_{j=1}^{k}\beta_{ij} X_i X_j + \sum_{i=1}^{k}\beta_{iI} X_i^2$$

where Y is catalase production, $\beta_0$, $\beta_i$, $\beta_{iI}$ and $\beta_{ij}$ were offset term, linearity misregistration, second-order deviation coefficient and interaction effect, respectively.

TABLE-1
PLACKETT-BURMAN DESIGN AND RESULT

| Run | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | Catalase production (U $mL^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | -1 | 1 | -1 | -1 | -1 | 1 | 1 | 1 | -1 | 1 | 7865.48 |
| 2 | 1 | 1 | -1 | 1 | -1 | -1 | -1 | 1 | 1 | 1 | -1 | 7561.75 |
| 3 | -1 | 1 | 1 | -1 | 1 | -1 | -1 | -1 | 1 | 1 | 1 | 8859.56 |
| 4 | 1 | -1 | 1 | 1 | -1 | 1 | -1 | -1 | -1 | 1 | 1 | 8489.00 |
| 5 | 1 | 1 | -1 | 1 | 1 | -1 | 1 | -1 | -1 | -1 | 1 | 5483.08 |
| 6 | 1 | 1 | 1 | -1 | 1 | 1 | -1 | 1 | -1 | -1 | -1 | 7739.42 |
| 7 | -1 | 1 | 1 | 1 | -1 | 1 | 1 | -1 | 1 | -1 | -1 | 8280.03 |
| 8 | -1 | -1 | 1 | 1 | 1 | -1 | 1 | 1 | -1 | 1 | -1 | 9478.00 |
| 9 | -1 | -1 | -1 | 1 | 1 | 1 | -1 | 1 | 1 | -1 | 1 | 12087.98 |
| 10 | 1 | -1 | -1 | -1 | 1 | 1 | 1 | -1 | 1 | 1 | -1 | 7065.99 |
| 11 | -1 | 1 | -1 | -1 | -1 | 1 | 1 | 1 | -1 | 1 | 1 | 8376.48 |
| 12 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | 9670.05 |
| Effect | -2091 | -1393 | | 300 | 79 | | -1310 | 877 | | -216 | 228 | |
| Coeff. | -1046 | -696 | | 150 | 39 | | -655 | 438 | | -108 | 114 | |
| S.E. | 193.2 | 193.2 | | 193.2 | 193.2 | | 193.2 | 193.2 | | 193.2 | 193.2 | |
| t-Value | -5.41 | -3.60 | | 0.78 | 0.20 | | -3.39 | 2.27 | | -0.56 | 0.59 | |
| p-Value | 0.012[a] | 0.037[a] | | 0.494 | 0.852 | | 0.043[a] | 0.108 | | 0.615 | 0.597 | |

$X_1$: Temperature, -1 (33 ºC), + 1 (37 ºC); $X_2$: initial pH, -1 (6), +1 (7.5); $X_4$: rotation speed, -1 (200 rpm), +1 (250 rpm); $X_5$: inoculum amount, -1 (0.04), +1 (0.06); $X_7$: nitrogen source, -1 (32 g $L^{-1}$), +1 (40 g $L^{-1}$); $X_8$: carbon source, -1 (24 g $L^{-1}$), +1 (30 g $L^{-1}$); $X_{10}$: volume in 250 mL flasks, -1 (50 mL), +1 (70 mL); $X_{11}$: fermentation time, -1 (28 h), +1 (36 h). $X_3$, $X_6$, $X_9$ was the dummy variables. Indicates model terms are significant.

TABLE-2
EXPERIMENTAL PLAN FOR OPTIMIZATION OF CATALASE PRODUCTION USING RSM

| Runs | $X_1$ | $X_2$ | $X_7$ | Catalase production (U $mL^{-1}$) | |
|---|---|---|---|---|---|
| | | | | Observed | Predicted |
| 1 | -1 | -1 | -1 | 11993.83 | 11597.41 |
| 2 | -1 | -1 | 1 | 12318.90 | 11653.97 |
| 3 | -1 | 1 | -1 | 10204.76 | 9836.14 |
| 4 | -1 | 1 | 1 | 8837.65 | 9128.87 |
| 5 | 1 | -1 | -1 | 10369.50 | 8761.06 |
| 6 | 1 | -1 | 1 | 10922.70 | 9974.10 |
| 7 | 1 | 1 | -1 | 6860.99 | 6208.71 |
| 8 | 1 | 1 | 1 | 7578.71 | 6657.91 |
| 9 | -2 | 0 | 0 | 10573.63 | 10485.12 |
| 10 | 2 | 0 | 0 | 3770.64 | 5177.82 |
| 11 | 0 | -2 | 0 | 10099.42 | 11250.71 |
| 12 | 0 | 2 | 0 | 6005.88 | 6173.25 |
| 13 | 0 | 0 | -2 | 9233.80 | 10088.80 |
| 14 | 0 | 0 | 2 | 10130.91 | 10594.57 |
| 15 | 0 | 0 | 0 | 10378.32 | 10023.95 |
| 16 | 0 | 0 | 0 | 10253.81 | 10023.95 |
| 17 | 0 | 0 | 0 | 9813.87 | 10023.95 |
| 18 | 0 | 0 | 0 | 9735.69 | 10023.95 |
| 19 | 0 | 0 | 0 | 9175.94 | 10023.95 |
| 20 | 0 | 0 | 0 | 9465.00 | 10023.95 |

$X_1$: Temperature, -2 (31 °C), -1 (33 °C), 0 (35 °C), +1 (37 °C), +2 (39 °C); $X_2$: initial pH, 2 (5.5), -1 (6.25), 0 (7), +1 (7.75), +2 (8.5); $X_3$: nitrogen source, -2 (28 g $L^{-1}$), -1 (32 g $L^{-1}$), 0 (36 g $L^{-1}$), +1 (40 g $L^{-1}$), +2 (44 g $L^{-1}$).

TABLE-3
ANALYSIS OF VARIANCE (ANOVA) FOR THE SELECTED QUADRATIC MODEL

| Source | DF | SS | MS | F | Pr > F |
|---|---|---|---|---|---|
| $X_1$ | 1 | 28167456 | 28167456 | 25.14104 | 0.000526 |
| $X_2$ | 1 | 25780727 | 25780727 | 23.01075 | 0.000727 |
| $X_7$ | 1 | 255806.3 | 255806.3 | 0.228322 | 0.643044 |
| $X_1 \times X_1$ | 1 | 7553841 | 7553841 | 6.742229 | 0.026647 |
| $X_1 \times X_2$ | 1 | 312907.7 | 312907.7 | 0.279288 | 0.608696 |
| $X_1 \times X_7$ | 1 | 668718.4 | 668718.4 | 0.596869 | 0.457646 |
| $X_2 \times X_2$ | 1 | 2704860 | 2704860 | 2.414241 | 0.151287 |
| $X_2 \times X_7$ | 1 | 291721.2 | 291721.2 | 0.260378 | 0.620934 |
| $X_7 \times X_7$ | 1 | 158641.8 | 158641.8 | 0.141597 | 0.714559 |
| Model | 9 | 65689206 | 7298801 | 6.514591 | 0.003576 |
| Error | 10 | 11203773 | 1120377 | – | – |
| Total | 19 | 76892980 | – | – | – |
| $R^2$ = 85.43 % | | Adj-$R^2$ = 72.32 % | | | |

SS: sum of square; DF: degree of freedom; MS: mean square.

The quality of fit of the second-order model equation was expressed by the coefficient $R^2$ and its statistical significance was determined by an F-test. The significance of the effect of each variable on catalase production was measured using a t-test.

## RESULTS AND DISCUSSION

**Optimized inducer for catalase production:** Glucose (22 g $L^{-1}$) was substituted with L-malic acid, citric acid, L-glutaminic acid, succinic acid, respectively having equimolar carbon concentration for testing their catalase production. The result is shown in Fig. 1. Catalase production of citric acid was the highest compare to that of other inducers.

**Screening the important influent factors on catalase production:** Fixed citric aid as the suitable inducer, Plackett-Burman design was used to screening those important influent factors on catalase production. The experimental design and result are shown in Table-1. *p* value less than 0.05 indicated that the factor was significant effect on catalase production. So temperature, initial pH and corn steep liquor powder were chosen for the next study (Table-1).

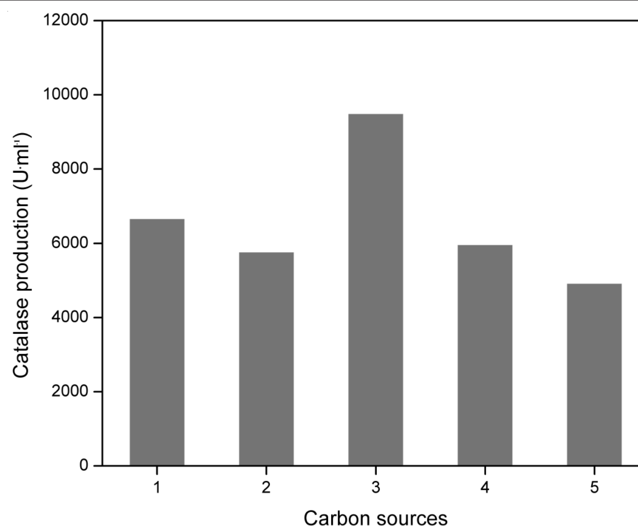


Fig. 1. Effect of various inducers on catalase production. 1, L-malic acid; 2, succinic acid; 3, citric acid; 4, L-glutaminic acid; 5, glucose

**Optimization of screened variables using central composite design:** Base on Plackett-Burman experiment, those values of three significant factors were determined for obtaining the highest catalase production by response surface methodology (RSM) using central composite design (CCD). The experiment design and results are displayed in Table-2. The relation between catalase production and three significant factors can been presented in a following regression equations:

$$Y_1 = 10023.59-1326.826 * X_1 - 1269.368 * X_2 + 126.4432 * X_7 - 548.1212 * X_1 * X_1 - 197.7713 * X_1 * X_2 + 289.119 * X_1 * X_7 - 327.9934 * X_2 * X_2 - 190.9585 * X_2 * X_7 + + 79.43311 * X_7 * X_7$$

where $Y_1$ is catalase production (U $mL^{-1}$), $X_1$, $X_2$, $X_7$ are tempreture, intial pH, corn steep liquor powder concentration, respectively.

The statistical significance of regression equations was checked by F-test and the analysis of variance (ANOVA) for response surface quadratic model was summarized in Table-3. The analysis of variance of the quadratic regression model indicated that the model was highly significant, as was supported by model F-value with a very low *p* value [($P_{model}$ > F) = 0.000526]. Coefficient correlation was $R^2$ (85.43 %), as indicated that only 14.57 % of the catalase production could not be explained by the model and 85.43 % catalase production could be contributed to those independent variables. This suggested the model could well predict catalase production.

Values of 'Prob > F" less than 0.0500 indicate model terms are significant. $X_1$, $X_2$ and the quadratic terms ($X_1 * X_1$) have significant effect on catalase production. $X_7$, the interaction term ($X_1 * X_2$, $X_2 * X_3$ and $X_1 * X_3$) and the quadratic terms ($X_2 * X_2$ and $X_7 * X_7$) have not significant effect on catalase production.

The fitted response for the above regression model was plotted in Figs. 2-4. Three-dimensional graphs were generated

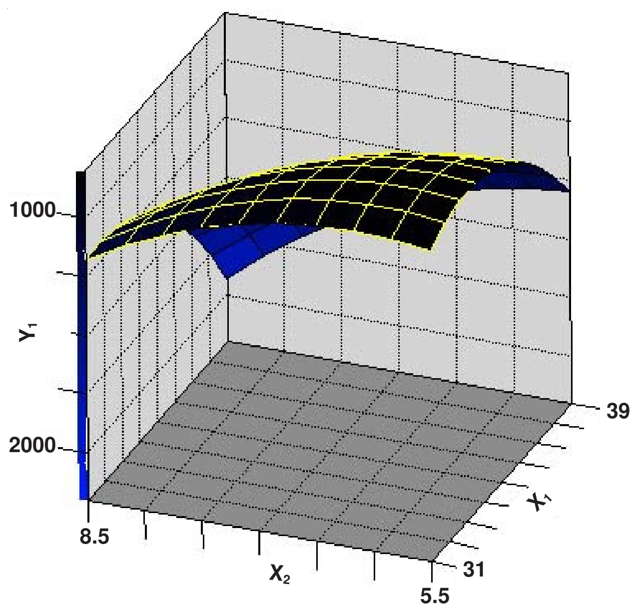


Fig. 2. Effect of temperature ($X_1$) and intial pH ($X_2$) on catalase production ($Y_1$) (supplemented with corn steep liquor powder of 33.8 g $L^{-1}$)

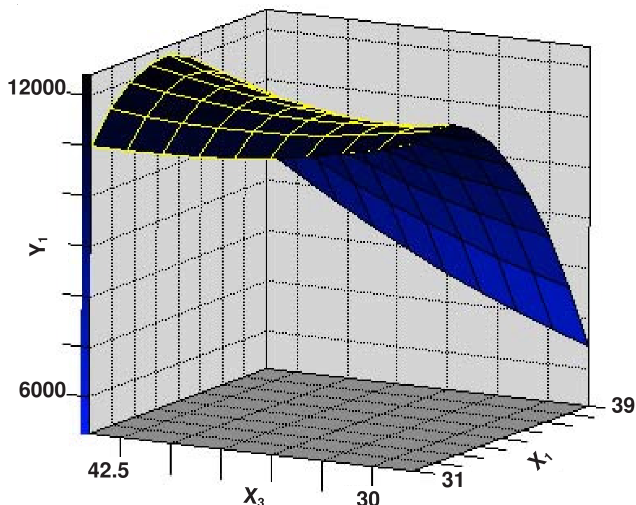


Fig. 3. Effect of temperature ($X_1$) and corn steep liquor powder ($X_3$) on catalase production ($Y_1$) (supplemented with intial pH of 5.91)

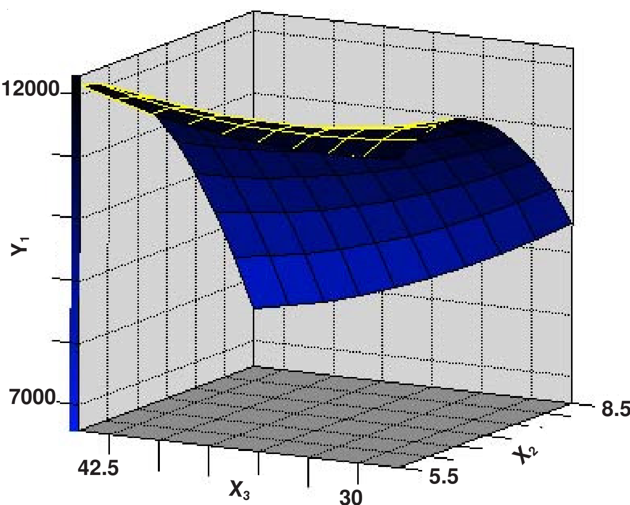


Fig. 4. Effect of intial pH ($X_2$) and corn steep liquor powder ($X_3$) on catalase production ($Y_1$) (supplemented with temperature of 32.8 ºC)

for the pair-wise combination of the three factors, while keeping the other one at their optimum levels for catalase production.

The three dimensional graph can intuitively understand the effect of each factor on catalase production. Temperature and initial pH is very important factors on effect microorganism growth. We found that high temperature and initial pH sharply decreased catalase production (Figs. 2-4). It is very similar in the report of Caridis *et al.*[9]. Corn steep liquor power was usually chosen as a industry nitrogen source for producing enzyme. High concentration of corn steep liquor reduced the catalase production, as displayed in Figs. 2 and 3. It was also found in the study of Caridis *et al.*[9].

For obtaining the highest predicted catalase production, one-order partial derivatives of nonlinear model of each significant factor were calculated using mathematical software. The optimum temperature, initial pH and corn steep liquor powder was 32.8 g $L^{-1}$, 5.9, 33.8 g $L^{-1}$, respectively. The maximum predicted catalase production was 11632.88 U $mL^{-1}$.

**Verification of the optimum condition:** Time course of fermentation for production of catalase was performed in the optimum condition and the result is present in Fig. 5. The highest catalase production (12182.74 U $mL^{-1}$) closely matches the predicted value and was only 4.72 % more than the predicted value. Finally, 28.27 % enhancement in catalase production was achieved in optimized conditions as compared to that under unoptimized conditions. Many reported[2-5,8,9] production of catalase by microorganism did not exceeded 3000 U $mL^{-1}$. Although the level of catalase production in the study was lower than that of *Rhizobium radiobacter* Strain 2-1[20], it was still rather high. Therefore *Serratia marcescens* SYBC08 sounded good potential in the production of catalase.

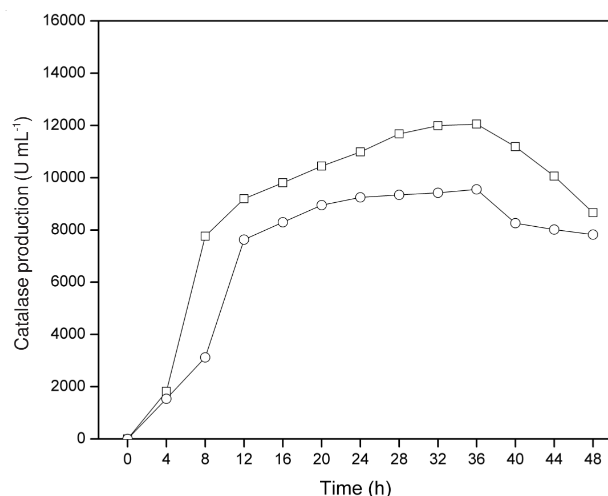


Fig. 5. Time courses of the fermentation process by *Serratia marcescens* SYBC08 cultivated in culture conditions. Under optimized conditions: (□); underunoptimized conditions: (○)

## Conclusion

For enchancing catalase production, citric aicd was chosen as a suitable inducer. The fermentation conditions and medium was optimized by Plackett-Burman and RSM designs. When *Serratia marcescens* SYBC08 was cultured in optimum conditions with temperature (32.81 ºC), initial pH (5.91) and corn steep liquor powder (33.8 g $L^{-1}$), the highest catalase production

(12182.74 U $mL^{-1}$) was 28.27 % increase as compared to that under unoptimized conditions.

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## REFERENCES

1. I.I. Yumoto, H. Iwata, T. Sawabe, K. Ueno, N. Ichise, H. Matsuyama, H. Okuyama and K. Kawasaki, *Appl. Environ. Microbiol.*, **65**, 67 (1999).
2. X. Shi, M. Feng, Y. Zhao, X. Guo and P. Zhou, *Biotechnol. Lett.*, **30**, 181 (2008).
3. G.L. Yan, Z.Z. Hua, L. Yin, D.R. Liu and J. Chen, *Biotechnol. Lett.*, **27**, 683 (2005).
4. Z. Hua, G. Yan, G. Du and J. Chen, *Biotechnol. J.*, **2**, 326 (2007).
5. H.S. Ko, H. Fujiwara, Y. Yokoyama, N. Ohno, S. Amachi, H. Shinoyama and T. Fujii, *J. Biosci. Bioeng.*, **99**, 290 (2005).
6. P.C. Loewen, J. Switala and B.L. Triggs-Raine, *Arch. Biochem. Biophys.*, **243**, 144 (1985).
7. T.L. Trivett and E.A. Meyer, *J. Bacteriol.*, **107**, 770 (1971).
8. A. Gromada and J. Fiedurek, *J. Basic. Microbiol.*, **37**, 85 (1997).
9. K.-A. Caridis, P. Christakopoulos and B.J. Macris, *Appl. Microbiol. Biotechnol.*, **34**, 794 (1991).
10. A. Masumeh and K. Gholam, *Asian J. Chem.*, **21**, 2131 (2009).
11. M.R. Swain and R.C. Ray, *J. Basic Microbiol.*, **47**, 417 (2007).
12. M.J. Richards, J.R. Edwards, D.H. Culver and R.P. Gaynes, *Infect. Control. Hosp. Epidemiol.*, **21**, 510 (2000).
13. K.L. Patterson, J.W. Porter, K.B. Ritchie, S.W. Polson, E. Mueller, E.C. Peters, D.L. Santavy and G.W. Smith, *Proc. Natl. Acad. Sci. (USA)*, **99**, 8725 (2002).
14. S.A. Adamo, *J. Insect. Physiol.*, **50**, 209 (2004).
15. A.A. Khardenavis, A. Kapley and H.J. Purohit, *Waste. Manag.*, **29**, 1409 (2009).
16. F.J. Romero, L.A. Garcia, J.A. Salas, M. Diazand and L.M. Quiros, *Proc. Biochem.*, **36**, 507 (2001).
17. J.H. Fu, H.Q. Huang, K. Meng, T.Z. Yuan, B. Yao, Y.H. Shi and P.K. Ouyang, *Enzyme Microb. Technol.*, **42**, 187 (2008).
18. R.F. Beers Jr. and I.W. Sizer, *J. Biol. Chem.*, **195**, 133 (1952).
19. M.T. Nakayama, Nakajima-Kambe, H. Katayama, K. Higuchi, Y. Kawasaki and R. Fuji, *J. Biosci. Bioeng.*, **106**, 554 (2008).

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