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# Optimization of no-cook technology at very high gravity for rice-based ethanol production

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**Abstract.** The application of raw-starch hydrolysis enzymes to ethanol production by no-cook technology at very high gravity (VHG) (311.5 g/L) from rice would reduce the energy consumption compared to traditional process. In this study, the Taguchi design and the desirability function were used to identify the optimized production process for economic and technological targets. Three factors: dosage of alpha- and gluco-amylase enzyme (Stargen 002 – A), dosage of protease enzyme (Fermgen – B) and dosage of yeast (C) were investigated. With the expected Desirability = 0.74, the no-cook process from rice would be optimized with following dosage: Stargen 002, Fermgen and yeast at 700 µL/L (1140 GAU/kg raw material), 151 µL/L (431 SAPU/kg raw material) and 0.25 g/L (6.25 × 106 cells/mL), respectively. After 120 h of fermentation, ethanol concentration reached 17.45 ± 0.07 % v/v, corresponding to ethanol yield of 86.08 ± 0.2 %, and the cost of raw material was at 3225.5 VND/L of rice slurry.

*Keywords*: no cook technology, very high gravity, taguchi design, multi-response optimization, design-expert

*Classification numbers:* 1.3.1

## 1. INTRODUCTION

For long time, rice has been used as material for alcoholic beverage production in Viet Nam. Most Vietnamese distilleries used Simultaneous Saccharification and Fermentation (SSF) process including liquefaction step at 95 - 105 °C and SSF step in a single reactor at 30 - 32 °C for the production of potable ethanol, that requires a high-energy input and equipment investment cost.

No-cook process (also known as Simultaneous Liquefaction, Saccharification and Fermentation (SLSF)) for ethanol production from starchy material is a technology using blend gluco-amylase and alpha-amylase enzyme to hydrolyze starch at low temperature. This no-cook technology has been previously studied for different starchy substrates (corn, cassava, broken rice, etc...) at normal gravity [1 - 3]. Moreover, regarding the demand of increasing fermentation

productivity and reducing consumption energy, no-cook process is combined with very high gravity (VHG) technology. This application could lead to a new way of saving energy and equipment cost for ethanol production. In Viet Nam, SLSF-VHG process has been applied for broken rice at pilot scale [4]. After 120 h of fermentation, the theoretical ethanol yield of 86.3 % and 83.2 % was achieved at lab scale and pilot scale (25 L), respectively. However, in that study, enzyme and yeast dosages were carried out as recommendation of the provider, and have not been optimized.

In this study, our approach is to optimize some parameters (enzyme and yeast dosage) of no-cook technology from rice to achieve higher economical efficiency process for ethanol production.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Broken rice is of OM variety from Southern of Viet Nam. Broken rice was grinded into flour (< 0.3 mm). Starch and protein content of broken rice flour were at 80.4 ± 0.5 % and 7.5 ± 0.2 %, respectively.

Commercial dry yeast Ethanol Red (*Saccharomyces cerevisiae*) was kindly provided by Fermentis (France). The average cell number of live cells was at $2.5 \times 10^{10}$/gram.

Different kinds of commercial enzymes were used in this work, including: Stargen 002 (alpha-amylase and gluco-amylase,optimal pH: 4.0 - 4.5, optimal temperature: 20 - 40 $^{o}$C, activity: 570 GAU/g, provided by Dupont, USA), Fermgen (protease, optimal pH: 4.0 - 5.0, optimal temperature: 28 - 35 $^{o}$C, activity:1,000 SAPU/g, provided by Dupont, USA), Amigase Mega L(gluco-amylase, optimal pH: 4.0 - 4.5, optimal temperature: 55 - 60 $^{o}$C, activity: 36,000 AGI/g, provided by DSM Food, Netherland).

$KH_2PO_4$ (China) was used as the phosphate nutrient for yeast.

The price of raw materials used in the study is described in the Table 1.

*Table 1*. Price of materials used in the study.

| **Material** | Broken rice | Ethanol Red | Stargen 002 | Fermgen | Amigase Mega L | $KH_2PO_4$ |
|---|---|---|---|---|---|---|
| **Price*** | 8,000 VND/kg | 275,000 VND/kg | 350,000 VND/L | 400,000 VND/L | 180,000 VND/L | 55,000 VND/kg |

* The price is applicable at the time of study

### 2.2. Methods

#### *2.2.1. No-cook at very high gravity technology from rice*

In this study, we adapted the SLSF-VHG process from Chu-Ky *et al.* [4] with some modifications (Figure 1): Broken rice flour was mixed with tap water to achieve a concentration of 311.5 g/L dry solid in a final volume of 1 L. The pH was adjusted to 4.5 using $H_2SO_4$. For all experiments, three commercial enzymes (Stargen 002, Fermgen and Amigase Mega L) were

added simultaneously to the slurry with $KH_2PO_4$ and yeast. Active dry yeast was hydrated in tap water at 35 $^oC$ for 15 min before adding to the slurry. The concentration of Amigase Mega L and $KH_2PO_4$ was at 0.035 % w/w and 0.59 g/L, respectively.

The SLSF-VHG processes from broken rice were carried out at 311.5 g/L in for 120 h.

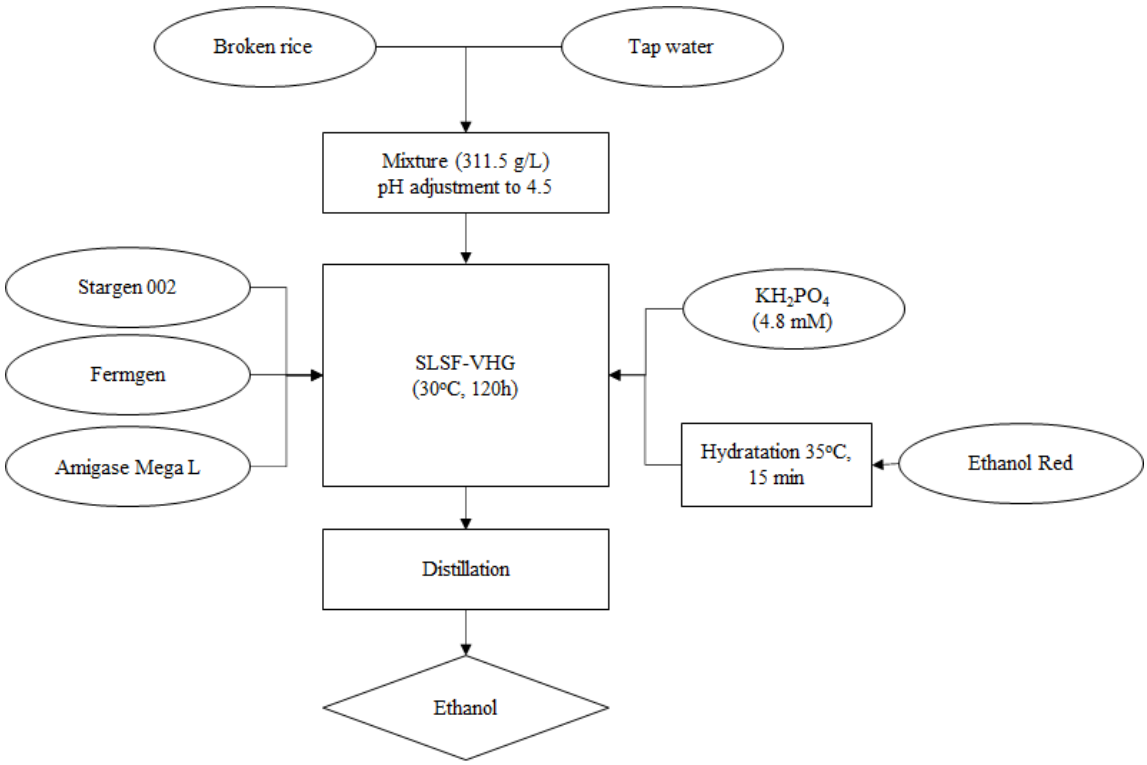


*Figure 1*. SLSF-VHG process from rice for potable ethanol production.

*2.2.2. Design of experiments with Taguchi*

In this study, Taguchi design was used to determine the optimum setting of the SLSF-VHG process. Three levels array was selected for optimization of three parameters: dosage of Stargen 002 (A), dosage of Fermgen (B) and dosage of yeast (C) (Table 2). Hence, Taguchi L9 orthogonal array, with 9 experiments, was applied [5]. The layout of Taguchi L9 orthogonal array was shown in Table 3.

Design-Expert 7.1 (Stat-Ease Inc.) using response surface method (RSM) was used to design the experiments and analyze the experimental data to establish the most influential parameter for the desired outputs (ethanol yield -$Y_1$ and raw material cost – $Y_2$) and to recognize the best operating conditions to achieve the optimal value of individual anticipated output.

To verify the ”lack of fit” of the model, two experiments at the center of the model were carried out with the dosage of Stargen 002 (A), Fermgen (B), and Yeast (C) were at 525 µL, 175 µL, 0.50 g, respectively.

The model in term of code factors for the desired output is given below:

$$Y_i = b_o + b_1A + b_2B + b_3C + + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$$

where A, B, C are the coded forms of dosage of Stargen 002 (A), dosage of Fermgen (B) and dosage of yeast. AB, AC, BC are the interaction terms, and $A^2$, $B^2$, $C^2$ are the squared terms of the independent variables.

*Table 2.* Process parameters and their levels using Taguchi L9 Orthogonal Array.

| Parameters | Levels | | | |
|---|---|---|---|---|
| | Code | -1 | 0 | +1 |
| Stargen 002 (µL) | A | 350 | 525 | 700 |
| Fermgen (µL) | B | 105 | 175 | 245 |
| Yeast (g) | C | 0.25 | 0.5 | 0.75 |

*Table 3.* Layout of Taguchi L9 Orthogonal Array design.

| **Exp No.** | **Taguchi L9 Orthogonal Array** | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | Stargen 002 (A) (µL) | Fermgen (B) (µL) | Yeast (C) (g) |
| 1 | -1 | -1 | -1 | 350 | 105 | 0.25 |
| 2 | -1 | 0 | 0 | 350 | 175 | 0.50 |
| 3 | -1 | 1 | 1 | 350 | 245 | 0.75 |
| 4 | 0 | -1 | 0 | 525 | 105 | 0.50 |
| 5 | 0 | 0 | 1 | 525 | 175 | 0.75 |
| 6 | 0 | 1 | -1 | 525 | 245 | 0.25 |
| 7 | 1 | -1 | 1 | 700 | 105 | 0.75 |
| 8 | 1 | 0 | -1 | 700 | 175 | 0.25 |
| 9 | 1 | 1 | 0 | 700 | 245 | 0.50 |

*2.2.3. Analytical procedures*

To measure reducing sugar, fermentation beer was filtrated, then reducing sugar was determined by using DNS (3,5-dinitro salicylic acid) method [6]. Residual sugar was measured by the same method after acid hydrolysis (HCl 2 % for 120 min at 100 °C) of fermentation beer [7]. Ethanol was distilled from fermentation beer, and then ethanol concentration was determined using Portable Density/Specific Gravity Meter DA-130N (Kyoto Electronics Manufacturing, Japan). Ethanol yield was calculated as the ratio of actual ethanol concentration with the theoretical ethanol concentration [8].

## 3. RESULTS AND DISCUSSION

### 3.1. Exprimental results of Taguchi L9 Orthogonal Array

Ethanol concentration and yield from Taguchi L9 Orthogonal Array experiments were presented in Table 4. The lowest ethanol yield (78.01 %) was observed when the dosage of enzymes and yeast were at lowest level (experiment No. 1), while the highest ethanol yield was at 86.89 % (experiment No. 7) when the dosage of Stargen 002 and yeast were at highest level and the dosage of Fermgen was at lowest level. In previous study with SLSF-VHG process from

rice [4], the ethanol yield achieved was at 86.3 % at lab scale when using enzymes at recommended dosage of provider. Interestingly, this yield was similar to the highest ethanol yield in this work. Stargen 002, a mixture of alpha-amylase and gluco-amylase, is the key enzyme for the SLSF-VHG technology, with the ability to hydrolyze starch at room temperature. Besides, the use of Fermgen as protease could provide more amino acids for the growth of yeast. To explore the influence of each factors (Stargen 002, Fermgen and Yeast) to the ethanol yield of this process, the obtained experimental yields were analyzed to produce a suitable regression model using Design-Expert 7.1 software.

*Table 4.* Experimental data of Taguchi L9 Orthogonal Array.

| **Exp No.** | **Taguchi L9 Orthogonal Array** | | | **Results** | | | |
|---|---|---|---|---|---|---|---|
| | Stargen 002 (A) (μL) | Fermgen (B) (μL) | Yeast (C) (g) | Reducing sugar at 120 h (g/L) | Residual sugar at 120h (g/L) | Ethanol concentration (% v/v) | Ethanol yield (%) |
| 1 | 350 | 105 | 0.25 | 7.4 | 38.2 | 15.8 | 78.01 |
| 2 | 350 | 175 | 0.50 | 6.5 | 29.1 | 16.5 | 81.46 |
| 3 | 350 | 245 | 0.75 | 5.2 | 23.6 | 16.9 | 83.44 |
| 4 | 525 | 105 | 0.50 | 6.1 | 31.2 | 16.3 | 80.48 |
| 5 | 525 | 175 | 0.75 | 2.5 | 16.4 | 17.4 | 85.91 |
| 6 | 525 | 245 | 0.25 | 4.6 | 27.1 | 16.7 | 82.45 |
| 7 | 700 | 105 | 0.75 | 3.1 | 14.3 | 17.6 | 86.89 |
| 8 | 700 | 175 | 0.25 | 3.1 | 16.8 | 17.4 | 85.91 |
| 9 | 700 | 245 | 0.50 | 3.8 | 24.5 | 16.8 | 82.94 |

### 3.2. RSM statistical analysis

*Table 5.* The results of regression of ethanol yield.

| Source | Variance | F-value | p-value (Possibility > F) |
|---|---|---|---|
| Model | 11.02 | 210.31 | 0.0001 |
| A | 27.43 | 523.38 | 0.0001 |
| B | 1.98 | 37.84 | 0.0035 |
| C | 3.99 | 76.28 | 0.0009 |
| AB | 2.80 | 53.44 | 0.0019 |
| $B^2$ | 10.63 | 202.78 | 0.0001 |
| $C^2$ | 5.20 | 99.20 | 0.0006 |
| Lack of fit | 0.07 | 154.98 | 0.0590 |

To verify the “Lack of fit” of the model, two experiments at the center were performed. As results, the ethanol yield was at 83.38 and 83.41 %. The results of regression of ethanol yield were studied and the experimental design and results were analyzed by selecting the appropriate model either linear, quadratic, and so forth (Table 5). The F-value of the model was found at

210.31 (p = 0.0001), indicated that the generated models can statistically significantly representing the observed experiment data. The high $R^2$ value at 0.9968, and the high adjusted $R^2$ value, *i.e.*, at 0.9921, shows the model is adequate to predict ethanol yield in different conditions. The "Lack of fit" value was at 154.98 (p = 0.059), meaning that the model is totally compatible with the experiments.

The best fit models in terms of coded factors (1) and actual factors (2) for the ethanol yield obtained are given below:

$$Y = 83.38 + 2.14A + 0.58B + 1.07C - 1.15AB - 2.06\,B^2 + 1.57C^2 \quad (1)$$

$$Y = 58.153 + 0.029A + 0.205B - 20.777C - 9.401E\text{-}5*AB - 4.201E\text{-}4*B^2 + 25.053C^2 \quad (2)$$

where A, B, C and D are the coded forms of Stargen 002, Fermgen and yeast dosage, respectively, AB, AC, AD, BC, BD and CD are the interaction terms, and $A^2$, $B^2$, $C^2$ and $D^2$ are the squared terms of the independent variables. In the above equation, positive coefficients have positive effect to ethanol yield, whereas negative coefficients will decrease the ethanol yield. As shown in Table 5, it can be found that the variable with the most significant effect on the ethanol yield of SLSF-VHG process was the dosage of Stargen 002, A (F value: 262.42), followed by quadratic term of Fermgen, $B^2$ (F value: 81.05) and yeast, C (F value: 37.49).

Regarding the affect of each factor to the ethanol yield (Figure 2), the ethanol yield was most influenced by the dosage of Stargen 002 (A) and Yeast (C). The higher those dosages, the higher ethanol yield was achieved. In contrary, the higher dosage of Fermgen, the lower ethanol yield was obtained.

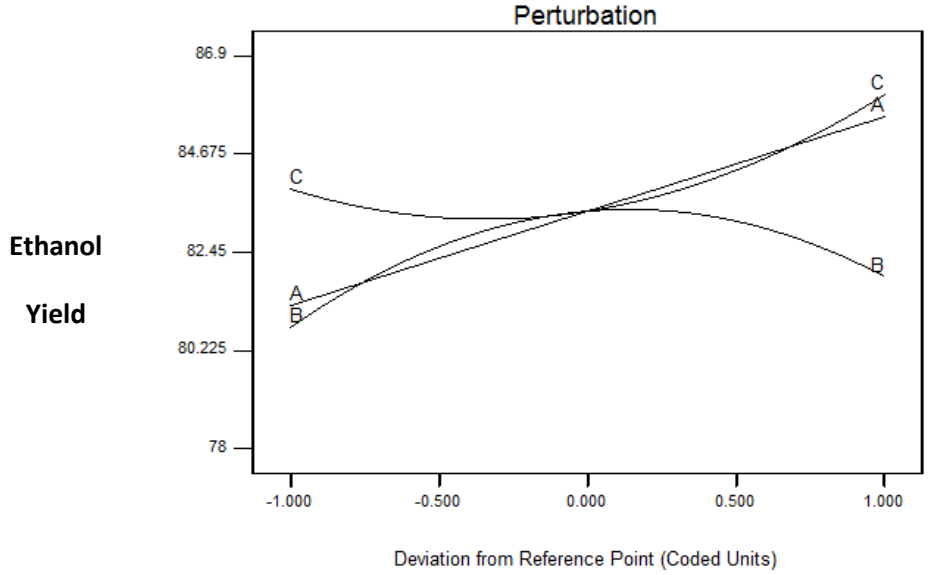


*Figure 2.* Influence of factors to ethanol yield.

RSM is also applied to understand the interaction relationship between the test variables affecting the selected process response. Figure 3 shows the 3D response surface plots are important tools in interpreting the interaction and correlation of two different independent variables or factors on ethanol yield while keeping the other factors at a constant value ofarbitrary zero. Figure 3a demonstrates that ethanol yield increased with the increasing in Stargen 002 dosage from 350 to 700 µL, and the Fermgen dosage from 105 to 175 µL. The highest ehanol yield (85.6 %) was obtained when Stargen 002 dosage at 700 µL and the

Fermgen dosage at 175 µL. Figure 3b illustrates that the highest ehanol yield (88.2 %) was obtained when Stargen 002 dosage at 700 µL and yeast dosage at 0.75 g/L. As the amount of Stargen 002 increases, more consumable sugars were produced, thus the yeast could accelerate the ethanol production rate. Moreover, the production of amino acids thanks to the activity of protease (Fermgen) could help the growth of yeast, leads to an increase of ethanol. Besides, a high amount of yeast supplied from the beginning of the fermentation could reduce the risk of contamination. The present study shows that an increase of Stargen 002 and yeast dosage have positive effect on the improvement of ethanol yield.

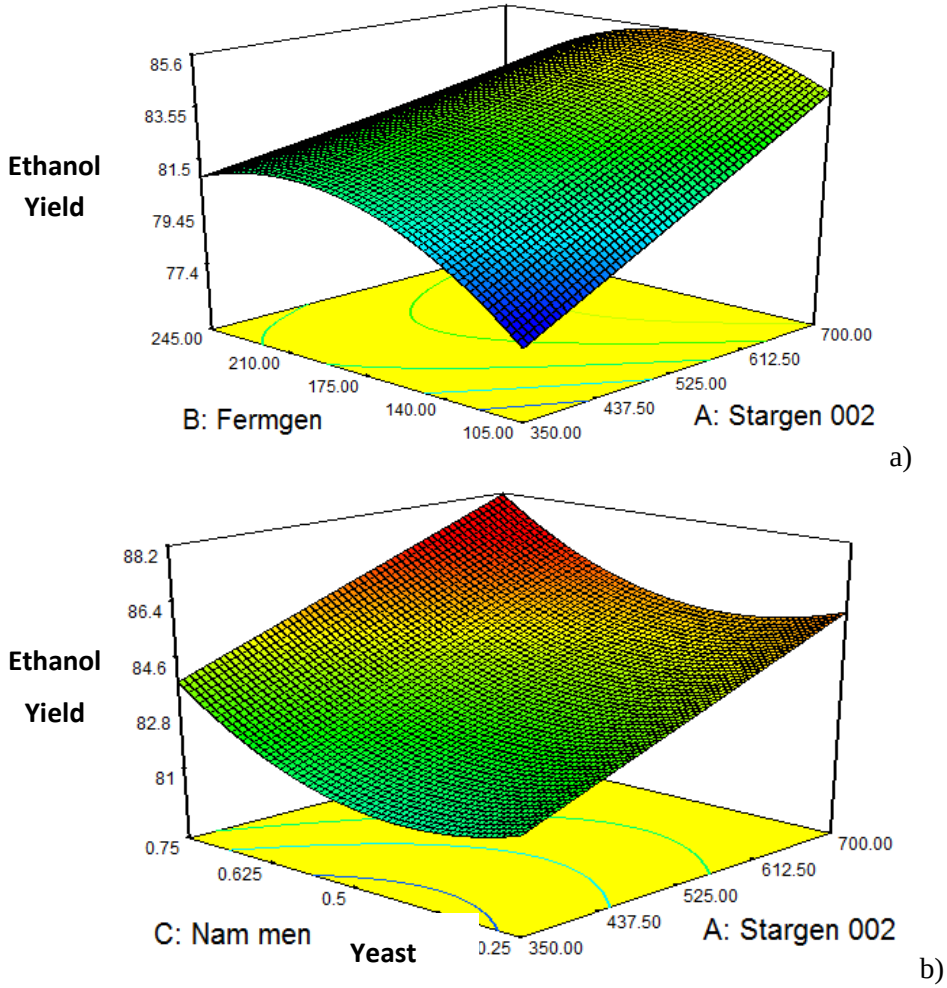


*Figure 3.* 3D response surface of ethanol yield.

### 3.3. Optimization of the process

The desirability function was applied to introduce an effective method to obtain optimum process condition. The ethanol yield ($Y_1$) and raw material cost ($Y_2$) were presented below:

$$Y_1 = 83.38 + 2.14A + 0.58B + 1.07C - 1.15AB - 2.06B^2 + 1.57C^2$$

$$Y_2 = 3242.60 + 61.25A + 28.00B + 68.75C.$$

In this study, we would like to obtain a high ethanol yield with low raw material cost. Figure 4 showed the optimal condition of the model. When the Desirability = 0.74, the optimum SLSF-VHG process will be performed with Stargen 002, Fermgen and Yeast dosage at 700 µL/L (1140 GAU/kg raw material), 151 µL/L (431 SAPU/kg raw material) and 0.25 g/L (6.25 × $10^6$ cells/mL), respectively. Under these conditions, the ethanol yield will achieve 85.98 % and the raw material cost is at 3225.5 VND/L of rice slurry.

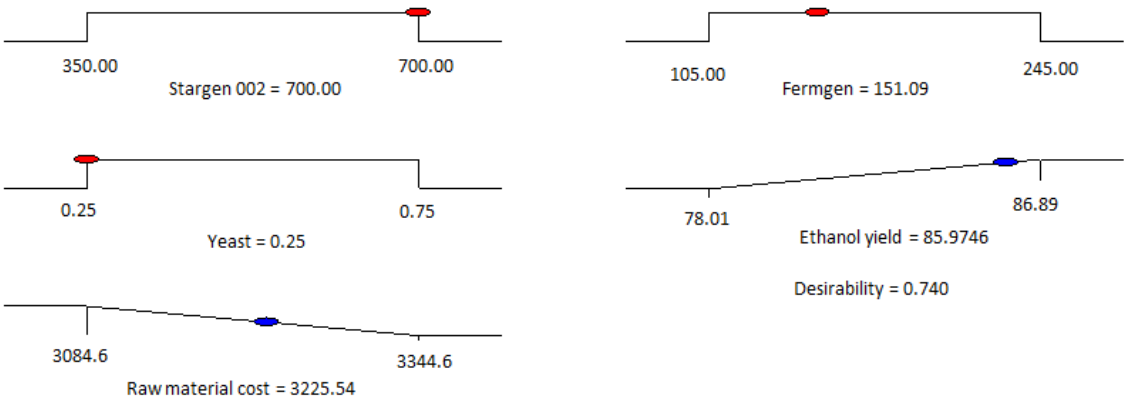


*Figure 4.* The optimal condition of the model.

To verify the accuracy of the optimization, SLSF-VHG process was carried out with three independent replicates. The results showed that the SLSF-VHG process is in perfect agreement with the value of running the optimization model (Table 6).

*Table 6.* Verification of optimal condition.

| No | Stargen (µL) | Fermgen (µL) | Yeast (g/L) | Ethanol yield (%) | Raw material cost (VND) |
|---|---|---|---|---|---|
| 1 | 700 | 151 | 0.25 | 86.25 | 3225.5 |
| 2 | 700 | 151 | 0.25 | 86.13 | 3225.5 |
| 3 | 700 | 151 | 0.25 | 85.85 | 3225.5 |
| Average | | | | 86.08 ± 0.21 | |
| Expected | | | | 85.98 | |
| t | | | | 0.83 | |
| Critical value $t_{b,\alpha = 5\ \%,\ f = 2} = 4.303$ | | | | | |

In a study focusing on the production cost of alcohol from traditional fermentation method [9], the fermentation time was long (11 days) and the ethanol yield obtained was remained low (72.99 %). The net production cost was at 22,484 VND/L of ethanol 40°. In another previous study [4], the ethanol yield was at 86.4 % with raw material cost of 3511.55 VND/L of rice slurry, corresponding to the net cost of 20,066 VND/L of ethanol 96°. After the optimization, the ethanol yield and raw material cost were at 86.08 % and 3225.5 VND/L of rice slurry,

corresponding to the net cost of 18,639 VND/L of ethanol 96$^{o}$. Thus, compared to the previous study using SLSF technology [4], the optimal process reduces 7.6 % of raw material cost, corresponding to a reduction of the net cost at 1527 VND/L of ethanol 96$^{o}$. Moreover, compared to the traditional fermentation method, we observed a huge reduction of production cost of 3845 VND/L.

## 4. CONCLUSION

At lab scale, using Taguchi design and the desirability function, the optimal conditions for SLSF-VHG process from broken rice was obtained: Stargen 002, Fermgen and Yeast dosage at 1140 GAU/kg raw material, 431 SAPU/kg raw material and $6.25 \times 10^6$ cells/mL, respectively, in which the ethanol yield achieved 86.08 %. The raw material cost was decreased by 7.6 %. This no-cook process is attractive enough to apply at pilot scale before introducing to potable ethanol industry in Viet Nam.

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***CRediT authorship contribution statement.*** Phuong Vu Thi: Methodology, Investigation, Writing-original draft; Chinh-Nghia Nguyen: Writing – original draft, Writing – review and editing; Son Vu Hong: Formal analysis, Writing – review and editing. Son Chu-Ky: Supervision, Funding acquisition, Project administration, review.

***Declaration of competing interest.*** We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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