Production of Fumaric acid from Oil Palm Empty Fruit Bunch

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Abstract

Oil palm empty fruit bunch (EFB)**,** one of the most abundant agricultural residues in Thailand**,** is an attractive lignocellulosic material for value added agricultural products. They could be used as a raw material for cheap renewable feedstock to produce acids and many other value-added products. This research is to investigate the potential of EFB as a raw material for fumaric acid production through separate hydrolysis and fermentation (SHF) by the selected fungal isolate K20. Steam explosion was used in this study to pretreat the lignocellulosic material. A Cellic CTec2 enzyme (25 FPU/ml) was used to hydrolyze the pretreated material into glucose. Response surface methodology (RSM) based on central composite design (CCD) was applied to optimize the effect of the medium composition including solid loading, $(NH_4)_2SO_4$ and Na_2CO_3 on fumaric acid concentration. The optimized medium consisted of (g/L): solid loading 166.70, $(NH_4)_2SO_4$ 0.29 and Na_2CO_3 3.07. The maximum predicted value of fumaric acid concentration of 5.18 g/L was obtained, which corresponded with the observed values of 5.30 g/L.

Keywords : *Fumaric acid, oil palm empty fruit bunch, isolation and screening, separate hydrolysis and fermentation (SHF).*

1. Introduction

Thailand is a potential country for oil palm production. Therefore, oil palm planting area has been increased within a few years. For growing of oil palm, oil palm empty fruit bunch must be removed about twice per month. Also, there are many oil palm empty fruit bunch residues and they are an attractive raw material fumaric acid production due to their annual amount and chemical compositions. They contain cellulose, hemicellulose and lignin which slightly different to other lignocellulose residues. Pretreatment is an important tool for practical cellulose conversion process. It is required to alter or remove the structure of lignocellulosics materials and compositional impediments to hydrolysis in order to improve the rate of enzyme

hydrolysis and increase yields of fermentable sugars from cellulose (1).

In this work, oil palm empty fruit bunch was targeted for production of fumaric acid through fermentation. Fumaric acid is a four-carbon unsaturated dicarboxylic acid which has many potential industrial applications, ranging from the manufacture of synthetic resins and biodegradable polymers to the production of intermediates for chemical synthesis (2). Currently, fumaric acid is produced in two ways including chemical conversion from maleic anhydride and biological conversion by fungi (3). However, as petroleum is becoming scarcer, it has attracted increasing attentions on producing fumaric acid by fermentation (4)(5) pointed out that the high cost of raw materials is one of the greatest obstacles restraining the industrialization of fumaric acid fermentation. According to former studies, most of the commonly used feedstocks for fumaric acid production, such as purified sugars, starchy materials and cellulosic material, encounter various issues that impeding them from being an ideal raw material. Purified sugars are expensive for industrial utilization, and using sugars and starchy materials as feedstocks can directly compete with humans for food and break the equilibrium of supply and demand of grain. Furthermore, cellulosic material could not be directly used for fermentation. It needs to be processed by acid or cellulase in order to release fermentable sugars (6). The pretreatment processes are complicated and costly. Hence, searching for economic carbon source is crucial. This work deals with the fermentative production of fumaric acid from oil palm empty fruit bunches matherials as substrates using the selected strain.

2. Materials and Methods

2.1 Substrate

Oil palm empty fruit bunch collected from Suksomboon Palm Oil Industry Chonburi Province, Thailand, sun-dried and ground to a particle size smaller than 1 inch.

2.2 Pre-treatment of Substrate

2.2.1 Steam explosion pretreatment

Two hundred gram of oil palm empty fruit bunches were steam-exploded at 210 °C for 4 min.

2.2.2 Water extraction pretreatment

After the steam exploded fibers were extracted with 80 ˚C water for 30 min. The fibers remaining were filtered and washed by tap water until colorless.

2.2.3 Alkali extraction pretreatment

After the water extracted fibers were soaked with 15% (w/v) sodium hydroxide at 90˚C for 60 min. The fibers remaining were filtered and washed by tap water until colorless.

2.3 Enzymatic hydrolysis

Fibers were soaked in citrate buffer solution (pH 4.8) and added with Cellic CTec2 enzyme (25 FPU/mL) at 50˚C, 150 rpm for 24 h. The fibers hydrolysate obtained was used for fermentation.

2.4 Microorganism and culture conditions

2.4.1 Isolation of Fungi

Fungal isolates were made from soil samples by serial dilution agar on Potato dextrose agar plates.

2.4.2 Screening for Acid Production

Selected strains for screening of acid production by point inoculation on

acid medium and incubated for five days for the formation of yellow zone around the mycelial growth.

Selected fungal for fumaric acid production

Formation of yellow zone were selected for fumaric acid production, inoculum was added to production medium and incubated at 30 ˚C, 200 rpm for 96 h. fumaric acid determination using HPLC.

2.5 Fermentation medium

2.5.1 Inoculum preparation

The spore suspension of *Rhizopus oryzae* K20 used as inoculum was obtained from 7th day grown culture on PDA slant at room temperature. The spores were suspended with sterile distilled water. Number of spores was counted microscopically on haemacytometer. The spore inoculum concentration of 10^7 spores/mL, the spore suspension was inoculated into a 100 mL of growth medium at 30 ˚C, 200 rpm for 24 h, were used as inoculums.

2.5.2 Fermentation for fumaric acid production

The inoculums was inoculated into production medium and incubated at 30 ˚C, 200 rpm for 120 h. The production medium was composed of (L) 0.6 g KH_2PO_4, 0.25 g $MgSO_4.7H2O$, 0.04 g $ZnSO_4$, 1.25 g $(NH_4)_2SO_4$, and 10 g Na_2CO_3 and EFB hydrolysate was used as the sole carbon source for fumaric acid production.

2.6 Analytical method

Fumaric acid was analysed by HPLC Aminex HPX-87H column and 0.005 M sulphuric acid as the mobile phase at 60 °C and a flow rate of 0.6 mL/min.

2.7 Statistical analysis and modeling

The data obtained from RSM of fumaric acid production was subjected to the analysis of variance (ANOVA).

2.8 Key medium components screened for fumaric acid production

Experiments were carried out by the one factor design to select the suitable factors for maximum fumaric acid production. The medium components (carbon, nitrogen and neutralizing agent) were selected for fermentation. The optimal concentrations of the selected key factors were further determined to obtain a higher production of fumaric acid.

2.9 RSM: CCD as the experimental design

In order to evaluate the effect of factors on the response surface in the region of investigation, a three-factor-five-level CCD was performed. Based on the best results of the one factor design, the ranges and levels of three variables. solid loading (X_1), $(NH_4)_2SO_4$ (X_2) and Na_2CO_3 (X_3) are listed in (Table 1).

3. Results and Discussion

Optimization of Solid loading, $(NH_4)_2SO_4$ and Na_2CO_3 using RSM

Table 1. Experimental range and levels of independent variables used in CCD

CCD Independent variables	Range and levels				
	-1.68	-1	0	1	1.68
Solid loading (g/L), X_1	65.91	100	150	200	234.09
$(NH_4)_2SO_4$ (g/L), X_2	0.13	0.2	0.3	0.4	0.47
Na_2CO_3 (g/L), X_3	1.32	2	3	4	4.68

Table 2. Experimental design and result of CCD of response surface methodology

Run no.	X_1	X_2	X_3	Fumaric acid (g/l)	
	Solid loading (g/L)	$(NH_4)_2SO_4$ (g/L)	Na_2CO_3 (g/L)	Experimental	Predicted
1	-1	-1	-1	1.35	1.67
2	1	-1	-1	1.83	3.09
3	-1	1	-1	1.73	3.09
4	1	1	-1	3.38	4.51
5	-1	-1	1	3.33	3.09
6	1	-1	1	4.37	4.51
7	-1	1	1	1.66	1.67
8	1	1	1	2.30	3.09
9	-1.68	0	0	1.52	1.75
10	1.68	0	0	4.10	4.13
11	0	-1.68	0	2.07	1.70
12	0	1.68	0	2.00	1.70
13	0	0	-1.68	4.30	4.97
14	0	0	1.68	4.42	4.97
15	0	0	0	4.91	4.97
16	0	0	0	4.90	4.97
17	0	0	0	4.97	4.97

A three-variable-five-level matrix of CCD was employed to determine the optimized conditions and the interactive effects. solid loading, $(NH_4)_2SO_4$ and Na_2CO_3 were selected as the factors for CCD. The fumaric acid concentrations for each individual run along with the predicted responses are summarized in (Table 2).

The highest fumaric acid concentration of 4.97 g/L when the concentrations of solid loading, $(NH_4)_2SO_4$ and Na_2CO_3 were 150, 0.3, and 3 g/L, respectively. Also, the lowest fumaric acid concentration was 1.35 g/L, when the concentrations of solid loading, $(NH_4)_2SO_4$ and Na_2CO_3 were 100, 0.2, and 2 g/L, respectively. (Table 2).

Table 3. Analysis of variance (ANOVA) for the quadratic model

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value
Model	28.06	9	3.12	11.46	0.002
Residual	1.90	7	0.27		
Total	29.96	16			

Coefficient of determination R^2=0.9390, Adjusted R^2=0.8606, Coefficient of variation (CV)=16.68%

The response data were analyzed in the Design-Expert software. The application of RSM yielded the following regression equation, which is an empirical relationship between fumaric acid and the test variables in coded units:

$$Y = 4.96 + 0.6X_1 - 0.14X_2 + 0.26X_3 + 0.096X_1X_2 - 0.056X_1X_3 - 0.71X_2X_3 - 0.85X_1^2 - 1.12X_2^2 - 0.30X_3^2 \quad \ldots\ldots\ldots(1)$$

where Y is the fumaric acid produced as a function of solid loading (X_1), $(NH_4)_2SO_4$ (X_2) and Na_2CO_3 (X_3). The statistical significance of the above equation was checked by the F test, and the ANOVA for the response surface quadratic model is shown in (Table 3). The model F value of 11.46 and values of probability $(P)>F$ (0.002) indicated that the model terms were significant. The regression equation showed that the R^2 was 0.9390 (Table 3). This result indicates that approximately 93.90 % of the variability in the dependent variable can be explained by this model. The R^2 value is always between 0 and 1. The closer the R^2 the effects of solid loading and $(NH_4)_2SO_4$ on the production of fumaric acid. Fumaric acid production was increasing with increased the $(NH_4)_2SO_4$ from 0.2 to 0.3 g/L. When $(NH_4)_2SO_4$ at a higher con-

The response surface curves were then plotted to study the interaction among different factors, and to determine the optimal concentration of each factor for maximum fumaric acid production. As shown in (Figure 1a),

centration (>0.3 g/L), fumaric acid production was declined. As, fumaric acid production was increasing with increased the solid loading from 100 to 166.7 g/L. When solid loading at a higher (>166.7 g/L), fumaric acid production was declined.

The effects of solid loading and Na_2CO_3 are shown in (Figure 1b). Fumaric acid production was increasing with increased the Na_2CO_3 from 2 to 4 g/L. As, fumaric acid is to 1.0, the stronger the model and the better it predicts the response. The adjusted R^2, which corrects the R^2 value for the sample size and for the number of terms, was 0.8606.

production was increasing with increased the solid loading from 100 to 166.7 g/L. When solid loading at a higher (>166.7 g/L), fumaric acid production was declined.
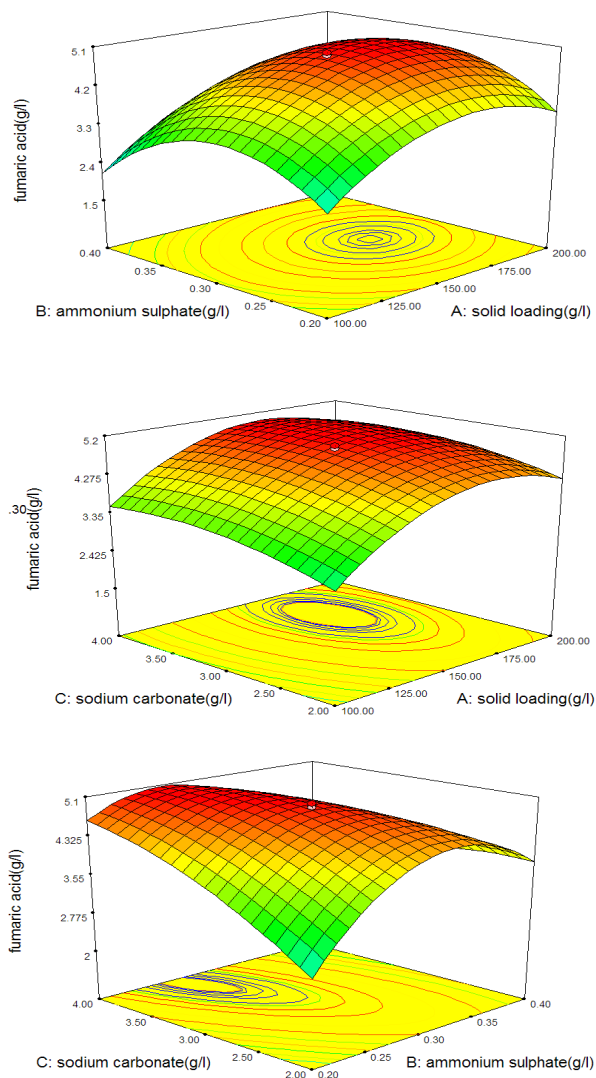
Figure 1. Response surface plots and contour plots,
        (a) Solid loading and $(NH_4)_2SO_4$
        (b) Solid loading and Na_2CO_3
        (c) $(NH_4)_2SO_4$ and Na_2CO_3

The effects of $(NH_4)_2SO_4$ and Na_2CO_3 are shown in Figure 1c. Fumaric acid production was increasing with increased the Na_2CO_3 and fumaric acid production was increasing

with increased the $(NH_4)_2SO_4$ from 0.20 to 0.27 g/L. when $(NH_4)_2SO_4$ at a higher concentration (>0.27 g/L), fumaric acid was declined.

Table 4. Confirmation experiments

No	Solid loading (g/L)	$(NH_4)_2SO_4$ (g/L)	Na_2CO_3 (g/L)	Fumaric acid concentration			
				Actual (g/L)	Predicted (g/L)	Residual (g/L)	Error (%)
1	162.52	0.30	3.07	5.26	5.17	0.09	1.71
2	166.70	0.29	3.07	5.30	5.18	0.12	2.26
3	159.94	0.29	3.07	5.28	5.15	0.13	2.46
4	175.34	0.29	3.07	5.27	5.15	0.12	2.27

3.2 Validation of model RSM

The model was validated for the three variables within the design space. The conditions and results four experiments are listed in Table 4. The result shows that under the following conditions: solid loading 166.7 g/L, $(NH_4)_2SO_4$ 0.29 g/L and Na_2CO_3 3.07 g/L, the concentration of fumaric acid (5.30 g/L) nearly reached the optimized concentration, 5.18 g/L. The predicted values and actual experimental values were compared, and then the residual was calculated in Table 4. It was observed that the percentage errors between the actual and predicted values for fumaric acid production varied from 1.17% to 2.46%. Therefore, the empirical models were reasonably accurate, and the RSM analysis is indeed a useful technique to predict and optimize the fermentation media. Usually, it is necessary to check the adequacy of the model to ensure 5.30 g/L. The result shows that response surface methodology was proved to powerful that it provides maximum approximation on relationship between response and factors. The residuals from the least squares are an important tool for judging the model adequacy.

4. Conclusion

Among the variable studied, solid loading was found to be significantly effecting fumaric acid production from isolate K20. The interactions between the variables were also significant. Under optimum conditions (solid loading 166.70 g/L, $(NH_4)_2SO_4$ 0.29 g/L and Na_2CO_3 3.07 g/L), the observed value of 5.30 g/L corresponded with predicted value of 5.10 g/L. The highest fumaric acid concentration obtained from isolate K20 using EFB as substrate was 5.30 g/L. The result shows that response surface methodology was proved to be a powerful tool optimization of fumaric acid production. Morever, EFB could be a potential raw material for fumaric acid production and could scale up to industry scale. Based on this work, focusing on fumaric acid production on fermentor is designing to obtain higher fumaric acid concentration.

5. Acknowledgement

6. References

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