Bioconversion of Cassava Stem to Ethanol Using *Aspergillus fumigatus* and *Saccharomyces cerevisiae*

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Cassava stem was bioconverted to ethanol using microorganisms. First, cassava stem was pretreated by in ways, alkaline solution alone (ASA), microwave treatment combined with alkaline solution (MTCAS), and ultrasonic treatment combined with alkaline solution (UTCAS). The compositions of cassava stem pretreated by different methods were analyzed, and the results showed that the cassava stem pretreated by MTCAS was more suitable for saccharification and subsequent ethanol production. The pretreated cassava stem was subjected to simultaneous saccharification and ethanol production using *Aspergillus fumigatus* and *Saccharomyces cerevisiae*. Response surface methodology was used to optimize various process parameters including fermentation temperature, initial pH, fermentation time, rotational speed and substrate concentration. A bioconversion yield of 70 mg/g was obtained at the optimum conditions of fermentation, viz, temperature 35 °C, initial pH 5.6, fermentation time 132 h, rotational speed 155 rpm, and substrate concentration 4.6 wt%. An experiment under optimum conditions confirmed the model predictions. The results suggest that pretreatment with MTCAS and simultaneous fermentation with *A. fumigatus* and *S. cerevisiae* would be a good choice for the bioconversion of lignocellulosic biomass to bioethanol. Considering the cost advantage, using microbial fermentation instead of pure enzyme hydrolysis is more advantageous in 2nd generation bioethanol production.

Keywords: Bioconversion; Cassava stem; Pretreatment; Respond surface methodology

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INTRODUCTION

Today, environmental pollution, the greenhouse effect, and global climate change are urgent and sensitive issues (Septia *et al.* 2018; Cinthia *et al.* 2019; Intaramas *et al.* 2018). It is well known that the use of a renewable resource to replace traditional fossil fuels would be a good alternative to solve these problems (Jin-Ho and Volker 2017; Germec and Turhan 2018; Niethammer *et al.* 2018; Zhou *et al.* 2018). In particular, the bioconversion of lignocellulosic biomass to bioenergy, such as biofuel and bioethanol, is considered a potential way to substitute traditional energy (Pattiya *et al.* 2012; Shen *et al.* 2017; Yang *et al.* 2018).

For this reason, bioethanol production from lignocellulosic biomass, usually agricultural wastes, is gaining increasing research interest (André *et al.* 2018; Singh *et al.* 2018). In general, there are three steps involved in the bioconversion of lignocellulosic biomass to bioethanol: pretreatment, saccharification, and ethanol production.

Pretreatment with alkali is a traditional method to remove the lignin from lignocellulosic biomass (Zhu *et al.* 2005). However, it is inefficient due to its high loss of

cellulose and hemicellulose as well as being time consuming. Further improvements are required, and many researchers are doing great work in this field. The purpose of saccharification is to convert cellulose and hemicellulose into fermentable sugars. Cellulase from microorganisms is often used in the saccharification process. However, the production costs of bioethanol will be raised greatly due to the use of an enzyme. It would be an effective alternative method to use microorganisms that can produce cellulase and hemicellulase instead of a commercial enzyme. Ethanol production is the last step in which saccharification products are fermented and fermentable sugar is converted into ethanol by yeast.

Cassava is a starchy crop belonging to the Euphorbiaceae family (Martin *et al.* 2017). It is cultivated in many countries across Africa, Asia, and South America (Veiga *et al.* 2016). According to the Food and Agriculture Organization's (FAO) estimates, 233 million tons of cassava was produced worldwide in 2008, and the amount has been growing for nearly a decade (Pattiya 2011; Pattiya *et al.* 2012). As the main agricultural waste product of the cassava industry, cassava stem is a good source of lignocellulosic biomass that can be converted into bioethanol (Tanaka *et al.* 2019). Various researchers have reported the process and conditions of bioethanol production from cassava stem. Kouten *et al.* (2016) reported that pretreated cassava stems and peelings *via* thermohydrolysis and fermentation with cellulase can obtain a satisfactory saccharification yield (Kouteu *et al.* 2016). Kamalini *et al.* (2018) used a response surface methodology (RSM) with a Box-Behnken design (BBD) that was employed to investigate the optimum conditions for a microwave-assisted alkaline pretreatment of cassava stem (Kamalini *et al.* 2018). However, most of these studies focused on the process of saccharification of cassava stem, and few studies applied the last step of bioconversion, which is ethanol production.

To bioconvert the cassava stem into ethanol in an efficient and cost-effective way, the pretreatment, saccharification, and ethanol production of cassava stem were studied in this work. Three methods of pretreatment were compared, these included pretreatment by alkaline alone (AA), microwave treatment combined with alkaline solution (MTCAS), and ultrasonic treatment combined with alkaline solution (UTCAS). *Aspergillus fumigatus* and *Saccharomyces cerevisiae* were used for the saccharification and ethanol production in one fermentation process. Additionally, the level of fermentation factors were optimized using RSM. An outline of the work is shown in Fig. 1.

EXPERIMENTAL

Raw Material

Cassava stem was obtained from local agricultural fields in Dongxiang, Jiangxi province, China. The cassava stem were cut into 2-cm length pieces and baker-dried to a 5 wt% moisture content at 105 ℃. After naturally cooling to room temperature, they were milled to pass through a 40-mesh screen. The obtained powders were conditioned in sealed plastic bags and stored at ambient temperature (25 ± 3 ℃) until further use.

Fermentation strain

The fungus *Aspergillus fumigatus* (CICC 2434) that can produce cellulase and hemicellulase was used as the fermentation strain for saccharification. The yeast *Saccharomyces cerevisiae* (CICC 1023) that can convert fermentable sugar into ethanol was used as the fermentation strain for ethanol production. They were purchased from the

China Center of Industrial Culture Collection (CICC; Beijing, China) and were plated in malt-agar medium (5° Bé, degree Baumé). The *A. fumigatus* was incubated at 45 °C, and the *S. cerevisiae* was incubated at 30 °C for colony formation. *A. fumigatus* suspensions were prepared using sterile water. The spore count was adjusted to 2×10^6 spores/mL. *S. cerevisiae* inoculi were prepared using malt juice culture. The number of viable spores was adjusted to 5×10^8 Colony-forming Units (CFU)/mL.

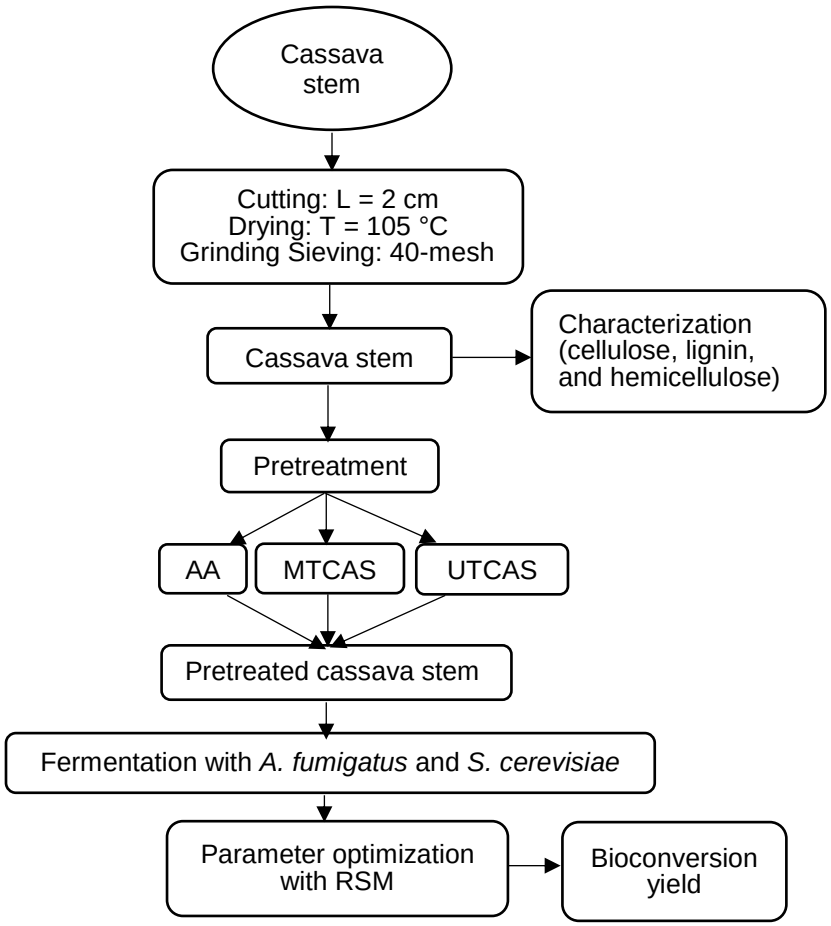

Fig. 1. The outline of experimental process

Pretreatment

Pretreatment by alkaline solution alone (ASA)

Samples (20 g) of cassava stem powder were suspended in 200 mL of NaOH aqueous solution and boiled in a 500-mL beaker (treatment temperature 100 ℃) for different times, as designated in Table 1. The residues were collected and extensively washed with tap water until neutral pH. Then, the material was dried and ground into a fine powder.

Pretreatment by microwave treatment combined with alkaline solution (MTCAS)

A total of 20 g of cassava stem powder were suspended in 200 mL of NaOH aqueous solution in the round bottom flask positioned at the center of a microwave reaction station (SINEO MAS-II Plus; Shanghai Xinyi Microwave Chemical Technology Co., Ltd., Shanghai, China) for microwave treatment (treatment temperature 100 ℃), as designated

in Table 1. The residues were collected and extensively washed with tap water until neutral pH. The residues were collected and then treated as mentioned above.

Pretreatment by ultrasonic treatment combined with alkaline solution (UTCAS)

A total of 20 g of cassava stem powder were suspended in 200 mL of NaOH aqueous solution in a 500-mL beaker, and the beaker was positioned into an ultrasonic extractor (Scientz EXC933; Ningbo Xinzhi Technology Co., Ltd., Ningbo, China) for ultrasonic treatment (ultrasonic frequency 60 KHz, treatment temperature 100 ℃), as designated in Table 1. The residues were collected and then treated as mentioned above.

Table 1. Process Parameters and Experimental Design of Pretreatment

Pretreatment Method								
AA			MTCAS			UTCAS		
Run	NaOH (wt%)	Time (min)	Run	NaOH (wt%)	Time (min)	Run	NaOH (wt%)	Time (min)
1	1	20	10	1	10	19	1	10
2	1	30	11	1	20	20	1	20
3	1	40	12	1	30	21	1	30
4	2	20	13	2	10	22	2	10
5	2	30	14	2	20	23	2	20
6	2	40	15	2	30	24	2	30
7	3	20	16	3	10	25	3	10
8	3	30	17	3	20	26	3	20
9	3	40	18	3	30	27	3	30

Bioconversion of Cassava Stem to Ethanol

The bioconversion of cassava stem was completed in a 500-mL conical flask containing pretreated cassava stem. Pretreated cassava stem was sterilized at 121 °C for 30 min and cooled. *Aspergillus fumigatus* and *Saccharomyces cerevisiae* were inoculated for saccharification and ethanol production, respectively, and incubated in a constant temperature incubator shaker (ZQZY-75AN; Shanghai Zhichu Instrument Co., Ltd. Shanghai, China) for bioconversion. After the bioconversion, the fermentation broth was filtrated to remove the biomass. The bioethanol produced was determined using the potassium dichromate method (William and Reese 1950). The various factors that influence the bioconversion yield, including temperature, initial pH, time, rotational speed, and substrate concentration, were studied using RSM.

The bioconversion yield Y (mg/g) was calculated using the following Eq. 1,

$$Y = m / n \quad (1)$$

where m is the ethanol formed (mg) and n is the pretreated cassava stem (g).

Methods

Response surface methodology

Response surface methodology was used to study the influence of various process parameters, including fermentation temperature (A), initial pH (B), fermentation time (C), rotational speed (D), and substrate concentration (E) on the bioconversion yield from cassava stem.

A Box-Behnken design with 46 experiments (40 axial and 6 central points) was elaborated to study the effect of independent variables on the responses (bioconversion

yield) and interaction of factors. The ranges of selected process parameters are shown in Table 2. The choices of factors, as well as their levels, were determined according to the authors' preliminary research.

Table 2. Minimum and Maximum Values of Various Factors Selected for Optimization of Bioconversion Yield from Cassava Stem

Variable Codes	Variables	Level		
		-1	0	1
A	Fermentation temperature (°C)	25	30	35
B	Initial pH	4	5	6
C	Fermentation time (h)	72	108	144
D	Rotational speed (rpm)	120	150	180
E	Substrate concentration (wt%)	3	4	5

Analytical methods

The moisture was measured as the weight loss of 1 g cassava stem dried at 105 ℃ for 24 h. The cellulose content was determined *via* the HNO_3–ethanol method. The lignin content was assayed using the 72 wt% H_2SO_4 method. The hemicellulose content was analysed according to the two-brominating method (Liu 2004; Zhu *et al.* 2005).

Statistical analysis

The Student's t-test permitted verification of the statistical significance of the regression coefficients. The Fisher's test for analysis of variance (ANOVA) was performed on the experimental data to evaluate the statistical significance of the model. Design Expert 11.0 software (StatEase, Inc., Minneapolis, MN, USA) was employed to determine and evaluate the coefficients of the acquired full regression model equation and their statistical significance.

RESULTS AND DISCUSSION

The chemical compositions of cassava stems pretreated using different methods are presented in Table 3. The proportions of cellulose, hemicellulose, and lignin of cassava stems without pretreatment (Run 0) were 28.8 wt%, 19.2 wt%, and 10.2 wt%, respectively.

The Tukey test was used for the statistical analysis for the significant difference of the data set for each run. There is a significant difference between the mean values followed by different letters in the column, and the significance level is 5%.

Pretreatment

To facilitate the later saccharification step and obtain as high as possible fermentable sugars yield, the lignocellulose needs to be pretreated to remove the lignin and increase the cellulose and hemicellulose content.

Table 3. Results of Pretreatment Experiments of Cassava Stem

Run	Cellulose (wt%)	Hemicellulose (wt%)	Lignin (wt%)	Run	Cellulose (wt%)	Hemicellulose (wt%)	Lignin (wt%)
0	28.8 ± 0.2^{a}	19.2 ± 0.4^{a}	10.2 ± 0.7^{d}	14	48.1 ± 0.4^{d}	22.5 ± 0.7^{b}	4.80 ± 0.6^{ab}
1	33.6 ± 0.4^{b}	20.1 ± 0.4^{a}	7.9 ± 0.9^{d}	15	50.1 ± 0.5^{d}	23.1 ± 0.4^{c}	4.0 ± 0.2^{a}
2	42.5 ± 0.6^{c}	21.0 ± 0.5^{ab}	6.2 ± 1.3^{c}	16	48.0 ± 0.3^{d}	22.2 ± 0.4^{b}	5.0 ± 0.2^{ab}
3	43.9 ± 0.2^{c}	21.4 ± 0.3^{ab}	5.7 ± 0.8^{bc}	17	50.7 ± 0.6^{d}	23.4 ± 0.4^{c}	4.20 ± 0.3^{a}
4	43.8 ± 0.3^{c}	21.1 ± 0.2^{ab}	5.9 ± 0.6^{bc}	18	52.1 ± 0.5^{d}	24.0 ± 0.6^{d}	3.60 ± 0.4^{a}
5	44.2 ± 0.8^{c}	21.4 ± 1.1^{ab}	5.0 ± 0.8^{ab}	19	36.4 ± 0.3^{bc}	19.6 ± 0.4^{a}	8.2 ± 0.6^{d}
6	45.8 ± 0.3^{c}	21.8 ± 0.4^{b}	4.70 ± 0.2^{a}	20	40.9 ± 0.5^{bc}	20.8 ± 0.3^{a}	7.20 ± 0.9^{c}
7	47.0 ± 0.6^{cd}	22.1 ± 0.5^{b}	4.50 ± 0.1^{a}	21	44.6 ± 0.4^{c}	21.7 ± 0.3^{b}	5.80 ± 0.4^{b}
8	48.2 ± 0.5^{d}	22.4 ± 1.3^{b}	4.4 ± 0.8^{a}	22	42.1 ± 0.4^{c}	20.3 ± 0.1^{a}	7.0 ± 0.4^{cd}
9	48.6 ± 0.3^{d}	22.9 ± 0.5^{bc}	4.0 ± 0.2^{a}	23	44.9 ± 0.7^{c}	22.0 ± 0.4^{b}	5.4 ± 0.3^{b}
10	40.5 ± 0.2^{bc}	20.5 ± 0.5^{a}	7.0 ± 0.7^{cd}	24	46.2 ± 0.6^{c}	22.7 ± 0.6^{bc}	4.8 ± 0.4^{ab}
11	47.5 ± 0.5^{c}	21.4 ± 0.3^{ab}	5.70 ± 0.8^{bc}	25	46.0 ± 0.6^{c}	21.1 ± 0.5^{ab}	5.50 ± 0.1^{b}
12	48.7 ± 0.6^{d}	22.1 ± 0.4^{b}	4.5 ± 0.4^{a}	26	47.8 ± 0.3^{c}	22.8 ± 0.8^{c}	4.4 ± 0.2^{a}
13	43.2 ± 0.6^{c}	21.4 ± 0.1^{ab}	6.2 ± 0.7^{bc}	27	49.5 ± 0.3^{d}	23.7 ± 0.5^{c}	4.0 ± 0.2^{a}

From Table 3, it can be found that within the same pretreatment method and at the same NaOH concentration, the content of cellulose and hemicellulose increased and the lignin decreased with increased treatment time. Furthermore, within the same pretreatment method with the same treatment time, the content of cellulose and hemicellulose increased and the lignin decreased with increased NaOH concentration.

When compared with different pretreatment methods, it was found that when pretreated with the same NaOH concentration and treatment time (20 or 30 min), MTCAS exhibited the highest cellulose and hemicellulose content and the lowest lignin content. The results of experiment run 15, run 17, and run 18 were more suitable for the request of saccharification. The pretreatment process of run 18 was used in subsequent experiments of bioconversion. Thus, cassava stem were pretreated in 3% NaOH solution at 100 ℃ for 30 min in the microwave reaction station. Under these conditions, the pretreated cassava stem for bioconversion containing 52.1 wt% cellulose, 24 wt% hemicellulose, and 3.6 wt% lignin can be obtained.

RSM Analysis

In the present work, the relationship between the bioconversion yield and five process variables was developed using RSM. The BBD was used to optimize various parameters affecting the bioconversion yield of cassava stem. The experimental design, experimental, and predicted values of bioconversion yield are shown in Table 4. Variance analyses (ANOVA) are shown in Table 5.

Table 4. Box-Behnken of RSM in Actual Value for Optimization of Bioconversion Yield from Cassava Stem

Exp. Order	Process Parameters					Y_A	Y_P	Exp. Order	Process Parameters					Y_A	Y_P
	A	B	C	D	E				A	B	C	D	E		
1	25	4	108	150	4	11.55	11.97	24	30	6	144	150	4	37.18	39.26
2	35	4	108	150	4	6.38	5.73	25	25	5	108	120	4	11.88	14.79
3	25	6	108	150	4	18.15	17.21	26	35	5	108	120	4	11.33	11.58
4	35	6	108	150	4	40.26	38.25	27	25	5	108	180	4	16.17	16.51
5	30	5	72	120	4	3.85	1.01	28	35	5	108	180	4	36.85	34.52
6	30	5	144	120	4	13.31	12.12	29	30	5	72	150	3	5.39	5.19
7	30	5	72	180	4	5.17	4.27	30	30	5	144	150	3	5.39	0.46
8	30	5	144	180	4	32.78	33.53	31	30	5	72	150	5	13.2	18.70
9	30	4	108	150	3	5.94	7.13	32	30	5	144	150	5	63.03	63.80
10	30	6	108	150	3	3.85	2.58	33	25	5	108	150	3	4.95	4.92
11	30	4	108	150	5	20.9	22.12	34	35	5	108	150	3	10.23	13.97
12	30	6	108	150	5	65.67	64.43	35	25	5	108	150	5	49.17	44.99
13	25	5	72	150	4	7.15	7.28	36	35	5	108	150	5	51.15	50.74
14	35	5	72	150	4	4.95	5.06	37	30	4	108	120	4	3.41	3.17
15	25	5	144	150	4	16.5	17.84	38	30	6	108	120	4	12.65	14.02
16	35	5	144	150	4	33.55	34.87	39	30	4	108	180	4	7.26	7.47
17	30	5	108	120	3	3.96	4.62	40	30	6	108	180	4	32.56	34.38
18	30	5	108	180	3	6.38	7.22	41	30	5	108	150	4	36.3	36.78
19	30	5	108	120	5	34.21	33.30	42	30	5	108	150	4	34.65	36.78
20	30	5	108	180	5	56.1	55.37	43	30	5	108	150	4	37.95	36.78
21	30	4	72	150	4	2.2	0.19	44	30	5	108	150	4	36.41	36.78
22	30	6	72	150	4	2.75	2.96	45	30	5	108	150	4	37.51	36.78
23	30	4	144	150	4	4.4	4.26	46	30	5	108	150	4	37.84	36.78

A: fermentation temperature (°C); B: Initial pH; C: Reaction time (h); D: Rotational speed (rpm); E: Substrate concentration (wt%); Y_A: Actual value of bioconversion yield (mg/g); Y_P: Predicted value of bioconversion yield (mg/g)

Modelling

The second-degree polynomial model for the bioconversion yield is given as Eq. 2 (In terms of coded factors).

$$Y_{Bioconversion\ yield} = 36.78 + 3.70 \times A + 9.44 \times B + 10.09 \times C + 6.17 \times D + 19.21 \times E - 6.95 \times A^2 - 11.54 \times B^2 - 13.57 \times C^2 - 10.48 \times D^2 - 1.17 \times E^2 + 6.82 \times A \times B + 4.81 \times A \times C + 5.31 \times A \times D - 0.83 \times A \times E + 8.06 \times B \times C + 4.02 \times B \times D + 11.72 \times B \times E + 4.54 \times C \times D + 12.46 \times C \times \mathrm{E} + 4.87 \times D \times E \quad (2)$$

where *A*, *B*, *C*, *D*, and *E* are fermentation temperature (°C), initial pH, fermentation time (h), rotational speed (rpm), and substrate concentration (wt%), respectively.

The Model F-value of 120.76 implies that the model is significant. There was only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob. > F" less than 0.0500 indicate the model terms are significant. In this case, A, B, C, D, E, A^2, B^2, C^2, D^2, AB, AC, AD, BC, BD, BE, CD, CE, and DE are significant model terms. The substrate concentration (E) was the most significant variable for the production

of ethanol from cassava stem due to its higher F value (977.87) and lower p-value (< 0.0001).

Values greater than 0.1000 indicate that the model terms are not significant. In this case, E^2 and AE were insignificant model terms. The lack of fit F-value of 4.51 implies that there is a 5.11% chance that a lack of fit F-value this large could occur due to noise. Lack of fit is bad because the model needs to fit. The "Pred R-Squared" (R^2_{Pred}) of 0.9604 is in reasonable agreement with the "Adj R-Squared" (R^2_{Adj}) of 0.9816. The "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The authors' ratio of 38.690 indicates an adequate signal. The authors' model can be used to navigate the design space.

Effect of process variables on the bioconversion yield

From this regression model, all five variables showed positive effects on the bioconversion yield. This indicated that increasing the level of these variables at the range of experimental design will improve the bioconversion yield. The substrate concentration (E) was the most significant variable for the bioconversion yield from cassava stem due to its higher F value (977.87) and lower p-value (< 0.0001).

Table 5. ANOVA for Response Surface Quadratic Model of Bioconversion Yield

Source	Sum of Squares	DF	Mean Square	F Value	Prob. > F
Model	14581.63	20	729.08	120.76	< 0.0001
A	218.89	1	218.89	36.26	< 0.0001
B	1425.63	1	1425.63	236.14	< 0.0001
C	1629.74	1	1629.74	269.95	< 0.0001
D	608.49	1	608.49	100.79	< 0.0001
E	5903.62	1	5903.62	977.87	< 0.0001
A^2	421.35	1	421.35	69.79	< 0.0001
B^2	1162.39	1	1162.39	192.54	< 0.0001
C^2	1606.29	1	1606.29	266.07	< 0.0001
D^2	958.06	1	958.06	158.69	< 0.0001
E^2	12.01	1	12.01	1.99	0.1706
AB	186.05	1	186.05	30.82	< 0.0001
AC	92.64	1	92.64	15.34	0.0006
AD	112.68	1	112.68	18.66	0.0002
AE	2.72	1	2.72	0.45	0.5080
BC	259.69	1	259.69	43.02	< 0.0001
BD	64.48	1	64.48	10.68	0.0031
BE	548.96	1	548.96	90.93	< 0.0001
CD	82.36	1	82.36	13.64	0.0011
CE	620.76	1	620.76	102.82	< 0.0001
DE	94.77	1	94.77	15.70	0.0005
Lack of Fit	143.00	20	7.15	4.51	0.0511
R^2	0.9898		R^2_{Adj}	0.9816	
R^2_{Pred}	0.9604		Adeq. Precision	38.69	

From Table 5, it is observed that the interaction effect of fermentation time (C) and substrate concentration (E) showed a highly significant effect on yield than other interactions because it has a high F-value of 620.76 and low p-value of (< 0.0001). This result indicated that increasing the variables (C and E) will result in increased yield. Moreover, the interaction between fermentation temperature (A) and substrate concentration (E) was not significant, which may have been attributable to the selected range of fermentation temperature (25 to 35 ℃) that resulted in either decreased enzyme activity produced by the fermentation strain or limited growth of the fermentation strain.

According to the BBD, the experimental bioconversion yield of 71.4 mg/g was obtained at optimum conditions of fermentation temperature 35 ℃, initial pH 5.6, fermentation time 132 h, rotational speed 155 rpm, and substrate concentration 4.6 wt%. This result was validated at its optimal conditions in triplicates and the experimental results match well with the predicted values from the model equation.

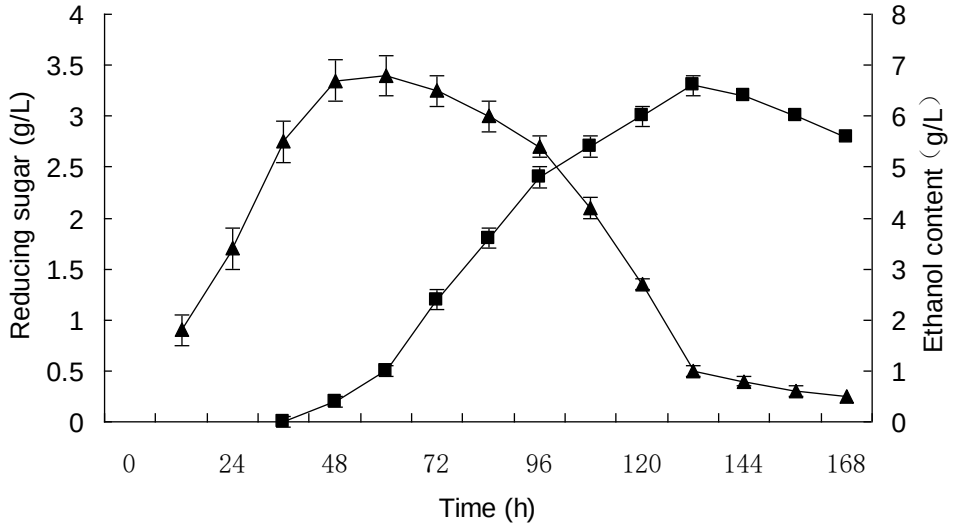

Fig. 2 The fermentation kinetic plot of production of reducing sugar and ethanol. Results are the average of three replicates, and bars indicate standard error of three replicates. (▲ reducing sugar ■ ethanol content)

A. fumigatus was found to produce cellulase and hemicellulase early. However, there are few studies on the possible industrial application of enzymes from this fungus. It was of interest in the current study to examine the feasibility of using *A. fumigatus* cellulase and xylanase to convert lignocellulosic biomass into fermentable sugars.

Cassava stem was selected as a substrate for bioconversion because of its local and abundant availability. Lignocellulosic biomass cannot be bioconverted by enzymes or microorganisms in a high yield without a pretreatment procedure because the lignin in the plant cell wall is a barrier to enzyme action (Kouteu Nanssou *et al.* 2016). In the present study, cassava stem was pretreated *via* microwave combined with alkaline prior to fermentation. This treatment was effective in fractionating the hemicellulose and lignin components (Zhu *et al.* 2005).

During bioconversion of cassava stem, fermentation temperature, initial pH, fermentation time, rotational speed, and substrate concentration had a significant effect on bioconversion yield ($P < 0.01$). The saccharification and ethanol production of cassava

stem was the synergism result of microorganism growth and the effect of enzyme, so the variation of pH, temperature, and rotational speed will significantly affect the bioconversion yield. The suitable pH and temperature for microorganism growth and for the enzyme activity is different. Most of the fungal and yeast growth and their metabolites are suitable for the pH range of 4 to 6. In general, the suitable pH for enzyme activity produced by *A. fumigatus* is 5 to 6. Fermentation time showed a positive effect on the bioconversion yield, which means that the bioconversion yield will increase as the fermentation time increases within the experimental range. It is known that increasing the substrate concentration will enhance the overall bioconversion yield from cellulose (Tanaka *et al*. 2019). In the current work, a higher bioconversion yield with higher substrate concentration was obtained. This was similar to some of the results of previous works (Ang 2013), where the enzyme concentration in fermentation broth of *A. fumigatus* was directly affected by the substrate concentration. However, some other factors, such as physical properties and cellulose microstructure, that were not discussed in this experiment may also affect the bioconversion yield.

The conventional technique for the optimization of a multifactorial system is to deal with one factor at a time. However, this type of method is time-consuming and also does not reveal the alternative effects between components. In general, experimental results were enhanced by the optimization of the RSM more than the conventional optimization methods (Kamalini *et al*. 2018).

Figure 3 and figure 4 showed the response surface plots (Contour and 3D) of the experiment.

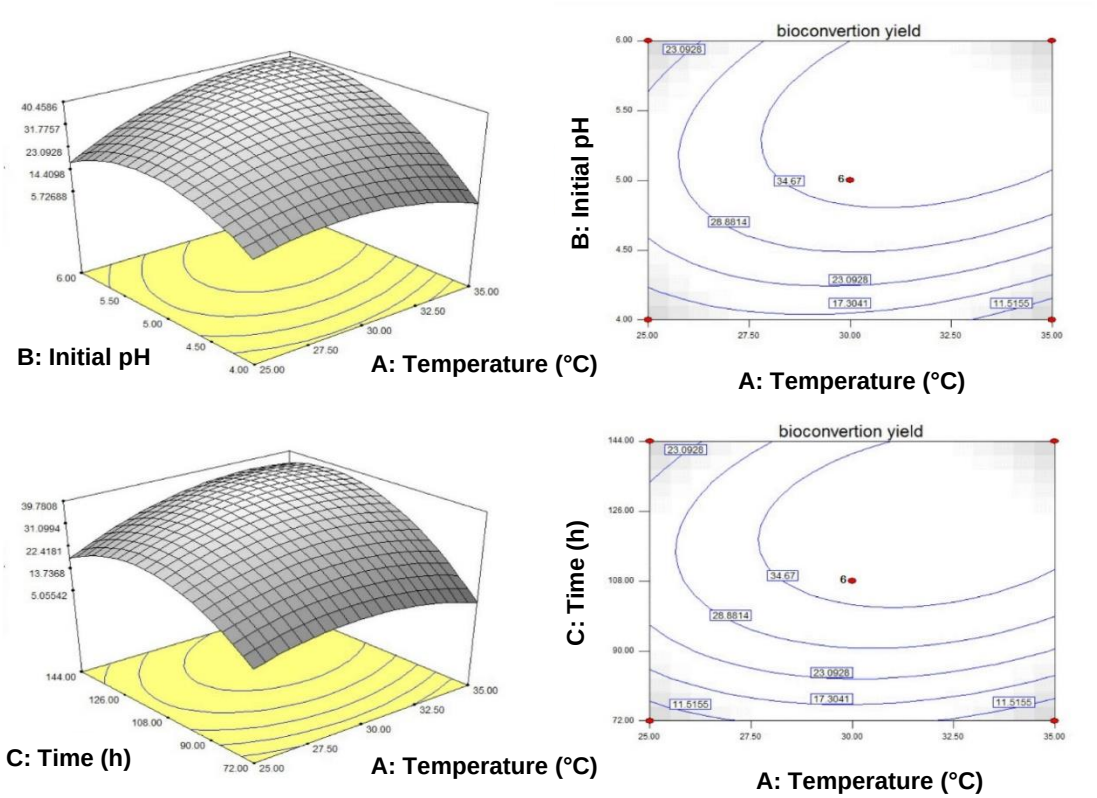

Fig. 3. Response surface plots (Contour and 3D) showing the interactive effects of temperature (°C) and initial pH (AB) as well as temperature (°C) and time (h) (AC) on the Bioconversion yield

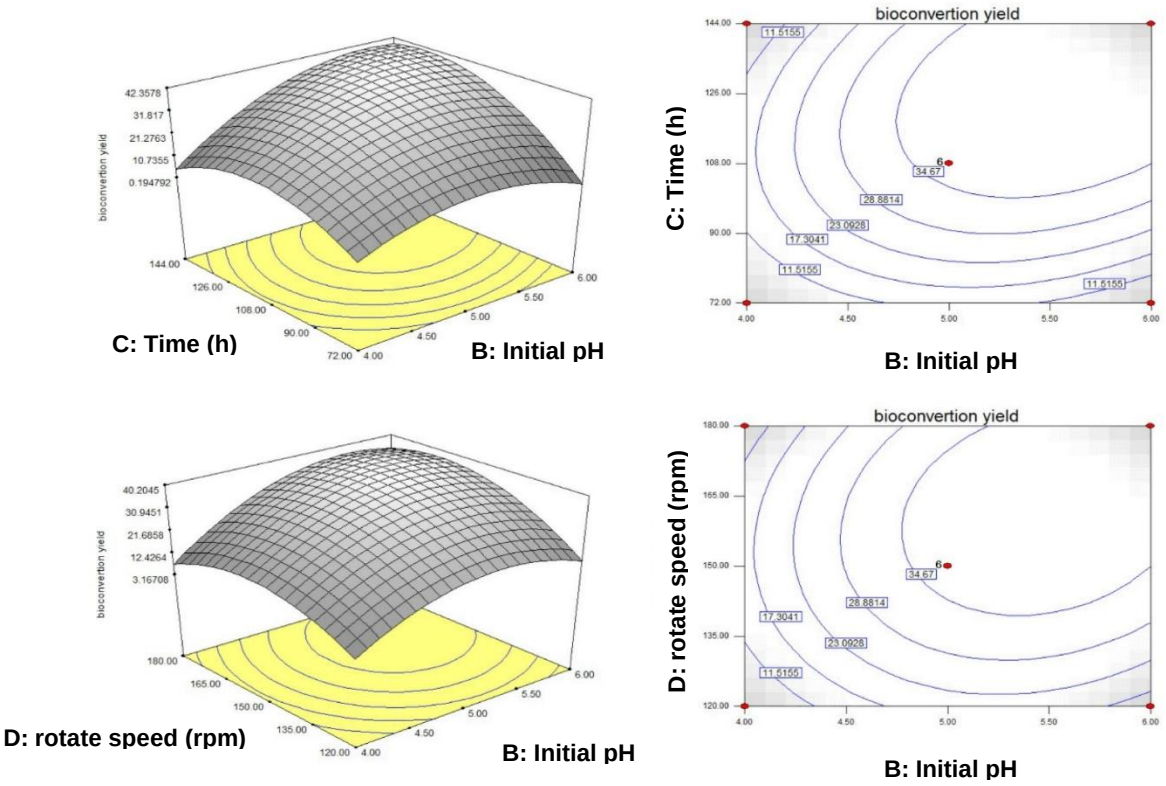

Fig. 4. Response surface plots (Contour and 3D) showing the interactive effects of initial pH and time (h) (BC) as well as initial pH and rotate speed (rpm) (BD) on the Bioconversion yield

Discussion

Cassava stems are principally composed of cellulose, hemicellulose, and lignin. Many studies have shown that the lignin-hemicellulose matrix surrounding the cellulosic fraction will act as a physical barrier preventing the access of cellulase on the cellulose surface and thereby affecting the efficiency of lignocellulosic conversion (Alvira *et al.* 2010; Hsu *et al.* 2010). So the pretreatment is necessary to alter the physical and chemical properties, thereby enhancing enzymatic hydrolysis.

Various researchers have reported different pretreatment methods that can enhance the bioethanol production (Alvira *et al.* 2010; Nanssou *et al.* 2016). Among these methods, alkaline pretreatment was shown to be more effective and advantageous since it use low-cost chemicals and operate at lower temperatures (Balat 2011). However, this method usually takes a long time. Microwave and ultrasonic treatments have been studied as assistants to conventional pretreatment methods (Aguilar-Reynosa *et al.* 2017; Moodley and Kana 2017). Kamalini *et al.* (2018) investigated the application of response surface methodology on the effect of alkaline NaOH pretreatment on cassava stem powder under microwave conditions. The maximum reducing sugar of 41±2 mg/L was obtained under the optimal process parameters. The relatively high result of 6.6±2 g/L of reducing sugar was obtained during the fermentation process in the present work.

CONCLUSIONS

1. Bioconversion of cassava stem to ethanol using *Aspergillus fumigatus* and *Saccharomyces cerevisiae* in one process is feasible. The bioconversion yield of 70 mg/g can be obtained at a fermentation temperature of 35 °C, initial pH 5.5, fermentation time 132 h, rotational speed 155 rpm, and substrate concentration 4.6%.
2. The pretreatment by microwave treatment combined with alkaline solution on cassava stem powder was more suitable for the saccharification and subsequent ethanol production.
3. The RSM was a good way to optimize the bioconversion process.
4. *Aspergillus fumigatus* is the suitable strain for the saccharification of cellulose due to its production capability of cellulase and hemicellulose.

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