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Optimization of Ethanol Production from Mango Peels using Response Surface Methodology

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Abstract. This study aims to optimize bioethanol production from mango peels using Response Surface Methodology (RSM). The effect of temperature (25–40^{o}C), yeast concentration (6–14 g/ml) and fermentation time (48–96 hours) on bioethanol yield was investigated. Prior to the fermentation process, mango peels were treated with 0.25–1% (w/v) sulphuric acid. Optimum glucose yield was obtained at 0.25% (w/v) sulphuric acid. RSM using 3-factor 2-level central composite design (CCD) was employed to evaluate and optimize the synthesis parameters. Based on numerical optimization, the optimum fermentation conditions were at 38^{o}C using 6 g/ml yeast for 48 hours, giving a yield of 7.34 g/ml bioethanol.

Introduction

Over the last few decades, the escalating price of fossil fuel as well as increased green house gas emissions have prompted the development of biofuel technology as an alternative renewable fuel resource. Today, bioethanol contributes to approximately 3% of the total road transport fuel globally [1]. There are various ways of deriving biofuel; via chemical reactions, fermentation, or heat to breakdown the starches, sugars, and other molecules in plants [2].

Fermentation is a metabolic process in which a microorganism, such as yeast converts a carbohydrate, such as starch or a sugar, into an alcohol or an acid. The most commercially used yeast for ethanol production is *Saccharomyces cerevisiae* [3]. Prior to the fermentation process, biomass is generally pre-treated with acids or enzymes to produce sugars. The pretreatment process decreases the crystallinity of biomass while removing lignin and other inhibitors [4].

Mango is one of the most important fruits marketed in the world with a global production exceeding 26 million tons in 2004 [5]. It is grown naturally in over 90 countries worldwide and is known to be the second largest produced tropical fruit crops in the world [6]. Mango peels, which consists of 20–25% of total fruit weight, is a byproduct of the mango processing industry. These peels are not utilized commercially, hence are invariably discarded as wastes [7,8].

In this work, waste mango peels were utilized as biomass for bioethanol production. The fermentation temperature, time and yeast concentration were varied in order to produce bioethanol in sufficient yield. The relationship between parameters and response (yield) was studied; to determine the optimal fermentation conditions using a central composite design (CCD) and response surface methodology (RSM) analysis. In designing an experiment, RSM promotes low cost in terms of experimentation as it uses a statistical model to estimate a reduced number of runs for statistically acceptable result.

Methodology

Hydrolysis of Mango Peels. Mango peel powder (10 g) was added to deionized water (90 ml), followed by addition of sulphuric acid (0.25M, 0.5M, 0.75M or 1.0M). The resulting solution was sterilized in an autoclave at 121°C for 15 min. The sugar content in the sample was analyzed using refractometer. The sample with highest sugar content was subsequently selected for fermentation.

Preparation and Propagation of Yeast Cells. Dried yeast powder was added to a sterilized Erlenmeyer flask containing 50 ml glucose yeast extract and incubated at 30°C for 48 hours at 100 rpm. The cells were then centrifuged for 10 min at 4°C.

Fermentation Process. Hydrolysate was neutralized and supplemented with a concentrated nutrient solution, to reach a final concentration of 0.1% (w/v) yeast extract. The hydrolysate was agitated at 250 rpm and heated to 80°C for 30 min. The fermentation process was carried out according to the runs generated from RSM (Table 1). Samples were drawn at the end of the experiment and analyzed for sugar and ethanol content.

Experimental Design. 3-factor 2-level CCD was employed in this study, consisting of 15 experimental runs and performed in a random order. The parameters for study were: reaction temperature (25–40^{o}C), yeast concentration (6–14 g/ml) and fermentation time (48–96 hours). Table 1 shows the process factors and actual experimental design.

Results and Discussion

Pretreatment. Fig. 1 presents the sugar content of samples by varying the concentrations of sulphuric acid during hydrolysis process. It was found that optimum glucose yield can be obtained at 0.25% (w/v) sulphuric acid.

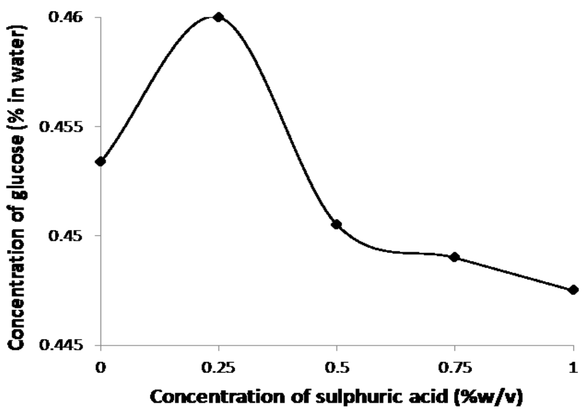


Fig. 1: Sugar content after pretreatment

Model Fitting and Analysis of Variance (ANOVA). Expermental data for bioethanol production using mango peels is given in Table 1. Fitting of the data to various models and their ANOVA suggested that quadratic polynomial model most adequately describe the fermentation process. The quadratic polynomial is given in Eq. 1.

$$y\left(\frac{g}{ml}\right) = 12.698 - 0.293x_1 - 0.315x_2 - 0.048x_3 - 0.0059x_1x_2 + 0.0031x_1x_3 + 0.0057x_2x_3 + 0.0042x_1^2 + 0.021x_2^2 - 0.00055x_3^2 - 0.00014x_1x_2x_3 \quad (1)$$

The model F-value was 313.68, which implies that the model is significant. Values of Prob > F less than 0.05 indicate model terms were significant. All variables and interaction were significant (Table 2). The pure error was very low, suggesting good reproducibility of the data obtained. The coefficient of determination of R^2=0.998 indicates that the quadratic polynomial model was adequate to represent the actual relationship between the response and significant variables.

Mutual effect of parameters. Based on statistical analysis of the experimental data, the interaction between independent process factors and response was portrayed graphically using three-dimensional surface countour plot, as depicted in Fig. 2(a), (b) and (c).

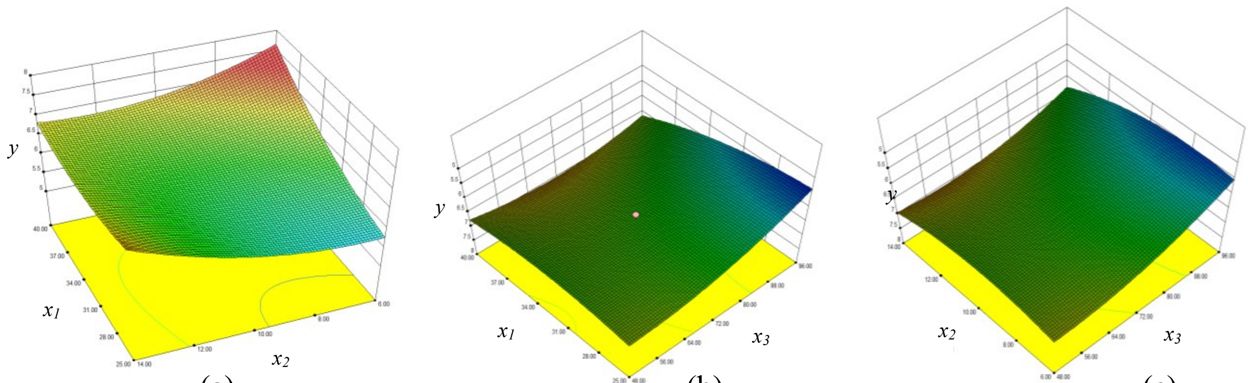
Table 1: Design of experiment and actual values for RSM analysis

Run	Temp. x_1 [°C]	Yeast Conc. x_2 [g/ml]	Time x_3 [hours]	Ethanol Conc. y [g/ml]
1	33	10	72	6.46
2	25	14	96	6.46
3	33	10	72	6.38
4	33	10	106	5.28
5	33	10	72	6.43
6	40	14	48	6.62
7	22	10	72	6.46
8	33	16	72	7.19
9	33	4	72	7.04
10	33	10	72	6.46
11	40	6	96	7.34
12	43	10	72	7.34
13	33	10	38	6.31
14	25	6	48	6.74
15	33	10	72	6.41

Table 2: ANOVA for variables pertaining to ethanol yield

Source	Mean square	*F* value	Prob > *F*
Model	0.37	313.68	<0.0001 [a]
Temp. x_1	0.39	330.94	<0.0001 [a]
Yeast Conc. x_2	0.011	9.62	0.0362 [a]
Time x_3	0.53	453.38	<0.0001 [a]
x_1x_2	0.45	384.32	<0.0001 [a]
x_1x_3	0.18	156.97	0.0002 [a]
x_2x_3	0.029	25.08	0.0074 [a]
x_1^2	0.32	272.02	<0.0001 [a]
x_2^2	0.67	576.27	<0.0001 [a]
x_3^2	0.57	489.24	<0.0001 [a]
Pure Error	0.0012		

[a] Significant at "Prob > *F*" less than 0.05.
[b] Insignificant at "Prob > *F*" more than 0.05.



(a) (b) (c)

Fig. 2: Response surface plot showing the effect of (a) temperature and yeast concentration (b) temperature and fermentation time (c) yeast concentration and fermentation time, on ethanol yield

Fig. 2(a) shows the effect of temperature and yeast concentration on ethanol yield. The maximum production of ethanol was obtained at 5.28-7.34 g/ml. High ethanol yield was obtained between 37-40°C as optimum condition for yeast growth was between 37-46 °C. Yeast will start to degrade at temperature above 49 °C [9]. It was found that higher yeast concentration and low temperature gave increased ethanol yield.

Based on Fig. 2(b), the maximum production of ethanol occured at higher temperature and fermentation time between 64-72 hours. According to Arumugam and colleagues [10], the optimum fermentation time to yield maximum production of ethanol ranged from 42-48 hours. For this study, the presence of inhibitory product in the sample may be the reason for the need of longer fermentation time to achieve higher ethanol yield. As shown in Fig 2(c), ethanol yield was found to slightly increase with increasing fermentation time and yeast concentration.

Response surface optimization. Numerical optimization was performed to predict the optimal conditions for bioethanol synthesis. Table 3 shows that the most desirable condition was at 38 °C, using 6 g/ml yeast for 48 hours.

Table 3: Numerical optimization

Reaction condition	Temperature [°C]	Yeast concentration [g/ml]	Fermentation time [hours]	Predicted yield [g/ml]	Desirability
1	38	6.00	48	7.3401	0.974
2	25	13.30	48	7.1916	0.963
3	37	6.00	48	7.1604	0.955

Conclusion

This research aims to maximize ethanol production from mango peels using RSM. Mango peels were selected as the source of biomass due to their high sugar content which is preferable for bioethanol production via fermentation. The ethanol yield was influenced by fermentation temperature, yeast concentration and time. The optimum condition to produce 7.34 g/ml of ethanol from 10 g mango peels was at 38 °C, 6 g/ml yeast and 48 hours fermentation time.

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