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Utilization of waste Plantain (*Musa paradisiacal*) peels for Bio-Ethanol Production: Acidic Hydrolysis Optimization using response surface methodology.

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Abstract - *In this present study, production and hydrolysis of bio ethanol using acidic solvent from plantain (**Musa Paradisiacal**) peels has been discussed. The process variables (acid conc., temperature and time) were optimized by Response surface methodology. Samples were fermented with **Saccharomyces cerevisiae** strain using the following operating conditions: 2.0 (v/v percentage) of acid conc., 95 degree Celsius of temperature and 20 min of time and produced maximum ethanol yield of 23.18 percentage. The predicted values were in correlation with experimental values. To explain the effect of independent variables on ethanol yield 3D surface plots were generated.*

***Key Words*: Plantain peels, Bio ethanol, hydrolysis, RSM.**

1. INTRODUCTION

The essential power for all human activities such as cooking, heating, lighting, health, food production and storage, education, mineral extraction, industrial production and transportation is Energy. [1] [2]. Consumption of energy has increased steadily over the last century, since the growth of population and industrial revolution. One such major resource required to meet this increased energy demand is crude oil. [3]

Bio ethanol is a promising alternative fuel for energy demand worldwide. It can be obtained from many fruit peels due to their high reducing sugar content. Bio ethanol is domestically produced liquid fuel from renewable resources known as biomass [4] [5]. Due to recent fluctuations in the market of conventional fossil fuels, ethanol is gaining momentum as a viable fuel source. Ethanol is used as an additive, gasoline and even as an alternative fuel source in addition to its common pharmaceutical and beverage uses. Biofuels are generally produced by fermentation of agricultural wastes, fruit wastes, municipal and industrial wastes using Saccharomyces cerevisiae (baker's yeast) as food for the microorganisms [3]. The complexity of the production process depends on the feedstock [6]. Plantain i.e. *Musa paradisiacal* is a commercial herbaceous plantof genus musa mainly cultivated for its edible fruit in most of the developing countries. The *Musa sepientum* and its residues are promising feedstock that can be used to produce ethanol at cheaper rate as compared to other agricultural products through hydrolysis, fermentation and distillation. [7]

In the present work, bio ethanol production employing of *Saccharomyces cerevisiae* strain from plantain peels. The effect of hydrolysis process variables (acid concentration, temperature and time) on ethanol production yield investigated using face centered-central composite response surface design (FCCD). Research reveals that no evidence has been found production of ethanol using plantain peels using FCCD. So, the present work discusses about bio ethanol production from plantain peels using fermentation of *Saccharomyces cerevisiae* strain and optimization of hydrolysis process variables using response surface methodology (RSM).

2. MATERIALS AND METHODS

2.1 Sample preparation and Chemicals used

Plantain peels were obtained from process industries, was chopped into small pieces and dried in an oven at 65˚C for 48 hr . The dried peels were powdered with an electric grinder to a mesh size of 250 μm, packed in polyethylene bags and stored at room temperature for further use. Analytical grade chemical, sulphuric acid (H_2SO_4), sodium hydroxide (NaOH), yeast extracts agar, urea, dextrose sugar (Mg $SO_4.7H_2O$), baker's yeast/yeast (*Saccharomyces cerevisiae* maintained on YEPDA (1% yeast extract, 2% peptone, 2% agar) slant stored at 4 ◦C) and double distilled water were used in the experiment.

2.2 Pre-treatment

A juice was prepared for each sample by adding 10:1 (v/w) ratio of distilled water to the sample in separate flasks. The lignocelluloses molecules must be broken down into free sugars before the fermentation required for alcohol production [8] [9]. The separate samples capped with aluminium foil were autoclaved at 15psi pressure for 30 min. After autoclaving the samples were allowed to cool and the soluble portion was separated from the insoluble using filtration. The insoluble portion was then allowed to hydrolyse, and the amount of sugar produced was measured. [2].

2.3 F-CCD Experimental Design

The Face centered central composite design (F-CCD) has been selected for the optimization of three variables (acid concentration (% v/v), temperature (˚C) and time (min)) on acidic hydrolysis process. After selection of process (independent) variables and their ranges, experiments were established based on an F-CCD and the complete design consists of 20 experiments. The total number of experiments was calculated from the following equation:

$$N = 2K (K - 1) + C_0 \quad \text{... (1)}$$

Where, K is number of factors and C_0 is the number of central point.

For predicting the optimal point after performing experiments, a second- order polynomial model was fitted to correlate relationship between independent variables and responses, which accounts for variations caused by linear and quadratic order effects as well as by interactions [10]. For the three factors, the equation is:

$$Y = \beta_0 + \sum_{i=1}^{K} \beta_i X_i + \sum_{i=1}^{K} \beta_{ii} X_i^2 + \sum_{i<j}^{K} \sum_{i=1}^{K} \beta_{ij} X_i X_j + \sum_{i\le j}^{K} \sum_{j\le k}^{K} \sum_{k}^{K} \beta_{ijk} X_i X_j X_k + \varepsilon \quad \text{...(2)}$$

where Y is the response; X*i* and X*j* are variables (i and j range from 1 to k); β_0 is the model intercept coefficient; β*i*, β*ii* and β*ij* are interaction coefficients of linear, quadratic and the second-order terms, respectively; k is the number of independent parameters (k = 3 in this study) [11].Statistical analysis of the experimental data was performed using the Stat ease Design Expert 8.0.7.1 statistical software (Stat-Ease Inc., Minneapolis, USA).

2.4 Acid hydrolysis

The insoluble component from pre-treatment steps of fruit peels were treated for acid hydrolysis in the reactor with diluted sulphuric acid (H_2SO_4).The process conditions were maintained according to the F-CCD coded level ranges shown table 1. After hydrolysis, pH adjustment was carried out with 1M NaOH until the pH reached a pH of 7. Insoluble particles were separated by filtration and lignin was removed from soluble portion. The soluble component was then added with the previously filtered solution from the pre-treatment step for the next procedure.

Table - 1: F-CCD Design summery of acidic hydrolysis

Process	**Levels**		
Variables	**-1**	**0**	**1**
Acid Concentration (% v/v)	0.5	1.5	2.5
Temperature (° C)	70	90	110
Time (min)	10	20	30

2.5 Fermentation

The sample was prepared for the fermentation process with *Saccharomyces cerevisiae.* 100 ml from the in column was added to 0.5 g of yeast (5 g/L yeast), S. cerevisiae, in 250-ml flask. The flask was covered with aluminum foil to prevent air entrance (the growth was made anaerobically). The conical flask was then placed in a shaking incubator for 24 h at temperature of 30 ˚C and 200 rpm, which was used for the fermentation process. This temperature was the working temperature throughout the entire fermentation process. At this step the pH was set to 5.0-5.5 (which is the optimum pH for the activity of S. cerevisiae) by the addition of 1MNaOH. The 25 ml freshly prepared yeast culture was added into each of the flasks (1:4 (v/v) of samples) and the flask's mouth was wrapped with aluminum foil. Finally, both samples were placed in shaker incubator at 200 rpm at 30 ˚C, for 3 days [2]. The samples were checked every day by adjusting the pH to between 5.0 and 5.5 [12]. Finally, the ethanol product from the plantain peel juice was distillated using a rotary evaporator.

3. RESULTS AND DISCUSSION

Pre-treated samples were treated with acid for hydrolysis .F-CCD design was used to optimize the process variables for acid hydrolysis of ethanol production. Process variables levels were fixed based on laboratory experiments. Fixed levels of F-CCD design summery of variables are shown in Table1. Based on the design summery, 20 experiment conditions has been generated to identify the optimum conditions.20 experimental and predicted values of F-CCD design shown in Table 2. Ethanol yield percentage varied from 12.25% to 22.91%, this could be due to changes in operating conditions of variables.

Table - 2: F-CCD analysis of experimental and predicted values

Run Order	Type	Acid Conc. (% v/v)	Temperature (°C)	Time (min)	Ethanol Yield (% v/v) Y_{Exp}	Y_{Pre}
1	Axial	1.5	110	20	20.81	20.88
2	Axial	0.5	90	20	16.01	16.32
3	Axial	1.5	70	20	20.59	20.65
4	Axial	2.5	90	20	22.69	22.51
5	Axial	1.5	90	10	19.91	19.97
6	Axial	1.5	90	30	20.6	20.66
7	Factorial	2.5	70	10	17.5	17.54
8	Factorial	0.5	110	10	12.61	12.53
9	Factorial	2.5	70	30	19.14	19.19
10	Factorial	0.5	70	10	12.21	12.14
11	Factorial	2.5	110	10	17.86	17.91
12	Factorial	0.5	110	30	12.34	12.26
13	Factorial	0.5	70	30	12.25	12.17
14	Factorial	2.5	110	30	19.2	19.24
15	Center	1.5	90	20	22.65	22.56
16	Center	1.5	90	20	22.31	22.56
17	Center	1.5	90	20	22.59	22.56
18	Center	1.5	90	20	22.91	22.56
19	Center	1.5	90	20	22.35	22.56
20	Center	1.5	90	20	22.81	22.56

3.1 Fitting the Model

To determine whether the model satisfies the assumptions of the analysis of variance (ANOVA), which is shown in Table 3, the probability (P-values) values were used as a model to check the significance of each coefficient which shows the interaction strength of each parameter. The smaller the P-values are, the bigger the significance of the corresponding coefficient. Probability values of "Prob > F" less than 0.0500 indicate the model terms are significant. Model p-value (<0.0001) shows highly significant with ethanol response. In this present study X_1, X_3, X_{13}, X_1^2, X_2^2 and X_3^2 are significant model terms. Full quadratic multiple regression analysis of the experimental data yielded the following regression equations for the recovery of bioethanol of acidic hydrolysis;

$$Y = 22.56 + 3.10X_1 + 0.11X_2 + 0.34\,X_3 - 0.00875X_1X_2$$

$$+ 0.40\,X_1X_3 - 0.076X_2X_3 - 3.15\,X_1^2 - 1.80\,X_2^2 - 2.24\,X_3^2 \quad \text{.......(1)}$$

Where Y is the yield of bioethanol %. The Predicted R^2 of 0.9984 is as close to the Adjusted R^2 of 0.9969 indicates a negligible block effect and are best fit for optimization.

Fitness of the experimental data with the selected model is indicated through the lack of fit data provided by ANOVA [13]. This study's lack of fit (0.18) value and its corresponding p-value (0.6833) clearly interprets that the adequate fit of model with experimental data. Degree of precision and reliability of the conducted experiments can be evaluated along with the deviations between the experimental and predicted values through CV values. Minimum CV values (1.15 %) of this experiment, interpret the low deviation between the experimental and predicted value, high degree of precision and a good deal of reliability of the experiment.

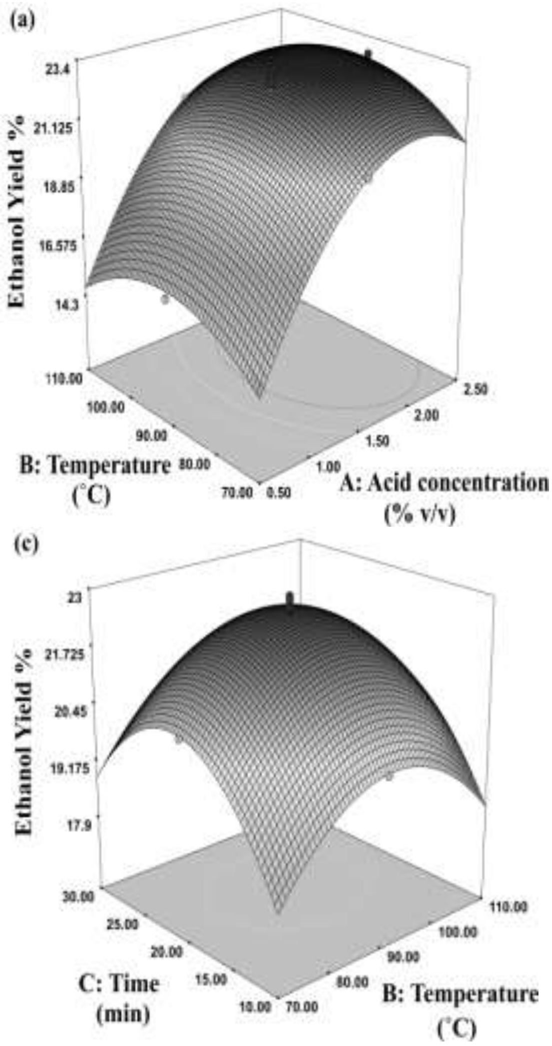
Table - 3: ANOVA for response surface quadratic model of ethanol yield (%)

Source	Sum of squares	df	F Value	p-value prob>F	remarks
Model	289.91	9	679.87	<0.0001	Significant
X_1-acid conc.	95.91	1	2024.37	<0.0001	
X_2-Temp	0.13	1	2.27	0.1317	
X_1-time	1.18	1	24.98	0.0005	
X_1X_2	0.0006	1	0.013	0.9117	
X1X3	1.29	1	27.18	0.0004	
X2X3	0.047	1	0.98	0.3451	
X12	27.25	1	575.09	<0.0001	
X22	8.89	1	187.58	<0.0001	
X32	13.83	1	291.94	<0.0001	
residual	0.473	10			
Lack of Fit	0.18	5	0.64	0.6833	Not significant
Pure error	0.29	5			
Cor total	290.38	19			
			R2	0.9984	
Std.Dev	0.22		AdiR2	0.9969	
mean	18.97		Pred R2	0.9942	
C.V%	1.15		Adeq Precision	67.738	
PRESS	1.67				

3.2 Effect of hydrolysis variables on ethanol Yield

3.2.1 Effect of acid concentration

Effect of acid concentration on ethanol yield is shown in Fig 1a and 1b.It was found that increasing the acid concentration level, production of ethanol yield could be increased. At lower concentration (< 1.5), cellulose might not get converted into simple glucose, and with a higher concentration (2.0 <), the cellulose might convert to other molecules that may not fermentable [14] [15]. Hence, acid concentration has a strong relationship for the yield of ethanol production ($p<0.0001$). This result is also similar to the experiment that was performed for the ethanol production from turnip, papaya, and apple and yield of ethanol, which obtained 1.5% (v/v). [16]



3.2.2 Effect of Temperature

Effect of temperature is represented in Fig 1b and 1c.It clearly shows that increasing the temperature up to 90 ˚C increases the ethanol yield. Beyond 90 ˚C yield starts to decrease gradually. This might be due to the possible formation of other molecules instead of the glucose formation at high temperature [17]. Moreover denaturisation of glucose occurs at elevated temperature. At lower temperature, the yield of ethanol is lower, because of the sample had not hydrolysed with sufficient temperature of the process.

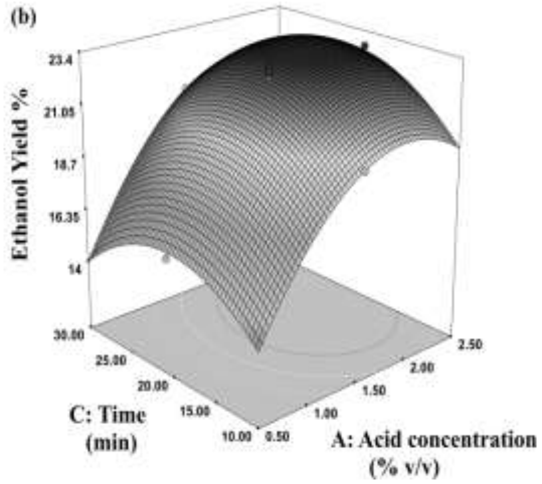


Fig -1: Effect of hydrolysis process variables on Ethanol yield %.(a)acid conc. and temperature. (b) Acid conc. and time (c) Temperature and Time.

3.2.3 Effect of Time

Independent variable time was high significant with ethanol yield % ($p<0.0005$).3D surface plots Fig 1b and 1c show the effect on ethanol yield. Increase the time of process, which results the production reducing sugar content. Neither acid concentration nor temperature of the process variables involved in hydrolysis, which reducing the ethanol yield beyond 20 min. At lower time of process, ethanol yield get started due to hydrolysis process has been initiated.

3.3 Optimization and validation of ethanol production

F-CCD design was used for optimization of maximum yield of bio ethanol. The optimum conditions were as follows: 1.97 (v/v %) of acid conc., 94.04 ˚C of temperature and 20.04 min of time. The bio ethanol yield obtained under these conditions are 23.27 %. Optimum conditions can be modified on considering practical application as 2.0 (v/v %) of acid conc., 95 ˚C of temperature and 20 min of time. The bio ethanol yield under modified optimum acidic hydrolysis is 23.18 ± 0.76% , these yield was found to be in well correlation with the predicted values.

4. CONCLUSION

Plantain (*Musa Paradisiacal*) peel is a suitable source for the production of ethanol. The experimental results show that at optimum parameters ,2.0 % v/v acid concentration, 95 ˚C temperature and 20 min retention time, 23.18 % of ethanol can be produced. Analysis of variance (ANOVA) correlation coefficients Pred R^2 0.9942 and Adj R^2 0.9969 indicates excellent evaluation of the experimental data by a second-order polynomial regression model. 3D surface plots clearly shown the effect of independent variables of acidic hydrolysis process on ethanol yield. The outcome of this research demonstrates that this process might represent a valid alternative to minimize the heavy waste burden of plantain peels. The waste from the food processing industry may bring serious environmental problems and can be minimized by the production of ethanol.

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