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Bioconversion of Coir Waste to Glucose for Bioethanol

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Abstract:
Although coir waste is resistant to natural degradation, it can be degraded with the help of important enzyme like cellulase produced by organisms like Aspergillus niger and Pleurotus sajor caju. The process produces glucose, which can be converted to ethanol through sequential saccharification and fermentation with the help of mixed fungal cultures. The present work aims at conversion of the used coir into glucose which can be used for producing bioethanol. The batch experiments are statistically designed and performed using Box-Benhken method of Response Surface Methodology to investigate the influence of major parameters viz. pH, temperature and substrate concentration on glucose production. The maximum glucose production of 1.44mg/ml was achieved under the conditions of substrate concentration 11.46 g/l , pH 5.79 and temperature 32.6 ^{o}C
Keywords — **Coir waste, Mixed culture, Optimization, Saccharification.**

I. INTRODUCTION

Coconut coir is composed of lignocellulosic materials such as cellulose, lignin, pectin and hemicellulose. Cellulosic biomass constitutes the most abundant organic molecules on earth [1]. All cellulosic waste materials can be converted into commercially important products such as citric acid, ethanol, methane, glucose syrups and singlecell proteins [2]. Bioconversion, particularly enzymatic hydrolysis, of these cellulosic materials into simple sugars, has been a subject of intensive research [3]. A well known by-product of the coconut is the husk which is the source of the coir fiber. About 70 % of the husk is “pith or waste” which is rich in lignocellulosic content [4]. The total quantity of coir waste in India is estimated to be 5,00,000 tonnes per annum. After the removal of the nuts, the coconut husks are being used as raw material for manufacturing coir fibre.

Coir waste, an agro industrial residual, is resistant to natural degradation; polyphenol leaching makes it unfit for the normal landfill practices either. It also pollutes the nearby receiving water body by changing the physico-chemical properties. Sustainable management of coir pith can be achieved by converting it into useful products through useful techniques.

Coir waste or coir pith is generally a mixture of dust, bits and fibres of lesser length which are rejected during processing centers year by year, disposal of which is a major problem. This coir waste accounts for about 50-60 percent of the total weight of the husk. The untapped potentials of coir waste, an abundantly available lignocellulosic byproduct of the coir industry, is today being recognized with its worth confirmed in various applications [5].

Coir waste can be used as a potential source for adsorption of textile dyes from textile effluent [6]. Dyes usually have a synthetic origin and complex aromatic structure which make them more stable and more difficult to biodegrade. The coir after decolorization can be degraded using Aspergillusniger and Pleurotus sajor caju. These organisms will degrade the coir and they will produce important enzyme like cellulase [7]. The degraded coir contains glucose. This can be converted to ethanol by Saccharomyces cerevisae [8].

II. MATERIALS AND METHODS

A. Cellulosic Waste

Coir waste was used as substrate for cellulase enzyme and glucose production. The ground coir material was used as microbial substrate.

B. Microbial Strains

Fungi were isolated from A.niger that was obtained from the surface of the decaying coconut and identified by their morphological, colony and molecular characteristics. It was cultured in the Potato dextrose agar slants. Pleurotus sajor caju obtained from Agricultural University, Mannuthy was inoculated into malt media.

C. Saccharification of coir waste

Biodegradation of coir waste was studied in solid state in Erlenmeyer flasks (250 ml) using cellulolytic fungi isolated from natural sources. Five grams of coir waste containing 60 % moisture was taken in individual Erlenmeyer flasks (250 ml). For mixed culture studies, the spore suspension was taken as 1: 1: 1 as inocula. The conical flasks were incubated at 28 ± 2 ℃ for a period of 30 days in the culture room. Separate flasks were maintained for studying the compositional changes in coir waste. Every 5 days of interval of study, the entire content of flask was withdrawn, filtered and was used in the analysis for measuring cellulase activity and total reducing sugars [9].

D. Determination of cellulase activity and total reducing sugars

Reducing sugars were determined using DNS method[10]. The supernatant from the degraded coir sample was collected and was centrifuged at 10,000rpm for 5mins to remove any unwanted debris. The supernatant after centrifugation was taken and1.5ml of the DNS solution was added to it. This was kept in a boiling water bath for 5mins. The test tubes were removed and while the solution was still hot, 0.5ml of Sodium Potassium Tartarate (40%) was added. The absorbance at 575nm was read.

1. Cellulase assay:

Cellulase activity was assayed by using Carboxymethyl-cellulose (CMC) as substrate. The reaction mixture contained 1ml of 1.0% (w/v) CMC in 0.1M solution of sodium acetate buffer, pH 5.0, and 0.5 ml of the cell-free culture supernatant. The mixture was incubated at 50 ^{0}C for 30 to 60 minutes. The reducing sugar released by the enzyme was measured as glucose equivalent using dinitrosalicyclic acid reagent. A unit of activity was defined as the amount of enzyme required to liberate1 mol of glucose per minute under the assay conditions.

E. Optimization of saccharification of Coir

Saccharification of coir waste by mixed fungal strain was cultivated with varying temperatures of 10^{0}C-50^{0}C, pH range 4.5-6.5, and substrate concentration 5-15 g/l to choose the appropriate temperature, pH and substrate concentration.

F. Experimental design

In the present study, the response surface methodology (RSM) has been used as a statistical technique to design experiments, build models and to determine the relation between the amount of glucose production and operating parameters such as Substrate concentration, pH, and Temperature. Table 1 gives the parameters and the operating ranges covered.

Table I

Level and range of variables chosen for degradation of coir waste

Sl Number	Variable	-1	0	1
1	Substrate Concentration (g/l)	5	10	15
2	pH	4.5	5.5	6.5
3	Temperature (^{0}C)	10	30	50

The Substrate concentration, pH, and temperature are referred by uncoded variables as X_1, X_2 and X_3 respectively. The variables in uncoded form are converted to coded form: x_1, x_2 and x_3 using the following equation:

$$x = \frac{X - ((X_{max} + X_{min})/2)}{((X_{max} - X_{min})/2)} \quad (1)$$

"The Box–Behnken experimental design of RSM has been chosen to find the relationship between the response functions and variables using the statistical software tool MINITAB 16 (PA, USA). In the Box–Behnken method a total number of 15 experiments are carried out to estimate the amount of glucose production. The interaction between the variables and the analysis of variance (ANOVA) has been studied by using RSM. The quality of the fit of this model is expressed by the coefficient of determination R^2" [11].

G. *Response surface methodology:*

The responses viz. amount of glucose production measured for different combinations of substrate concentration, pH and temperature for the 15 combinations of experimental conditions as proposed by the RSM design are reported in ***Table 2***. Each run was performed in triplicate to guarantee the reliability of results. In addition, a full quadratic model with regression coefficients was selected to fit the experimental data. Apart from the intercept, linear, and quadratic terms, this model also considers two-way interactions:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (2)$$

Where y is the amount of glucose production in mg/ml,β_0 is a constant,β_1 ,β_2 , β_3 are the regression coefficients for linear effects, β_{11}, β_{22}, β_{33} are the quadratic coefficients and β_{12}, β_{13}, β_{23} are the interaction coefficients. The coefficients of the model are given in Table 2.

With the help of the above model proposed by RSM it is possible to forecast the amount of glucose production at any combination of the three parameters in consideration within the experimental sphere of influence. The significance of regression coefficients for the amount of glucose production were analysed using p- and t-test.

Table 2

Glucose production (Responses) using different combinations of parameters

Sl. No	X_1(g/1)	X_2	X_3(^{0}C)	Glucose Production y(mg/ml)
1	5	4.5	30	0.35
2	15	4.5	30	0.79
3	5	4.5	30	0.35
4	15	6.5	30	1.12
5	5	5.5	10	0.56
6	15	5.5	10	1.02
7	5	5.5	50	0.66
8	15	5.5	50	1.11
9	10	4.5	10	0.72
10	10	6.5	10	1.06
11	10	4.5	50	0.85
12	10	6.5	50	1.14
13	10	5.5	30	1.38
14	10	5.5	30	1.37
15	10	5.5	30	1.36

Table 3

Statistical significance of coefficients

Factor	Coefficient of the model in coded factors	t value	p value	Significance level
β_0	- 8.56475	- 9.967	0	Significant
β_1	0.30779	8.803	0	Significant
β_2	2.63713	9.116	0	Significant
β_3	0.02371	2.885	0	Significant
β_{11}	-0.01134	-12.808	0	Significant
β_{22}	-0.22349	- 8.714	0	Significant
β_{33}	- 0.00030	- 4.620	0	Significant
β_{12}	0.0000	0.000	1.000	Not Significant
β_{13}	- 0.0000	- 0.000	1.000	Not Significant
β_{23}	- 0.00062	- 0.507	0.634	Not Significant

The 'p','t' and significant level are given in Table 3 and Table 4 respectively. It can be observed from the table that all terms except the interaction terms are considerably influencing the response.

$$Y_1 = -10.21 + 0.346X_1 + 3.1625X_2 + 0.030875X_3 - 0.01505X_1^2 - 0.27125X_2^2 - 0.00042X_3^2 \quad (3)$$

The above equation shows the coefficients of the model in uncoded factors. Finally Analysis of Variance (ANOVA) was used in the statistical analysis software (MINITAB 16 PA, USA)) for graphical analysis of the data to obtain the interaction between the process variables and the response. The quality of the fit polynomial model was expressed by the coefficient of determination R^2 and adjusted R^2 in the same program. Its statistical significance was checked by the Fisher's F-test in the same program. Model terms are evaluated by p-value (probability) with 95% confidence level. A high R^2 coefficient ensured smaller differences between the observed data and the fitted values thereby satisfactory representation of the second-order regression model to the experimental data. In the present study, the values of R^2 were 0.9982 for amount of glucose production.

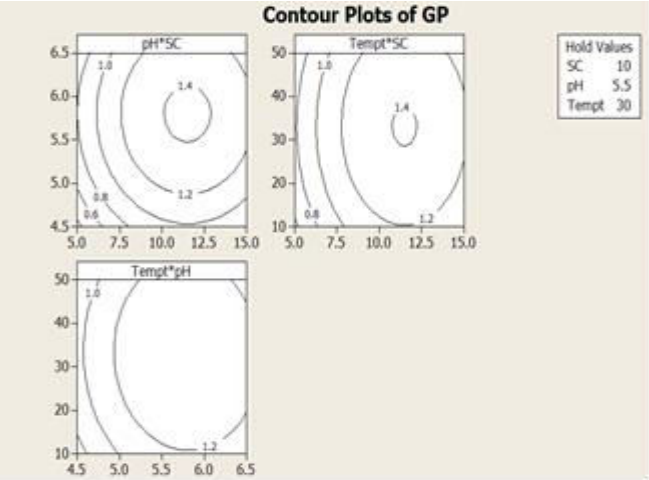

Fig. 1 Contour plot of variables

The plots are derived from the quadratic models of Eqs. (3) It was obvious that the effects of substrate concentration, pH and temperature on glucose production exhibited the same tendency. All the three plots evidently indicated that the response surfaces for glucose production show a clear peak, suggesting that optimum conditions for maximum glucose production were well inside the design boundary.[12] The optimum operating conditions as predicted by the response optimizer tool of MINITAB gave a maximum glucose production of 1.44 mg/ml with desirability of 96.238 for the parameter values of 11.46 g/l for substrate concentration 5.79 for pH and 32.62^{o}C for temperature. The optimization plot obtained is shown in figure 2.

This implies that the proposed quadratic regression model reasonably optimize the operating conditions and predict the maximum response.

The optimum substrate concentration was found to be 11.46 g/l. This may be due to the fact that mixed culture contain all the enzyme complex of cellulase enzyme and hence they can convert high concentration of coir in to sugars

. P^H has a decisive effect on the saccharification of coir waste. At the pH value of 4.5, there was very little saccharification. but it began to rise as the initial pH of the growth medium was increased and reached maximum at pH 5.79. Further rise in pH resulted in a gradual reduction of saccharification by the organism. Hence, pH of 5.79 was optimized for the maximum saccharification. After pH value of 5.79, the production of cellulases decreased which may be due to the fact that cellulase are acidic proteins and are greatly affected by the neutral pH values [13]. pH is among most important factors for any fermentation process and depends upon microorganisms because each microorganism possesses a pH range for its growth and activity [14]. .Increase and decrease in pH on either side of the optimum value resulted in decrease of growth product fermentation [15].

Temperature plays an important role in the amount of glucose produced. There was a gradual increase in saccharification as the temperature was increased. However, it showed maximum yield at 32.62^{0}C. As the temperature was further increased, there was a gradual reduction in the saccharification. This may be due to the fact that higher temperature denatures the saccharifying

.enzymes mainly cellulase[16]. High temperature may also lead to inhibition of microbial growth[17] showed that cellulases production and thus saccharification was maximum in flasks incubated at 32.62^{0}C and decreased with high temperature.

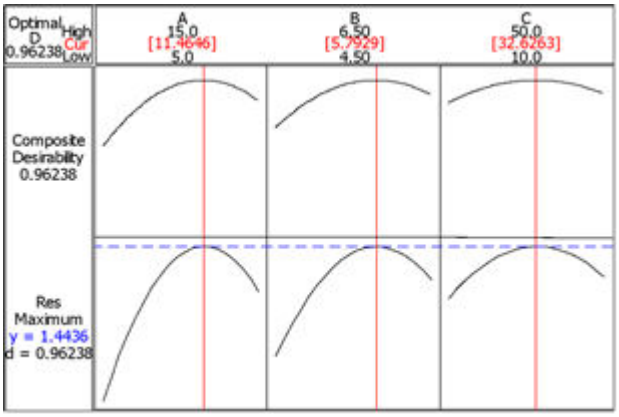

Fig. 2 Optimization plot for Glucose production

III. CONCLUSIONS

The coir waste is simply discarded after decolourisation process without any treatment which causes many problems to the water bodies as well as the environment. In our study degradation of the used coir was done with Aspergillus.niger and Pleurotus sajor caju. After degradation by microbes crude enzymes were formed as by products and finally glucose was produced by using yeast. The enzyme produced has wide range of application in different fields. By this work we could convert coir waste into glucose.

The Response Surface Methodology (RSM), a statistical tool for the design of experiments, was implemented using the software MINITAB16. RSM with a fifteen run Box-Behnken design was performed to investigate the influence of different parameters on the glucose production and a second-order regression model was generated. DNS Assay test was done to determine the glucose content. The amount of glucose produced was found to be function of the substrate concentration, pH and temperature.

The use of Box-Behnken design to create a set of experimental runs can reduce the number of runs needed to optimize the operating conditions in comparison with the one factor at a time experiment method. Under optimal conditions of process parameters (Substrate concentration = 11.46g/l, pH = 5.79, and Temperature= 32.62^{o}C, an optimal desirability of more than 96% and a maximum glucose production of 1.44 mg/ml were obtained.

The quadratic model proposed shows the existence of a high correlation between experimental and predicted values. ANOVA was conducted to test the significance of the second order model. It showed a high coefficient of determination value (Glucose production R^2 0.9982) thus ensuring a satisfactory conformity of the second-order regression model with the experimental data.

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