Optimization of Saccharification of Biological Pre-Treated Rice Straw by Response Surface Methodology

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Abstract

Pretreatment of rice straw facilitating the enzymatic saccharification process was performed by a ligninolytic fungal consortium, resulting in rice straw having 56.4% cellulose, 12.2% hemicelluloses, and 2.2% lignin. Using response surface methodology, the combined effect of substrate concentration, temperature and pH on reducing sugar yield from enzymatic saccharification of rice straw optimized substrate concentration, 5.0% (w/v); temperature, 55°C and pH, 4.8 yielding a maximum sugar content of 492.0 milligram per gram dry substrate (mg/gds) with a saccharification efficiency of 81.1%. The experimental results were in good agreement with predicted values. Therefore, the model could be successfully used to identify the effective combinations of the three factors for predicting reducing sugar yield.

Keywords
Rice straw, Fungal consortium, Saccharification, Bioethanol, Response surface methodology.

Introduction

Lignocellulosic biomass is an attractive and economical feedstock for bioethanol production because of its abundance and renewable nature (El-Ahmady El-Naggar et al., 2014). Rice straw consisting of cellulose (35-40%, w/w), hemicelluloses (20-30%, w/w) and lignin (10-15%, w/w) is one of the most abundant lignocellulosic wastes in the world (Binod et al., 2010). It has been estimated that in India, paddy is cultivated in about 43 million ha producing about 96 million tons of rice and 250 million tons of straw. The options for the disposition of rice straw are limited by the low bulk density, slow degradation in the soil, harbouring of rice stem disease and high mineral content (Kocher et al., 2016).

Nowadays, field burning, though an offense is the major practice for removing rice straw, but it increases the air pollution and consequently affects the public health (Wi et al., 2013).

Pretreatment is essential for removal of lignin and hemicellulose to reduce cellulose crystallinity and increase the porosity of biomass (Zhu et al., 2015). Post pretreatment, the amorphous cellulose is saccharified by the enzymes, and the resulting glucose is fermented to ethanol. Therefore, optimization of saccharification is an important step in the development of an efficient and cost effective saccharification strategy. Further, the traditional ‘one-factor-at-a-time approach’ is time consuming and does not reveal the interactive effects between the variables for optimization (Qi
et al., 2009). In this regard, response surface methodology (RSM) is an effective tool wherein many factors and their interactions affecting the response can be identified with fewer experimental trials [Box et al., 1978]. The RSM includes three major steps: (1) implementing a statistically designed experimental plan to the data, (2) developing a regression model to correlate experimental data, and (3) predicting the response of target variables to the process parameters using the regression model (Li et al., 2010; Vanderghem et al., 2010).

In the present study, rice straw pretreated with a ligninolytic fungal consortium was to identify the optimum saccharification conditions by using RSM based on a second order Central composite design (CCD).

Materials and methods

Microorganisms

A fungal consortium was developed using Pleurotus ostreatus, procured from Department of Microbiology, PAU Ludhiana and Phanerochaete chrysosporium MTCC 787 procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH) Chandigarh.

Development of ligninolytic consortia

The mycelia of P. ostreatus and P. chrysosporium were grown on PDA medium. The spores were obtained from one week old Petri plates by rinsing the plates with sterile distilled water and collecting the spores in sterile vials. The spore count was determined with the help of haemocytometer. The fungal spore suspension of 1x10^8 spores/ml was used for inoculation.

Enzyme production

The ligninolytic enzyme production from fungal consortium was carried out under solid state fermentation conditions using paddy straw (500 µ), moistened with lignin modifying enzyme (LME) basal medium (Pointing, 2010) to 80% moisture content. The experimental flasks were autoclaved at 121°C for 15 min, inoculated with fungal spore suspension (1x10^8 spores/ml) and incubated at 28°C in a BOD incubator.

Enzyme extraction and concentration

The extraction was performed by mixing the contents of experimental flask with 0.1 M potassium phosphate buffer (pH 6.0), added to achieve a solid/ liquid ratio of 1: 10 (w/v). The suspension was stirred at 120 rpm for 30 min in an orbital shaking incubator. The biomass was filtered using Whatman filter paper (No.1) and the filtrates were centrifuged for 10 min at 6000 rpm. The supernatants (crude enzyme extract) was subjected to ultrafiltration using a Polyethersulphone (PES) membrane of 10 KDa molecular weight cut off (CleaN Sep, Pvt. Ltd., Mumbai).

Enzyme assay

The concentrated enzyme extract was assayed for ligninolytic enzyme activity viz. laccase, lignin peroxidase and manganese peroxidase by the methods of Desai et al., (2011); Tien and Kirk (1988) and Paszczynski et al., (1988) respectively.

Pretreatment and proximate analysis of rice straw

The rice straw used in the present study was collected from Demonstration area, School of Renewable Energy Engineering, Punjab Agricultural University, Ludhiana. The washed and sun dried straw was chopped, ground to 2-10 mm and finally sieved to 30 mesh size. The rice straw was pretreated with concentrated (10 folds) enzyme extract under shake flask conditions using 2.5 g rice straw, 8.0 ml concentrated ligninolytic enzyme extract, supplemented with 10 mM Ca^{2+} ions and incubated at 45°C for an incubation period of 72 h (unpublished data). The pretreated biomass samples were analysed for Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) by the method of Goering and Van Soest (1970).

Saccharification of pretreated rice straw

Saccharification experiments were performed using commercial cellulase enzyme from
Arrowzymes (Banglore, India). The cellulase (30 FPU/g-substrate) was added to 0.1 M sodium citrate buffer (pH 4.8- 6.8) supplemented with 0.02% (w/v) sodium azide to inhibit microbial contamination, and then mixed to the substrate at a concentration of 5.0 -12.0% (w/v).

The experiments were carried out in 125 ml Erlenmeyer flasks containing 20 ml total reaction volume (the buffer–enzyme mixture). A surfactant, Tween 20 (0.2%) which can enhance the enzymatic conversion of lignocellulosic biomass and does not inhibit cell growth in the downstream fermentation process was used in these saccharification experiments (Sharma et al., 2014). The flasks were incubated at different temperatures (45-55°C) for 48 h in a rotary shaker at 100 rpm. Each sample taken from the saccharified solution was heated to 100°C immediately for 3 min to denature the enzymes, cooled to room temperature, and then centrifuged for 10 min at 6000 rpm. The supernatant was used for reducing sugar analysis (Miller, 1959). The saccharification (%) was determined using the following formula:

\[
\text{Saccharification (\%) = \frac{\text{Amount of reducing sugars formed (g)}}{\text{Amount of cellulose (g)}} \times 0.9 \times 100}
\]

**Statistical design of experiments**

To determine the best combination of parameters for optimizing the enzymatic saccharification of rice straw, a second order Central composite design (CCD) was employed. In CCD, the total number of experimental combination were \(2^k+2K+n_o\), where \(K\) is the number of independent variables and \(n_o\) is the number of repetitions of the experiment at the central point. The CCD contains a total of 20 experimental runs with five level full factorial design and replications of the central and the axial points. The substrate concentration (% w/v), temperature (°C) and pH were selected as independent variables and dependent variable selected was reducing sugar yield (mg/g dry substrate).

The Design-Expert software, version 9.0.4.1 developed by (Stat- Ease Inc., Minneapolis, MN, USA) was used to build and analyze the experimental design e.g. analysis of variance (ANOVA), determination of the estimated effects and interaction, regression equation which was fitted to the data. Three dimensional surface plots were drawn to show the effects of independent variables on response and a quadratic polynomial equation was proposed to describe the mathematical relationship between the variables and the response. The significance of the model was evaluated by determination of \(R^2\) and adjusted \(R^2\) coefficient. An experiment was also conducted to confirm the predicted optimum response using the selected optimum values of three variables. A second order polynomial equation was used to describe the effects of variables on the response:

\[
Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \epsilon
\]

Where, \(Y\) is the predicted response i.e., reducing sugar yield of enzymatic saccharification, \(\beta_0\) is a constant; \(\beta_1, \beta_2, \beta_3\) are the linear coefficients; \(\beta_{12}, \beta_{13}, \beta_{23}\) are the quadratic coefficients; \(\beta_{11}, \beta_{22}, \beta_{33}\) are the cross coefficients.

**Statistical analysis**

All the experiments pertaining to proximate analysis were carried out in triplicates with mean and standard deviation (SD) values calculated using MS Excel program.

**Results and Discussion**

**Proximate analysis of rice straw**

Pretreatment enhances saccharification rates and sugar yields of biomasses by decreasing the crystallinity of cellulose and breaking the lignin-hemicellulose sheath that surrounds the cellulose (Belal, 2013). Therefore, the rice straw was subjected to biological pretreatment i.e. ligninolytic fungal consortium consisting of *Pleurotus ostreatus* and *Phanerochaete chrysosporium*. The compositions of the rice straw before and after pretreatment were given in Table 1. The percent cellulose content increased to 56.4 % from 39.5 % of untreated rice straw. In contrast, the percent hemicellulose and lignin content after fungal consortium pretreatment
decreased to 12.2% and 2.4% from 23.3% and 11.5%, respectively, indicating that 47.6% and 79.1% lignin and hemicellulose can be removed by ligninolytic fungal consortium pretreatment. Similar to the present work, the rice straw has been reported to be pretreated with ligninolytic/ligninocellulolytic fungal consortium such as consortium of Cerrena unicolor and Chaetomium brasilense (Ramarajan and Manohar, 2017); consortium of Paenibacillus sp. and Aspergillus fumigatus (Matthews, 2016). According to Liao et al., (2005) either hemicellulose or lignin, prevent cellulose from being attacked by inhibiting the enzymes physically or chemically. The removal of these two fractions reduces the cellulose crystallinity and, the porosity and accessible surface area of substrate are increased, favouring the enzymatic attack (Mussatto et al., 2008).

**Development of a model for enzymatic saccharification of rice straw**

Three independent variables viz. substrate concentration % (w/v), temperature (°C) and pH and their values at different coded and actual levels were employed in the design matrix (Table 2). Table 3 shows the levels of selected variables for the CCD and summarizes the response values along with the predicted values.

The ANOVA was carried out to determine the significance of the model equation and the model terms (Table 4). The statistical significance of the above equation was checked by the $F$ test. The model $F$-value of 130.92 and a low probability value ($P < 0.0001$) showed that the model terms were significant. The standard deviation (12.0) and coefficient of variation, which is a measure of residual variation of the data relative to the size of the mean and is inversely related to the reliability of experiment (4.48%), were reasonably low and acceptable.

The coefficient of determination ($R^2$) value (0.99), a measure of the amount of variation around the mean, explained by the model indicated that only 1.0% of all variation for response could not be explained by the model and expresses a good fit. Figure 1 show that observed reducing sugars yield (the response) agreed well with the predicted data.

**Table 1 Chemical composition of untreated and pretreated rice straw samples**

| Samples              | Chemical composition (w/w, %) |  |
|----------------------|-----------------------------|---|
|                      | Cellulose                   | Hemicellulose | Lignin   |
| untreated rice straw | 39.5±0.20                   | 23.3±0.21     | 11.5±0.17 |
| pretreated rice straw| 56.4±3.1                    | 12.2±0.67     | 2.4±0.13  |

**Table 2 Coded values of the variables for the central composite design**

| Independent variables and symbols for actual values | Coded symbols | Actual values of coded levels |
|------------------------------------------------------|--------------|------------------------------|
| Substrate concentration % (w/v)                      | $X_1$        | -α (-1.68) -1 0 1 +α (+1.68) |
| Temperature (°C)                                    | $X_2$        | 4.5 5.0 8.75 12.5 11.25      |
| pH                                                   | $X_3$        | 4.12 4.8 5.8 6.8 7.48        |
**Table 3** Experimental design and results of CCD for saccharification of pretreated rice straw

| Run no. | Variable | Response |
|---------|----------|----------|
|          | Substrate concentration % (w/v) | Temperature (°C) | pH | Reducing sugars (mg/gds) |
|          | Observed values | Predicted values |
| 1 | 2.45 | 50.0 | 5.8 | 236.0 | 246.0 |
| 2 | 8.75 | 50.0 | 5.8 | 240.0 | 246.0 |
| 3 | 8.75 | 50.0 | 5.8 | 252.0 | 246.0 |
| 4 | 8.75 | 50.0 | 5.8 | 252.0 | 246.0 |
| 5 | 12.50 | 55.0 | 4.8 | 408.0 | 410.0 |
| 6 | 12.50 | 45.0 | 6.8 | 206.0 | 202.0 |
| 7 | 5.00 | 55.0 | 6.8 | 168.0 | 158.0 |
| 8 | 8.75 | 50.0 | 5.8 | 260.0 | 246.0 |
| 9 | 8.75 | 41.6 | 5.8 | 274.0 | 278.0 |
| 10 | 5.00 | 55.0 | 4.8 | 476.0 | 452.0 |
| 11 | 5.00 | 45.0 | 6.8 | 160.0 | 158.0 |
| 12 | 8.75 | 50.0 | 5.8 | 238.0 | 246.0 |
| 13 | 12.50 | 45.0 | 4.8 | 354.0 | 334.0 |
| 14 | 12.50 | 55.0 | 6.8 | 200.0 | 202.0 |
| 15 | 8.75 | 50.0 | 7.48 | 116.0 | 188.0 |
| 16 | 8.75 | 50.0 | 4.11 | 460.0 | 402.0 |
| 17 | 8.75 | 50.0 | 5.8 | 236.0 | 246.0 |
| 18 | 5.00 | 45.0 | 4.8 | 380.0 | 378.0 |
| 19 | 15.05 | 50.0 | 5.8 | 258.0 | 246.0 |
| 20 | 8.75 | 58.4 | 5.8 | 334.0 | 342.0 |

**Table 4** Analysis of variance (ANOVA) for the Quadratic Model with respect to saccharification of pretreated rice straw

| Source                | Reducing sugars (mg/gds) | Sum of squares | Degree of freedom | Mean square | F-value | Probability (P)>F |
|-----------------------|--------------------------|----------------|-------------------|-------------|---------|------------------|
| Model                 |                          | 1.791E+005     | 9                 | 19898.76    | 130.92  | < 0.0001         |
| Residual              |                          | 1519.94        | 10                | 151.99      |         |                  |
| Lack of Fit           |                          | 1052.60        | 5                 | 210.52      | 2.25    | 0.1968           |
| Pure error            |                          | 467.33         | 5                 | 93.47       |         |                  |
| Cor Total             |                          | 1.806E+005     | 19                |             |         |                  |
| Standard deviation    |                          | 12.0           |                   |             |         |                  |
| Coefficient of variation (CV) % | | 4.48           |                   |             |         |                  |
| R² value              |                          | 0.99           |                   |             |         |                  |
| Adjusted R² value     |                          | 0.98           |                   |             |         |                  |
| Predicted R² value    |                          | 0.95           |                   |             |         |                  |
| Adequate Precision    |                          | 41.32          |                   |             |         |                  |
Table 5: Significance of coefficients for the response model with respect to saccharification of pretreated rice straw

| Model terms | Reducing sugars (mg/gds) | Coefficient Estimate | Standard error | F-value | P-value |
|-------------|--------------------------|----------------------|----------------|---------|---------|
| β₀          | 245.94                   | 5.03                 |                |         |         |
| β₁          | 1.54                     | 3.34                 | 0.21           | 0.6547  |         |
| β₂          | 18.52                    | 3.34                 | 30.81          | 0.0002  |         |
| β₃          | -107.09                  | 3.34                 | 1030.48        | < 0.0001|         |
| β₁₁        | 2.83                     | 3.25                 | 0.76           | 0.4034  |         |
| β₂₂        | 22.99                    | 3.25                 | 50.10          | < 0.0001|         |
| β₃₃        | 17.33                    | 3.25                 | 28.47          | < 0.0001|         |
| β₁ β₂      | -7.00                    | 4.36                 | 2.58           | 0.1394  |         |
| β₁ β₃      | 21.50                    | 4.36                 | 24.33          | < 0.0001|         |
| β₂ β₃      | -18.50                   | 4.36                 | 18.01          | 0.0017  |         |

*p value less than 0.05 indicates model terms are significant

Table 6: Effect of variable’s interaction on enzymatic saccharification of pretreated rice straw

| Variable/Response | Goal       | Lower limit | Upper limit | Predicted value | Actual value |
|-------------------|------------|-------------|-------------|-----------------|--------------|
| A(Substrate concentration) | In range   | 5.0         | 2.5         | 12.5            | NA           |
| B (Temperature)   | In range   | 45.0        | 55.0        | 55.0            | NA           |
| C (pH)            | In range   | 4.8         | 6.8         | 4.8             | NA           |
| Reducing sugars (mg/gds) | Maximize   | 116         | 476         | 460.2           | 476.0 ±26.18 |

Table 7: Enzymatic saccharification conditions and yields from various studies on lignocellulosic biomasses

| Substrate                  | Pretreatment   | Reducing sugar yield (mg/gds) | Enzyme used                          | Reference                     |
|----------------------------|----------------|-------------------------------|--------------------------------------|-------------------------------|
| Parthenium sp.             | Fungal         | 485.6                         | Accellerase 1500                     | (Rana et al., 2013)           |
| Giant reed                 | Dilute sulphuric acid | 481.6                      | Celluclast 1.5 L and Novozyme-188    | (Shatalov et al., 2012)       |
| Switch grass               | Dilute sulphuric acid | 440.0                      | Commercial cellulase                 | (Ruan et al., 2013)           |
| Rice hull                  | Alkaline peroxide | 154.0                     | Celluclast 1.5 L and Novozyme 188    | (Saha and Cotta 2008)         |
| Rice straw                 | Steam explosion | 132.0                     | Celluclast 1.5 L, Periconia sp. bcc2871 | (Harnpicharnchai et al., 2009) |
| Rice hull                  | Lime           | 428.0                      | Celluclast, Novozyme 188 and Viscostar | (Saha and Cotta 2008)         |
| Corn stover, Miscanthus and wheat straw | Sodium hydroxide | 215, 258, and 280, respectively | Cellulase Onozuka | (Vintila et al., 2010) |
| Rice straw                 | Fungal consortium | 492.0                      | Arrowzyme                             | Present study                 |
Figure 1 The predicted versus actual plot for reducing sugar response

Figure 2 The 3-D response surface plots showing effects of various parameters on enzymatic saccharification of rice straw

2(a)
**Figure 3** Normal probability of internally studentized residuals for reducing sugars

**Figure 4** Plot of internally studentized residuals versus predicted response
For the developed model, the ‘predicted coefficient of determination’, $R^2$ value of 0.95 was in reasonable agreement with the ‘adjusted coefficient of determination’, $R^2$ value of 0.98. This indicated a good adjustment between the observed and predicted values. ‘Adequate precision’ represents the signal-to-noise (S/N) ratio and values $>4.0$ indicate that the model precision is Adequate. ‘Adequate precision’ ratio of 41.32 indicated an adequate signal.

Table 5 shows the F-test and the corresponding $P$-value along with the parameter estimate. The smaller the $p$-values, the bigger the significance of the corresponding coefficient. The parameter estimates and the corresponding $p$-values suggest that, among the independent variables, $X_2$ (temperature), and $X_4$ (pH) have significant effects on reducing sugars yield. The quadratic terms of $X_2$, and $X_3$ and interactions between $X_1$ and $X_3$, $X_2$ and $X_3$ have significant effects on reducing sugars yield. A statistically significant model only with significant terms can be written as follows:

$$Y=245.94+18.52*X_1+107.09*X_2+21.50*X_1*X_2-18.50*X_2^2+22.99*X_3^2+17.33*X_3^2$$

**Equation 2**

Where $Y$= reducing sugar yield, $X_1$-substrate concentration % (w/v), $X_2$-temperature (°C) and $X_3$- pH

### Effect of interaction of variables on reducing sugars yield

To examine the interaction of the variables and to determine the optimum level of each variable for maximum response, 3-D response surface curves were plotted against two experimental factors while maintaining the other factor constant at its central value. The two significant interactions (substrate concentration versus pH and temperature versus pH) are presented in Figure 2. The effect of interaction of substrate concentration and pH, when temperature is at central level (50°C) is shown in Figure 2(a). The reducing sugars increased with increase in substrate concentration up to 5.0% (w/v) and pH to 4.8, after which a decrease in the reducing sugars was observed. Enzymes posses’ ionic groups on their active sites which function in a suitable environment (acid or base). A change of pH in the medium leads to modification of enzyme in the ionic form of active site and its three-dimensional shape, as a result of which enzymes are active over a certain pH range. Most hydrolytic enzymes work best between the pH range of 3-5, with best performance of cellulase enzyme observed at pH 5 (Shuler and Kargi, 1992). Phuengjaayem et al., (2014) studied the effect of pH on saccharification of sweet sorghum bagasse and observed that maximum of 0.115 g glucose/g of dry solid was obtained at pH 5. Increased consistency, product inhibition and reduced surface contact between the enzyme and the substrate are responsible for the low reducing sugars content at high substrate concentrations. The extent of substrate inhibition is dependent on the ratio of the total substrate to total enzyme loaded (Xin et al., 2010; Wang et al., 2011). The effect of interaction of temperature and pH, with a substrate concentration (8.75%, w/v) at central level is presented in Figure 2 (b). The release of reducing sugars increased with an increase in temperature to 55.0°C from 45°C and decrease in pH to 4.8 from 6.8. This could be explained on the basis of gain of kinetic energy by the reactant molecules with increase in temperature and hence a more productive collision per unit time, resulting in better yields at high temperature (Segel, 1976). Shah et al., (2016) optimized the conditions for saccharification of laccase pretreated empty fruit bunch by RSM at enzyme concentration, 30 FPU/g; substrate concentration, 5.0% (w/v); temperature, 50°C; saccharification time, 24 h and pH 5, resulting in highest total sugars yield of 28.0% (w/w). The optimum parameters obtained for the hydrolysis of rice straw with cellulase from Aspergillus niger were pH 6.0; substrate concentration, 12% (w/v) and enzyme concentration, 10 U/g of rice straw. Under the optimum conditions, fermentable sugar content of 3.62g/l was obtained (Ong et al., 2012). Lai et al., (2016) reported the optimization of glucose production from ammonia pretreated oil palm trunk biomass via saccharification using two commercial enzymes namely, cellulast 1.5 (cellulase) from Trichoderma reesei and Novozyme 188 (β-glucosidase) from Aspergillus niger, with the help of RSM. Under the established optimum conditions of temperature, 44.23°C; pH 5.22 and enzyme ratio, 3:1, a maximum glucose yield of 4.964 g/l was obtained which was in close agreement with the value of...
4.958 g/l predicted by the model.

**Optimization of reducing sugars yield of rice straw**

Numerical optimization of saccharification process was carried out using Design Expert software, version 9.0.4.1, to evaluate the optimum values of different parameters from the developed model. In optimization, the desired goal was to maximize the reducing sugar yield with highest importance and the factors were selected to be within the mentioned range. The desirability values vary between 0 and 1 depending upon the proximity between predicted and the actual values. In the present experiment, a desirability of 0.956 was obtained. Response analysis predicted the maximum reducing sugars yield under the optimum process conditions i.e. when substrate concentration was 5.0% (w/v), temperature was 55° C and pH was 4.8 (Table 6). Validation experiment under these predicted optimum conditions yielded 476.0 mg/gds reducing sugars experimentally which was close to the predicted reducing sugars yield of 460.0 mg/gds. Thus, overall good agreement was observed between experimentally determined/observed responses and predicted optimum response.

**Confirmation of adequacy of model**

The residuals from the least squares are an important tool for judging the model adequacy to ensure that it provides maximum approximation on the relationship between factors and response. Normal probability was checked by plotting the normal probability plot of residuals. The normality assumption was satisfactory as normal residuals fall along a straight line as shown in Fig. 3. Figure 4 is the plot of residuals versus the predicted response. The residual plots of the model were randomly distributed without any trends which indicated good predictions of maximum response adequacy of the quadratic models.

**Effect of surfactant**

To enhance the rate of saccharification, Tween 20 (0.2%) was added in the experimental flask, which resulted in reducing sugar yield of 492.0 ±27.06 mg/gds compared to 460±25.3 mg/gds reducing sugars without surfactant addition. The final saccharification efficiency achieved from pretreated rice straw was 81.1%. Non-ionic surfactants like Tween 20 have proven to be effective in increasing cellulose hydrolysis. The mechanism underlying the enhancement of enzymatic cellulose hydrolysis is the increase of effective cellulosic surface area as well as increase in enzyme stability by reducing thermal denaturation (Kaar and Holtzapple, 1998; Erickson et al., 2002). For the saccharification of dilute acid pretreated rice hulls, addition of Tween 20 at 2.5g/L concentration enhanced the rice hulls saccharification by 3.5% (Saha et al., 2005). In another study, addition of Tween 80 resulted in increase in hydrolysis yield from 69 to 91% after 48 h for pretreated Lodgepole pine (Tu et al., 2009).

**Comparison of enzymatic saccharification conditions and yields with other lignocellulosic biomass**

The saccharification yield during enzymatic hydrolysis of different lignocellulosic biomass as reported by other workers is summarized in Table 7. The results revealed rice straw as a potential substrate in yielding highest amount of sugars under optimized conditions. The present study highlights the primacy of rice straw as a feedstock for bioethanol production as well as confirms the validity of RSM as compared to conventional methods of optimization. The pretreatment of rice straw by fungal consortium have the additional advantage of obtaining cellulose rich substrate in an eco-friendly way. In addition, use of rice straw substrate, an abundantly available agricultural residue without overhead costs makes the finding of this investigation a promising approach for bioethanol production.

**Acknowledgment**

The authors thank Mr. Shivkaran, Technical Service Manager, Nature Bioscience Pvt. Ltd., New Delhi for kindly providing the Arrowzyme.

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How to cite this article:
Pardeep Kaur, Gurvinder Singh Kocher and Monica Sachdeva Taggar. 2017. Optimization of Saccharification of Biological Pre-Treated Rice Straw by Response Surface Methodology. Int.J.Curr.Microbiol.App.Sci. 6(10): 1112-1123. doi: https://doi.org/10.20546/ijcmas.2017.610.135