Dilute acid pretreatment and enzymatic sachharification of cotton ginning waste

K U Chaudhary1*, A Padhiar2

1 Research Scholar, Shri M. M. Patel Institute of Sciences and Research, Kadi Sarva Vishwavidyalaya, Gandhinagar, 382015, India
2 Assistant Professor, Shri M. M. Patel Institute of Sciences and Research, Kadi Sarva Vishwavidyalaya, Gandhinagar, 382015, India

Abstract

Background/Objective: The objective is to study the effect of dilute sulfuric acid pretreatment and enzymatic saccharification of cotton ginning waste to produced fermentable sugars. The Box Behnken design (BBD) was used for optimization of physical parameters. Method: Cellulase production by Trichoderma sp. (A11) was carried out under solid state fermentation using wheat bran. Pretreatment were carried out with 3% (V/V) diluted sulfuric acid and sachharification with crude cellulase enzyme of cotton ginning waste. The proximate analysis of treated and untreated cotton ginning biomass was done by Goering and Van Soest method to find the cellulose, hemicellulose and lignin content. The total reducing sugar was measured by using Di-nitro salicylic acid (DNSA) method. Incubation time, moisture ratio and substrate loading was optimized by using BBD for sachharification of cotton ginning waste. Finding: Untreated cotton ginning waste biomass contain 40.7% (w/w) of cellulose and 17.3 % (w/w) of lignin. Under sulfuric acid treatment, the residual acid treated cotton ginning waste biomass was found to have 62.4 ± 4.1 % (w/w) cellulose and 9.9 ± 0.6 % (w/w) lignin. Acid treated cotton ginning waste produced 117.3 mg/g while as untreated cotton ginning waste produced 29.7mg/g total reducing sugar. After statistical optimization by using BBD total reducing sugar was increased to 328 mg/g experimentally, at an optimum 5% of substrate concentration, 10 FPU enzyme loading on 7th day of incubation period and at 5 pH. Application: The fermentable sugar which is produced from cotton ginning waste biomass which can be further utilized for bioethanol production.

Keywords: Cotton ginning waste; acid pretreatment; Cellulase; enzymatic sachharification
1 Introduction

Among the different conversion technologies from lignocellulosic biomass to ethanol, pretreatment followed by enzymatic hydrolysis and fermentation is claimed to be more beneficial. Pretreatment of cellulosic biomass in a cost effective manner is a major challenge of cellulose to ethanol technology research and development. Agricultural crop residues such as rice straw, wheat straw, sugarcane bagasse, sugarcane tops, cotton stalk, soft bamboo, bamboo processing wastes are considered as abundantly available feed stocks for bioethanol production globally, and particularly in tropical countries such as India.

Pretreatment of the feedstock, the first step in the process of making bioethanol from cotton ginning waste biomass required alteration of both biomass structure as well as chemical composition, so that hydrolysis of carbohydrate to monomeric sugars can be achieved more rapidly with higher yields. Various parameters such as enzyme loading, temperature, incubation time and substrate concentration are affected on efficiency of sachharification of pretreated substrate. Interactions between these factors and optimization of enzymatic hydrolysis plays an important role in improvement of substrates hydrolysis. Basically, statistically designed experiments such as RSM were used for optimization of physical and chemical parameters, estimation the regression coefficient in a mathematical model, predicting the response of model and checking the adequacy of the model. To determine how the process behaves in different situations, a mathematical model enables simple manipulation of variables to be accomplished. A model generally incorporates a number of parameters that can be affected the desired process. Mathematical modelling of enzymatic degradation of cellulose is highly challenging as it is necessary to balance complex biological processes with many variables.

The study was focused on efficient pretreatment and sachharification of cotton ginning waste which can be further utilized for bioethanol production. The cotton ginning wastes biomass were subjected to pretreatment and then saccharification was carried out by Trichoderma spp. for cellulase enzyme production. Improvement of efficiency of sachharification of pretreated cotton ginning wastes can be analysed statistically using BBD.

2 Material and Methods

Microbial strain

The locally isolated culture Trichoderma sp. (A11) (accession number MN203130.1) strain was used in the present study. Culture was maintained at 4C on PDA slants. This strain was previously isolated and studied for cellulase production in our laboratory.

Cotton Ginning waste

CGW was collected from local cotton ginning mills, Kadi district, Ahmedabad, India. All samples were stored in air-tight containers at room temperature for composition analysis. All chemicals of analytical grade, purchased from Sigma Aldrich and Merck, were used in this research.

Cellulase enzyme production and extraction from wheat bran

Crude cellulase enzyme was produced by Trichoderma sp. (A11) under solid state fermentation from wheat bran. Cellulase production under solid state fermentation was carried out in 250 ml Erlenmeyer flasks using 5 g of washed and dried wheat bran. Cellulase production was done using Mandel's media as the moistening agent and wheat bran as the substrate having ratio of 1:3 substrate to moistening agent at 35C. Inoculum was prepared from 7 days old culture that had been grown on PDA slants at 30C. Each flask was inoculated with 1ml of inoculum containing $1 \times 10^5$ spores/ml of Trichoderma spp. and incubated at 30 C. The flasks were incubated at 30 ± 2 C for 6 days. Enzymes were extracted from substrate flask by

https://www.indjst.org/
addition of 5 ml of cold 0.05 M acetate buffer (pH 4.8). The homogenate was filtered through muslin cloth and the filtrate was centrifuged at 5000 rpm at 4 C for 15min. The supernatant used as crude enzyme source and was analysed for endoglucanase activity (CMCase) and filter paper activity (FPase).

Enzyme assay
The activity of cellulolytic filter paper activity (FPase) and endoglucanase (CMCase) were determined according to Pankajkumar et al. [8]

Pretreatment of cotton ginning waste
Cotton ginning waste was washed with tap water for 30 min to remove impurities such as soil and dried at room temperature. The pretreatment of cotton ginning waste with dilute sulphuric acid (3% v/v) was carried out and untreated biomass was taken as control. The biomass-to-liquor ratio was maintained to 1:20. Pretreated cotton ginning waste and untreated cotton ginning waste biomass were washed with distilled water until pH 7 was obtained. The neutralized residues were dried at 60 C to a constant weight. The dry weights of fibres after pretreatment have been used for calculations. Finally, the residues were sealed in polybags and used for composition analysis and enzymatic hydrolysis.

Proximate analysis of the cotton wastes
The proximate analysis of the cotton ginning wastes were performed before pretreatment as control and after acid pre-treatment for cellulose, hemicellulose and lignin. Untreated and pre-treated cotton ginning waste substrates was estimated using protocol given by Goering and Van Soest method[9].

Enzymatic saccharification of acid treated and untreated cotton ginning waste
Enzymatic saccharification of acid treated and untreated cotton ginning waste was carried out using crude cellulase enzyme produced from wheat bran using *Trichoderma* A11. Crude cellulase from A11 were used at the level of 10 FPU/g during saccharification of acid treated and untreated substrates. In this method, enzymatic hydrolysis was performed in 500 ml screw cap Erlenmeyer flasks containing 5% pretreated and untreated substrates (w/v) and diluted enzyme in 0.05M sodium citrate buffer (pH 4.8) containing 10 mM sodium azide to prevent microbial contamination in a final volume of 100 ml. Controls were kept for each reaction in which the active enzyme was replaced with heat in-activated enzyme. After hydrolysis, the samples were filtered and centrifuged at 3000g for 10 min to remove unhydrolyzed residues. The reducing sugar (measured as glucose) content of the supernatant was determined using the 3, 5-dinitrosalicylic acid method (DNSA) at interval of 24 h[10].

Effect of temperature on enzymatic saccharification of cotton ginning wastes
The influence of temperature (30 C, 40 C and 50C) on enzymatic hydrolysis were studied using in-house produced cellulase. Samples were withdrawn after every 24 h of incubation. One ml sample were subjected for centrifugation and the supernatant was analysed for total reducing sugars by DNSA method.

Response surface methodology for optimization of enzymatic sachharification
Optimization of saccharification process was carried out using 3.0% acidic (diluted sulfuric acid (v/v)) treated cotton ginning waste as a substrate. Box–Behnken design (BBD) involves full factorial search by observing simultaneously, systematic and efficient variation of important components of the saccharification process. A Box–Behnken design in three factors having three centre runs (with a total of 15 experimental runs) was used for the optimization of saccharification process. The independent variables used for the analysis were substrate loading (%) (X1), Cellulase enzyme loading (U/g) (X2), and Time (days) (X3). Total reducing sugar (mg/g) was the dependent response variable and each of three independent variables was studied at three different levels shown in Table 1. All the experiments were carried out using 2.5% (w/v) substrate concentration at pH 4.8, at 50C with mild shaking (100 rpm). The contents of the flaks were analysed for reducing sugars after specific time planed in BBD ( Table 2 ).
Quadratic models considered as response surface model for predicting the optimal points were expressed according to Eqs. (1) and (2).

\[ x_i = (X_i - X_0) / \partial X_i \]  
(1)

Where \( X_i \) is the experimental value of the variable; \( X_0 \) is the midpoint of \( X_i \), \( \partial X_i \) is the step change in \( X_i \) and \( x_i \) is the coded value for \( X_i \), \( i = 1–3 \).

Released reducing sugar was analysed and response surface model given Eq. (2) were fitted using MINITAB 16.0.

\[ Y = \beta_0 + \Sigma \beta xi + \Sigma \beta ijX^2i + \Sigma \beta ijXiXi \]  
(2)

where \( Y \) is the predicted response variable, \( \beta_0 \), \( \beta_i \), \( \beta_{ii} \), \( \beta_{ij} \) are fixed regression coefficients of the model, \( x_i, x_j \) \( (i = 1,2,3,4,5\) and \( 6, i = j, i \neq j = 1,2,3,4,5,6) \) represent independent variables in the form of original values.

**Table 1.** Selected variables with range for BBD

| Variables                  | Symbols | -1   | 0   | 1   |
|----------------------------|---------|------|-----|-----|
| Substrate con. (%)         | X1      | 1:1  | 1:3 | 1:5 |
| Cellulase enzyme (FPU/g)   | X2      | 1:1  | 1:3 | 1:5 |
| Time (Days)                | X3      | 3    | 5   | 7   |

**Table 2.** Actual design with coded value of variable

| Run Order | Substrate con. | Enzyme (FPU) | Time (Days) | Total Reducing Experimental | Sugar (mg/g) Predicted |
|-----------|----------------|--------------|-------------|-----------------------------|------------------------|
| 1         | 2.5            | 2            | 6           | 22                          | 18.750                 |
| 2         | 7.5            | 2            | 6           | 14                          | 21.500                 |
| 3         | 2.5            | 10           | 6           | 97                          | 89.500                 |
| 4         | 7.5            | 10           | 6           | 311                         | 314.250                |
| 5         | 2.5            | 6            | 2           | 73                          | 80.125                 |
| 6         | 7.5            | 6            | 2           | 125                         | 121.375                |
| 7         | 2.5            | 6            | 10          | 73                          | 76.625                 |
| 8         | 7.5            | 6            | 10          | 270                         | 262.875                |
| 9         | 5.0            | 2            | 2           | 85                          | 81.125                 |
| 10        | 5.0            | 10           | 2           | 225                         | 225.375                |
| 11        | 5.0            | 2            | 10          | 113                         | 112.625                |
| 12        | 5.0            | 10           | 10          | 328                         | 331.875                |
| 13        | 5.0            | 6            | 6           | 276                         | 276.333                |
| 14        | 5.0            | 6            | 6           | 277                         | 276.333                |
| 15        | 5.0            | 6            | 6           | 276                         | 276.333                |

**Interpretation and data analysis**

The results of the experimental design was analysed and interpreted using MINITAB 16 (PA, USA) statistical software. Prediction of fermentation parameters and response contour plot generated by the model was also done by the same software. ANOVA was used to establish the significance of the model parameters.
3 Results

Proximate analysis of cotton ginning waste

Untreated cotton ginning waste biomass contain 40.7% (w/w) of cellulose and 17.3 % (w/w) of lignin. Under sulfuric acid treatment conditions, the residual acid treated cotton ginning waste biomass was found to have 62.4 ± 4.1 % (w/w) cellulose and 9.9 ± 0.6 % (w/w) lignin (Table 3). Hemicellulose is converted into cellulose and lignin was dissolved due to acid treatment, which increases crystallinity index of acid pretreated samples. Same study has been reported by Converse et al. (11) for microcrystalline cellulose, Sindhu et al. (2010) (12) for sugarcane bagasse and Satyanagalakshmi et al. (2011) (13) for water hyacinth. After pretreatment with acid, cellulose concentration was increases which is favourable for hydrolysis for cellulase enzyme. The National Renewable Energy Laboratory (NREL), overseers of the largest biomass ethanol development effort in the world, favour the use of dilute acid for substrates hydrolysis, according to their research 80% to 90% of hemicellulose sugars are recoverable by dilute acid technology (Aden A et al.,) (14). Acid pretreatment dissolved little lignin and various studies indicate that lignin is disrupted which will increasing cellulose susceptibility to enzymes (15).

| Table 3. Composition of untreated and pretreated cotton ginning waste |
|---------------------------------------------------------------|
| Cotton ginning waste | Hemicellulose | Cellulose | Lignin | Holocellulose |
|----------------------|---------------|-----------|-------|--------------|
| Untreated            | 21.1%         | 40.7%     | 17.3% | 72.7%        |
| 3% H2SO4 treated     | 5.3%          | 62.4%     | 9.9%  | 67.7%        |

All experiments were done in duplicate and average values are given.

Effect of pretreatment and incubation time on enzymatic sachharification of cotton ginning waste

Saccharification was carried out using 10 FPU g⁻¹ of substrate and the reducing sugars were measured by DNSA method after every 24 hrs of intervals. During study of incubation time course of enzymatic saccharification of the untreated and acid treated cotton ginning waste, a regular increase in sugar was observed till 7th day of incubation. However, after attaining the maximum rate of saccharification, the saccharification rate was decreased. Maximum reducing sugar from waste biomass after 7th day of incubation was 117.2 mg g⁻¹ for acid treated waste and 29.7 mg g⁻¹ untreated waste (Table 4). Thus the use of dilute acid pretreatment shows improvements in cellulose digestibility over those obtained for CGW by microbial pretreatment.

| Table 4. Effect of pretreatment on sachharification of cotton ginning waste: |
|-----------------------------------------------|
| Incubation time (Days) | Total Untreated | Reducing Sugar (TRS) (mg/g) |
|------------------------|-----------------|----------------------------|
|                        |                 | 3% H2SO4 treated           |
| Day 1                  | 11.3            | 12.7                       |
| Day 2                  | 15.7            | 25.6                       |
| Day 3                  | 17              | 54                         |
| Day 4                  | 21              | 60.6                       |
| Day 5                  | 23              | 58.4                       |
| Day 6                  | 25.7            | 64.74                      |
| Day 7                  | 29.4            | 117.23                     |
| Day 8                  | 29              | 97.65                      |
| Day 9                  | 27              | 84.78                      |

All experiments were done in duplicate and average values are given.
Effect of temperature on saccharification:

In order to evaluate the optimal temperature for enzymatic saccharification of cotton waste, the hydrolysis was performed at 30–55 C showing in Figure 1. The results indicate that the maximum enzymatic hydrolysis occurred at 50 C, corresponding to a degree of saccharification of reducing sugar is 251 mg/g in Cotton ginning waste shown in graph 1. However, a minor decrease in saccharification was observed at 55 C. The results also indicated that increasing the hydrolysis temperature to 55 C decreased the saccharification of substrates.

![Figure 1. Effect of temperature on cotton ginning waste](https://www.indjst.org/)

Effect of substrate loading on saccharification process

Enzymatic saccharification at high substrate loading have many advantages, like positive effect on economy of the process as it allows use of smaller reactor, produce concentrated sugar syrup and eventually lowers distillation cost. Up to 10% substrate loading was investigation by the cocktail of crude and pure cellulase for enzymatic sachharification (16). The results clearly indicate that there was decline in sugar yield with increase in substrate loading. Enzymatic saccharification at high substrate loading suffers some inherent problems such as concentrations of end products and inhibitors both difficult to optimum function of enzymes and hence reducing sugar is reduced. At 5 % substrate loading maximum 332 mg/g reducing sugar were obtained. After 10% reducing sugar was decline. At 10% substrate loading reducing sugar was 194mg/g which shown in Figure 2. High substrate loading produced concentrated sugar syrup which is highly advantageous for commercial bio-ethanol production process.
Optimization of saccharification of cotton ginning waste by response surface methodology using Box–Behnken design

Three variables viz. substrate con. (%), Cellulase enzyme (FPU/g), and pH were selected for response surface optimization and reducing sugar g g-1 was selected as response variable. The results of all the experimental runs (15 run) are shown in Table 1. The experimental results show that the variable used in the present study had strong effect on total reducing sugar production. By using MINITAB 16 statistical testing was carried, on the basis of these experimental values. As shown in Table 4 A, the analysis of the model was tested by Fisher’s ‘F’ and Student’s t-test. Analysis of variance (ANOVA) of reducing sugar indicate that the model was significant (P=0.000), mainly because of the square portion of the regression model. A P-value which is below 0.05 indicates the test parameters are significant and above 0.05 indicated parameters are non-significant.

Table 5. Analysis of variance

| Source           | DF | Adj SS  | Adj MS  | F-Value | P-Value |
|------------------|----|---------|---------|---------|---------|
| Model            | 9  | 174370  | 19374.4 | 331.28  | 0       |
| Linear           | 3  | 101466  | 33822.1 | 578.32  | 0       |
| Square           | 3  | 53920   | 17973.3 | 307.32  | 0       |
| Interaction      | 3  | 18984   | 6327.8  | 108.2   | 0       |
| Error            | 5  | 292     | 58.5    | -       | -       |
| Lack-of-Fit      | 3  | 292     | 97.3    | 291.75  | 0.003   |
| Pure Error       | 2  | 1       | 0.3     | -       | -       |
| Total            | 14 | 174662  | -       | -       | -       |

Fig 2. Effect of substrate loading in cotton
The fitted second-order response surface model as specified by Equation 2\(^{(17)}\) for total reducing sugar in coded process variables is as follows:

\[
Y(\text{TRS}) = -343.0 + 141.97 \times x_1 + 30.25X_2 + 7.59 \times 2 - 17.427X^2 - 3.526X^2 - 2.010X^2 + 5.550 \times 1 \times 2 + 3.625 \times 1 \times 3 + 1.172 \times 2 \times 3
\]

Where, \(Y\) is Total reducing sugar, and \(x_1, x_2\) and \(x_3\) are uncoded values of substrate con (%), Cellulase enzyme (FPU/g) and Time (days), respectively. A comparison of the experimentally obtained values with the predicted obtained values indicated that these data are in reasonable agreement as shown in Table 2. The parameter estimates and the corresponding P-value shows that selected variable substrate con (%), cellulase enzyme (FPU/g) and time had significant square effect (0.000, 0.000 and 0.000) on reducing sugar (Table 5 and Table 6). Also interaction between substrate and enzyme, substrate and time and enzyme and time had significant square effect (0.000, 0.000 and 0.004) on reducing sugar (Table 4 B). The \(R^2\) value provides a measure of variability in the observed response values that can be explained by the experimental factors and their interactions. Joglekar and May\(^{(18)}\) have suggested that for a good fit of the model, \(R^2\) should be at least 80%. Coefficient \(R^2\) for reducing sugar yield was observed to be 97.33%.

Application of RSM by Box-Behnken design predicted that maximum total reducing sugar produced with different optimum parameters of 5% substrate con., 10 FPU and on 7\(^{th}\) day. The model predicted highest (optimum) reducing sugar of 328mg/g (Figure 1). According to the predictive model of S. McIntosh et al; 2014\(^{(19)}\) investigated production of 273mg/g acid retreated CGT was observed.

The effects of substrate loading and incubation time on the sachharification of CGW are shown in Figure 3 when the enzyme unit factors was at their center points. At low levels of biomass loading (2.5%) and incubation time, the production of total reducing sugar was low. Significant improvement in the sachharification could be obtained by increasing biomass loading. When the biomass loading was set at 5.0%, the reducing sugar was reached a maximum 312mg/g treated biomass and further increase in biomass loading did not increase the sugar level. For enzymatic reaction, a fixed substrate concentration is required to reach the adsorption saturation of enzymes and further increase in substrate concentration results in a constant rate of product formation. Biomass loading is considered to be one of the major factors affecting the conversion rate of enzymatic hydrolysis of cellulose. High substrate concentration results in low hydrolysis yield due to product inhibition, enzymatic inactivation, and a decrease in the reactivity of cellulosic substrate with proceeding of hydrolysis process\(^{(20)}\).

From Figure 4 it was observed that total reducing sugar was high at 7\(^{th}\) day of incubation and 10 FPU enzyme loading. At low levels of enzyme loading, reducing sugar yield was low and with increase in enzyme...

---

Table 6. Coded coefficients

| Term             | Coef  | SE Coef | T-Value | P-Value |
|------------------|-------|---------|---------|---------|
| Constant         | 276.3 | 4.42    | 62.59   | 0       |
| Substrate con.   | 56.87 | 2.7     | 21.04   | 0       |
| Enzyme           | 90.88 | 2.7     | 33.61   | 0       |
| Time             | 34.5  | 2.7     | 12.76   | 0       |
| Substrate con. * | -108.9| 3.98    | -27.37  | 0       |
| Substrate con.   |       |         |         |         |
| Enzyme *Enzyme   | -56.42| 3.98    | -14.18  | 0       |
| Time *Time       | -32.17| 3.98    | -8.08   | 0       |
| Substrate con. * | 55.5  | 3.82    | 14.51   | 0       |
| Substrate con.*Time | 36.25| 3.82    | 9.48    | 0       |
| Enzyme *Time     | 18.75 | 3.82    | 4.9     | 0.004   |
loading, there was a significant increase in reducing sugar. Incubation time is an important factor affecting the enzymatic hydrolysis. It was inferred that the reducing sugar increased with increase in incubation time. Figure 5 explains the interaction between biomass loading and enzyme loading on reducing sugar liberation. It was observed that 5% substrate loading and 10 FPU enzyme loading gave highest amount of liberation reducing sugar.

**Fig 3.** Interaction effect of substrate concentration and incubation time on TRS

**Fig 4.** Interaction effect of enzyme loading and Incubation time on TRS
4 Conclusion

The acid pretreatment of cotton ginning waste decreases hemicelluloses (21.1% to 5.3 %) and lignin (17.3% to 9%) and the cellulose content was increased from 40.7% to 62.4% which was helped in enzymatic sachharification and improved the reducing sugar yield. Acid treated cotton ginning waste produced 117.3 mg/g while as untreated cotton ginning waste produced 29.7mg/g total reducing sugar. By statistical optimization of sachharification, total reducing sugar was enhanced from 117.3 to 328 mg/g experimentally. RSM based on BBD shown that substrate loading and enzyme loading was most significant effect on enzymatic saccharification of CGW. The high similarity between the experimental value (328 mg/g) and the predicted value (331 mg/g) of total reducing sugar shown that the RSM was an accurate and applicable tool to optimize the parameters.

References

1) Sonderegger M, Jeppsson M, Larsson C, Gorwa-Grauslund MF, Boles E, Olsson L, et al. Fermentation performance of engineered and evolved xylose-fermenting Saccharomyces cerevisiae strains. *Biotechnology and Bioengineering*. 2004;87(1):90–98. Available from: https://dx.doi.org/10.1002/bit.20094.

2) Pandey A, Biswas S, Sukumaran RK, Kaushik N. Study on the Availability of Indian Biomass Resources for Exploitation: A Report Based on Nationwide Survey. TIFAC, New Delhi. 2009. Available from: https://tifac.org.in/index.php/8-publication/240.

3) Qi B, Chen X, Shen F, Su Y, Wan Y. Optimization of Enzymatic Hydrolysis of Wheat Straw Pretreated by Alkaline Peroxide Using Response Surface Methodology. *Industrial & Engineering Chemistry Research*. 2009;48(15):7346–7353. Available from: https://dx.doi.org/10.1021/ie8016863.

4) Sindhu R, Kuttiraja M, Binod P, Janu KU, Sukumaran RK, Pandey A. Dilute acid pretreatment and enzymatic saccharification of sugarcane tops for bioethanol production. *Bioresource Technology*. 2011;102(23):10915–10921. Available from: https://dx.doi.org/10.1016/j.biortech.2011.09.066.
5) Shi J, Chinn M, Sharma-Shivappa R. Microbial pretreatment of cotton stalks by solid state cultivation of Phanerochaete chrysosporium. Bioresource Technology. 2008;99(14):6556–6564. Available from: https://dx.doi.org/10.1016/j.biortech.2007.11.069.

6) Narra M, Dixit G, Divecha J, Kumar K, Madamwar D, Shah AR. Production, purification and characterization of a novel GH 12 family endoglucanase from Aspergillus terreus and its application in enzymatic degradation of delignified rice straw. International Biodeterioration & Biodegradation. 2014;88:150–161. Available from: https://dx.doi.org/10.1016/j.ibiod.2013.12.016.

7) Mandel M, Resse E. Induction of cellulase in fungi in Trichoderma viride as influencing carbon source. Journal of Bacteriology. 1957;37:268–298.

8) Waghmare RP, Patil MS, Jadhav SL, Jeon BH, Govindwar SP. Utilization of agricultural waste biomass by cellulolytic isolate Enterobacter sp. SUK-Bio. Agriculture and Natural Resources. 2017. Available from: https://doi.org/10.1007/j.anres.2018.10.019.

9) Goering K, Soest PJ. Forage fiber analysis-agriculture research series. and others, editor. 1975.

10) Miller GL. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry. 1959;31(3):426–428. Available from: https://dx.doi.org/10.1021/ac60147a030.

11) Converse AO, Matsuno R, Tanaka M, Taniguchi M. A model of enzyme adsorption and hydrolysis of microcrystalline cellulose with slow deactivation of the adsorbed enzyme. Biotechnology and Bioengineering. 1988;32(1):38–45. Available from: https://dx.doi.org/10.1002/bit.260320107.

12) Sindhu R, Binod P, Satyanagalakshmi K, Janu KU, Sajna KV, Kurien N, et al. Formic Acid as a Potential Pretreatment Agent for the Conversion of Sugarcane Bagasse to Bioethanol. Applied Biochemistry and Biotechnology. 2010;162(8):2313–2323. Available from: https://dx.doi.org/10.1007/s12010-010-9004-2.

13) Satyanagalakshmi K, Sindhu R, Binod P, Janu KU, Sukumaran RK, Pandey A. Bioethanol production from acid pretreated water hyacinth by separate hydrolysis and fermentation. Journal of Scientific and Industrial Research. 2011;70:156–161.

14) Wooley R, Ruth M, Sheehan J, Ibsen K, Majdeski H, Galvez A. Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis: current and futuristic scenarios. National Renewable Energy Laboratory, Golden CO. Golden CO. 1999. Available from: http://www.doe.gov/bridge/home.html.

15) Grohmann K, Himmel M, Rivard C, Baker TM, J. Chemicalmechanical methods for the enhanced utilization of straw. In: and others, editor. Biotechnology Bioengineering Symposium; vol. 14. 1984;p. 137–157. Available from: http://works.bepress.com/irpindia/226/.

16) Kristensen JB, Felby C, Jørgensen H. Yield-determining factors in high-solids enzymatic hydrolysis of lignocellulose. Biotechnology for Biofuels. 2009;2(1). Available from: https://dx.doi.org/10.1186/1754-6834-2-11.

17) Lenth RV. Response-Surface Methods inR, Usingrsm. Journal of Statistical Software. 2009;32(7):1–17. Available from: https://dx.doi.org/10.18637/jss.v032.i07.

18) Joglekar AM, May AT. Product excellence through design of experiments. Cereal foods World. 1987;32:857–868. Available from: https://doi.org/10.12691/ajfst-3-4-2.

19) McIntosh S, Vancov T, Palmer J, Morris S. Ethanol production from cotton gin trash using optimised dilute acid pretreatment and whole slurry fermentation processes. Bioresource Technology. 2014;173:42–51. Available from: https://dx.doi.org/10.1016/j.biortech.2014.09.063.

20) Gregg DJ, Saddler JN. Factors affecting cellulose hydrolysis and the potential of enzyme recycle to enhance the efficiency of an integrated wood to ethanol process. Biotechnology Bioengineering. 1996;51.