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Use of fungal mixed culture for pretreatment of cotton gin waste to enhance the bioethanol production

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Abstract: **Due to stringent environment regulations, the disposal of cotton gin waste is one of the massive problems faced by cotton industries throughout the world. Cotton gin waste is a lignocellulosic biomass and this might be utilized to produce bioethanol which is a promising alternative energy sources for transportation fuel. The prime effort of the present investigation was to decompose or degrade the complex mixtures of cellulose, hemicellulose and lignin compounds of cotton gin waste. This pretreatment has enhanced the production of reducing sugar and bio-ethanol using mixed culture of efficient white rot fungi (*Trametes pubscens* and *Pycnoporus cinnabarinus*).It was examined that, in delignification process, solid state cultivation (SSC) having 57.5 % of lignin removal was found to be more effective than submerged cultivation (SMC) with 45.6 % of lignin removal.The corresponding cellulose and hemicellulose reduction were determined as 64 and 68.5% in SSC, whereas their values in SMC were 46.2% and 52% respectively.The conformations of delignification process with respect to fungal pretreated and untreated cotton gin waste have also been assessed using FTIR, XRD and SEM analysis. The optimization of process parameters for mixed culture further showed substantial improvement in delignification of 63.2% in SSC. In this study, optimization of parameters through response surface model highlights the importance of the mixed cultures for delignification of cotton gin waste.**

Keywords: *Cotton gin waste, mixed culture, white rots fungi, lignin, cellulose, hemicelluloses*

I. INTRODUCTION

In recent years, efforts have been put in place to produce bioethanol from lignocellulose biomass rather than energy crops due torise in food prices, consumption of land and water for their growth [1].Therefore, throughout the world, a huge quantity of cotton gin waste is generated in cotton mills. Globally, India is the second largest cotton producing country which produces undesired cotton gin waste [2].

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Cotton gin waste is one of the suitable lignocellulogic biomass, because of availability, sustainability and cost effective. Cotton gin trash/waste is very important to the researchers and the cotton producers due to the high production of waste and disposal problems [5]. Raw cotton processing generates cotton gin residue (CGR), which content immature bolls, cotton seed, hulls, burs, sticks, leaves, cotton lint and dirt [2]. About 218 kg of cotton fiber generate 68–91 kg of CGT [3]. Ginning one bale (227 kg) of spindle harvested seed cotton lint contributes 37 to 147 kg of waste [4]. The prime ingridants of cotton gin waste is cellulose, which binds tightly with lignin and hemicelluloses forming a complex materials [6], hence delignification/pretreatment is a vital step to release cellulose and hemicelluloses for their further conversion into sugar and subsequently ethanol. Existing pretreatment methods have largely been developed on the basis of physicochemical conversion such as steam explosion, dilute acid, alkali and oxidation or varied combinations, but these processes usually require high temperature and operating pressure [2,7]. In contrast, fungal pretreatment utilizes their enzyme systems to degrade lignin and hemicellulose compound of lignocellulosic biomass in comparatively low energy and mild environmental conditions [7]. Most of mixed cultures of white rot fungi were reported having more efficient in lignocellulolytic biodegradation in producing high activity enzymes due to their synergistic actions [8,9].Mixed fungal cultures could lead to a higher enzyme production through synergistic interactions, but the final results seems to depend on the particular species combination or on the mode of interaction among species, and on the micro-environmental or nutritional conditions in the substrate under colonization [10].Hence the present study explores the efficiency of fungal mixed cultures for the pretreatment processes through SSC and SMC which could improve the anatomical characteristic especially on the fiber morphology and its derived values. An effort had been made to optimize the key parameters like temperature, pH and shaking speed. The present investigation focuses on to determine the optimum pretreatment condition using solid state of cultivation by the help of response surface model (RSM). The study highlights the production of enhanced bioethanol by efficient delignification process using mixed cultures of white rot fungi. It will be also effective in controlling the environmental pollution.

II. MATERIALS AND METHODS

1. Biomass collection and preparation

Cotton gin waste was collected from Shree Ambica Agro Industries Ltd., Balangir, Orissa, India. The cotton fiber of the waste was reduced its length using milling pulverisette-5 (Fritsch Company). The impurities were removed by heating and washing with water followed by overnight drying at 60^0 C.

2. Strain and inoculation

The white rot fungal strains**,** *Trametes pubscens* (NCIM.No-1087) and *Pycnoporus cinnabarinus* (NCIM.No-1181) were collected from National Collection of Industrial Microorganisms, Pune, India. Fungal strains were inoculated on potato dextrose agar (PDA) plates and kept for incubation (4-5 days) at 35^0C.The biomass were finally stored in refrigerator for further use.

3. Components analysis of cotton gin waste

The moisture and ash fraction contents of the processed cotton waste was determined by the solid determination method of ASTM E 1754-95 (ASTM, 1995), ASTM E1721-95 (ASTM, 1995). Lignin degradation and analysis of carbohydrate fractions of cotton gin waste were done following the protocol in the book 'laboratory technique in sericulture' [11].

4. Fungal mixed culture pretreatment of cotton gin waste

The fungal pretreatment of cotton gin waste was carried out by submerge (SMC) and solid state (SSC) cultivation. In SMC pretreatment, 6g of air dried cotton waste was added with 108 ml acetate buffer (20mM, pH 4.5) and 1ml spore inoculums of mixed culture. For SSC cultivation, 6g of the cotton gin waste was mixed with 9.6ml acetate buffer and 6ml spore inoculums to obtain 75% substrate moisture content. The pretreatment experiments were carried out in 250ml Erlenmeyer flasks capped by a silicon stopper with inlet and exit lines connected to 0.2 µm filters. Sample without fungal strain was used as control. The cotton waste was autoclaved for 20mins at 121^0C and 15psi, with acetate buffer and then inoculated with spore suspension. Pretreatment was carried out in an air convection incubator at 35^0C with a shaking speed 100 rpm and flasks were flushed with oxygen (125ml min^{-1}) for 10 min in every 7days, starting from day 0 to 35 days.

5. FT-IR, XRD and SEM characterization of cotton gin waste

FT-IR spectra of dried cotton gin waste samples were recorded on FTIR spectrophotometer (Perkin Elmer-Version 5.3). The samples were mixed with KBr for uniform dispersion of the sample. Sample spectra were obtained over the range of 400-4000 cm^{-1} with a spectral resolution of 0.5 cm^{-1}.

The overall crystallinity of untreated and pretreated samples were determined by XRD PW 3040 equipment using Cu Kα radiation (α = 1.54 Å) at 30 kV and 20mA. The samples were scanned and intensity was observed at 2θ range from 20° to 70° with scanning speed of 3^0/min. Crystalline (%) was calculated as per the formula $[(I_{002} - I_{am})/I_{002}] \times 100$, where I_{002} represent maximum crystalline intensity peak at 2θ between 22° and 23° for cellulose I, and I_{am} represent minimum crystalline intensity peak at 2θ between 18° and 19° for cellulose I [12].
SEM images for both untreated and pretreated samples of cotton gin waste were obtained after drying followed by coating with platinum using JEOL JSM6480 LV SEM.

6. Optimization of pretreatment parameters

A three level response surface model (RSM) based on central composite design (CCD) was employed for optimization of pretreatment process using Minitab 16.2v software, which is the 20 combinations with 6 center point of three variables[2]. Statistical analysis was performed with 95% confidence level. Three different parameters selected for this study are (i) pH at three different levels (4, 4.5 and 5), (ii) rpm (100, 120 and 140) and (iii) temperature (30, 35 and 40). The optimization of pretreatment process has been conducted for 35 days in case of solid state cultivation, because it has shows maximum efficiency, based on the result of cellulose, hemicelluloses and lignin degradation of cotton waste. The pretreatment experiments were carried out in triplicates.

III. RESULT AND DISCUSSION

1. Composition and pretreatment results of cotton gin waste

The composition of cotton gin waste was 40.3%cellulose, 15% 19.8% lignin, hemicelluloses, 9% ash and 8.5% moisture content. Thus the total carbohydrate percentage has been analyzed as 56.3% (holocellulose). This high percentage of carbohydrates makes the cotton gin waste a potential feed stock for bio-ethanol production [2].

The lignin removal from the cotton gin waste by pretreatment process reveal the crystalline structure of cellulose, improves solubilization and thus facilitating substrate accessibility by hydrolytic enzymes [7, 13].Therefore a efficient/suitable pretreatment method is required before enzymatic hydrolysis in order to get maximum yield of sugar. Furthermore it has been reported that, washing and heating increases the cellobiose content with improved delignification after and before pretreatment [14]. All of the components decreased gradually with increase in time. In this study solid state cultivation with the mixed culture of fungi has shown the better pretreatment efficiency than submerged cultivation. The lignin removals of solid and submerge cultivation were 57.5% and 45.6% respectively. The corresponding cellulose and hemicellulose reduction were determined as 64% and 68.5%

in SSC, whereas their values in SMC were 46.2% and 52% respectively.

2. Characterization of cotton gin waste:

The FTIR image fig.1(a) shows the indicative peaks of cellulose and lignin are obtained at 1700 cm-1 to 1750 cm-1, 1726 and 1512 cm^{-1} as found in untreated sample but not in the pretreated sample .This may be due to reduction of compounds rich in carbonyl (C=O) i.e. mostly lignin, some amount of hemicelluloses and other extractives were removed during the pretreatment process. The absorption band at 2729 cm^{-1} is attributed to the stretching vibrations of hydroxyl (OH) groups in the untreated sample. Furthermore a difference in the intensity of absorption at ~2500 cm^{-1} band size was due to difference in absorbed water content between untreated and pretreated samples. This is explained with a change in degree of inter molecular H-bonding between OH group of cellulose and water. It can be expected that there would be increase in surface area and rearrangement of cellulose microfibrils which may provide a better accessibility to OH group by the enzymes in pretreated sample as similar study supports our results [15]. The OH groups may include sorbed water, aliphatic compounds, primary and secondary alcohols found in cellulose, hemicellulose and carboxylic acids in extractives [2].The shoulder near the OH stretching vibrations, 2900 cm^{-1}, is attributed to CH stretching vibrations and corresponds to the aliphatic moieties in polysaccharides (cellulose and survived hemicelluloses) of treated sample. The pure cellulosic are obtained at frequencies- 1431, 1372, 1318, 1281, 1165, 1059 and 897 cm^{-1}.

Cellulose macrofibrils have been observed more prominently with increased in pretreatment time fig.1 (b). The XRD for untreated and pretreated samples exhibited similar crystalline patterns. The widths at half height for the peaks at 2θ =17º and 26º were similar for all samples except a higher haziness in untreated sample, which suggested similarity in crystallite sizes[16].The cellulose crystallinity value of untreated sample of cotton gin waste was 18% while that of pretreated sample was 25.1% suggesting improvement in crystallinity of the sample. The crystallinity of the pretreated sample was increased due to removal of lignin and hemicellulose [2, 17]. It is expected that amorphous region present in between the regular crystalline region are subjected to enzyme attack after pretreatment.

Scanning electron micrographs (SEM) highlights the morphology of cellulose and hemicellulose fiber with different severities in fig 1(c).The untreated sample shows compact fibers distributed over the whole region. Whereas the pretreated sample was show partially degraded etched fibers indicating the influence of enzyme treatment. This shows an enhancement of surface area due to the removal of lignin and its associated compounds such as hemicellulose. A significant change on the surface property towards favorable interaction with enzyme has occurred due to pretreatment resulting cleavage of the amorphous region of cellulose with retention of crystalline

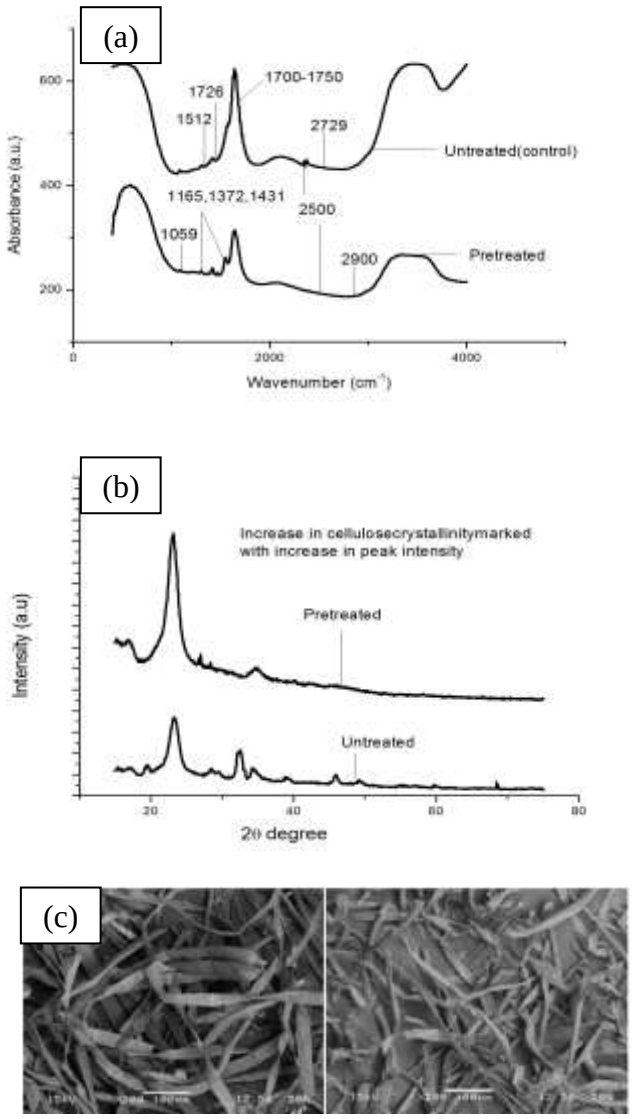

Fig.1. Characterization of control (untreated) and pretreated cotton gin waste through (a) FTIR spectra, (b) X-Ray diffraction diagram and (c) SEM images.

fraction. Additionally, lignin removal from pretreated sample increases degree of crystallinity[18]. Further gravimetric analysis shows an amount of 3.9% biodegradation of biomass during pretreatment.

3. Optimization study

The process parameters optimization would focus on the improvement of the economic feasibility of the process. The optimization study of three individual parameters i.e., pH, temperature and rpm has been carried out to obtain the maximum% lignin degradation of cotton gin waste by using central composite design. The percentage of delignification was obtained in the range of 54 to 63.2 %. The mathematical equation relating % lignin degradation to the different

independent variables is shown below in terms of coded factor:

$$Y1 = 62.62 + 0.37A_1 - 0.29A_2 + 0.66A_3 - 3.42A_1^2 - 2.52A_2^2 - 0.67A_3^2 + 0.36A_1A_2 - 0.78A_1A_3 - 0.13A_2A_3$$

Where, A1, A_2 and A_3 represent pH, temperature and rpm respectively. The individual action of all the three parameters studied, where the quadratic and interaction effects between the dependent variables were found to be significant from the regression model. The square and interaction effects between the variables were found to be statistically significant with a P- value less than 0 and 0.02 respectively. The regression model for pretreatment of cotton gin waste has been shown a high F-value (50.87) and a very low probability value (< 0.001) which show a significance of the model [19]. Although the square terms from the model shows more significance or effective with higher F-value 141.89. The quality of the model was evaluated by the coefficient R^2 and its statistical significance was determined by an F-test. In this pretreated sample the R^2 values obtained was as 0.9786 and hence justify the robustness of the model.

Fig. 2(a) shows the interaction of temperature and RPM at a constant pH, where as fig 2(b) shows the interaction between pH and RPM at constant temperature on lignin degradation of pretreated sample. The three dimensional plots show that the temperature at 35^0C and shaking speed at 100 rpm caused an increase in the lignin degradation (%), yielding a maximum lignin degradation value of 62.9 % after 35 days of solid state cultivation. However at a constant temperature the interaction between shaking speed and pH gives the maximum value of delignification. In Fig.2(c) the optimization has been performed with variation of temperature and pH, where as RPM is constant [20, 21]. From the experimental data, the above statistical model suggests the optimum predicted condition of pH, shaking speed and temperature 4.5, 122 rpm and 35^0C respectively for high percentage of delignification. In order to check the reliability of predicted response, triplicate experiment had been performed under optimum predicted conditions. From this design of experiments maximum reduction of cellulose and hemicelluloses were found to be 65.7% and 71.8%. Where delignification efficiency was 63.2 %, which is a good agreement with predicted value.

Table.1 Results of Submerge cultivation with respect to before and after optimization of pretreatment

Pre-treatment	Cellulose reduction%	Hemicelluloses reduction %	Delignification%
before optimization	64	68.5	57.5
after optimization	65.7	71.8	63.2

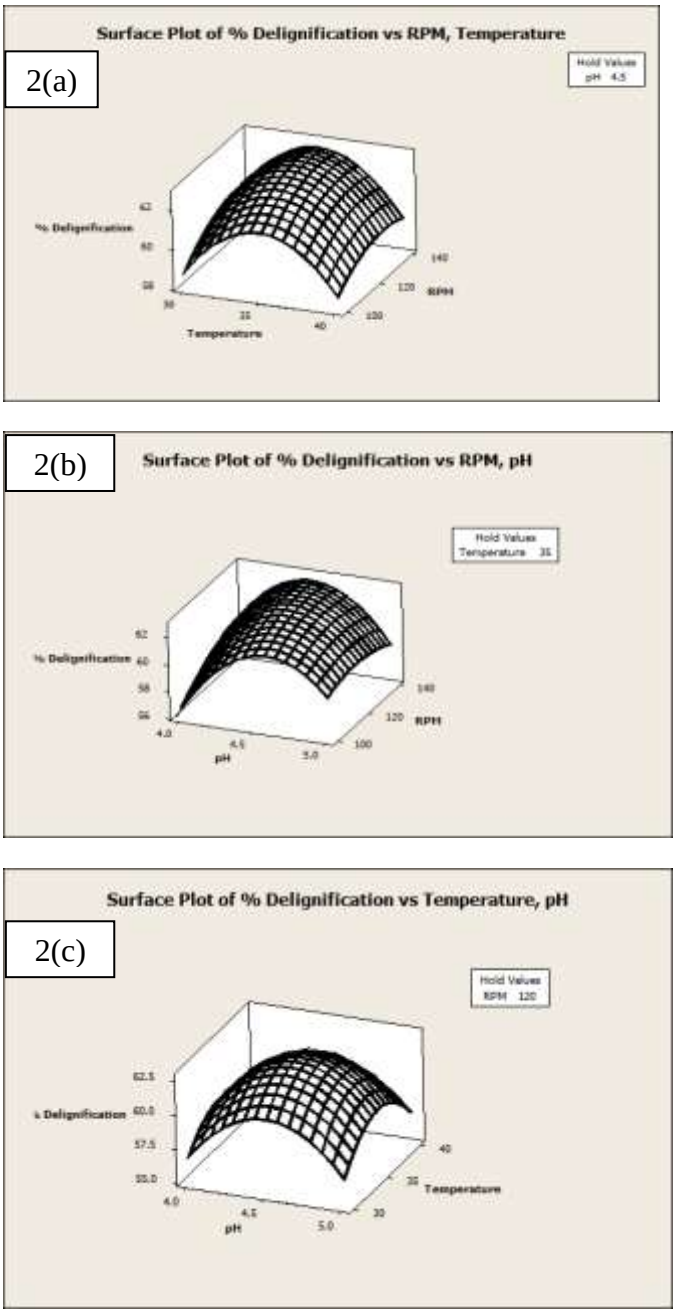

Fig.2. Response surface plots showing the effect of (a) temperature and shaking speed, (b) pH and shaking speed and (c) pH and temperature on pretreatment of cotton gin waste.

V. CONCLUSION

The present investigation is very useful for the cotton gin waste management particularly for India. The present study highlights the efficiency of fungal mixed culture pretreatment process for providing technical and economic feasibility to harness cotton gin waste. Mixed culture of fungi has been found to be highly efficient strains in producing lignocelluloses enzyme for delignification of cotton gin waste. Further, it was observed that solid state fermentation process

is more effective in terms of delignification efficiency than submerged fermentation. Such study will be highly effective in the line of intimation to clean up the environmental pollutions and need further scientific research for the sustainable management of cotton gin waste.

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