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RESEARCH ARTICLE

BIPHASIC ACID TREATMENT OF DEBARKED COTTON STALK: ONE NOVEL APPROACH TOWARDS BIOETHANOL PRODUCTION

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ABSTRACT

Lignocellulosic biomass includes wheat straw, corn stover, rice straw, cotton stalk etc. which are renewable resources of energy. These agricultural lignocellulosic wastes are attractive substrate for production of bioethanol. The main challenge for producing ethanol from lignocellulosic wastes is the conversion of lignin containing polymer in to delignified monomer. Acid hydrolysis is conventional process for this conversion. To avoid undesirable side reaction and minimize the environmental issues, the acid hydrolysis has divided in to two steps involving the treatment with concentrated acid in first step for decrystallization followed by dilute acid hydrolysis. In the present work effectiveness of different concentration of sulphuric acid on cotton stalk has been studied and solubilisation of fermentable sugar and specifically dextrose were checked at regular interval of time. The result of this study show that 75% H_2SO_4 at fixed sample-acid ratio of 1:2 (by weight) followed by dilution up to 1N, steam treatment at 121°C for 30 min. and heating up to four hour at 90°C gives maximum yield of glucose (0.24 g/g of biomass) and fermentable sugar (0.45 g/g of biomass).

INTRODUCTION

The decreasing reserves of conventional energy resources and increasing energy demands necessitates the development of alternative energy resources that are both renewable and environmental friendly. Ethanol produced from renewable biomass has been considered as one of the most promising biofuels which contributes to the reduction of negative environmental impacts, e.g. increased greenhouse gas emission (Siqueria *et al*. 2008). Biomass which includes animal and human waste, trees, shrubs, yard waste, wood products, grasses and agricultural residues such as wheat straw, corn stover, rice straw and cotton stalks etc. are renewable resources that store energy from sunlight in its chemical bond(Silverstein *et al*. 2007). These agricultural lignocellulosic wastes can be used for the production of bioethanol. The first step in bioconversion of lignocellulosic biomass to bioethanol is size reduction and pre-treatment (Balat *et al*. 2008).physical pre-treatment will increase the accessible surface area and size of pores, and decrease the crystallinity and degrees of polymerization of cellulose (Binod *et al*. 2010). Acid hydrolysis, particularly with sulphuric acid is widely used to treat lignocellulosic materials to obtained mono-sugars (Choi and Mathews, 1996). In the process acid first breaks the matrix structure of fibre into more accessible cellulose, hemicelluloses, and lignin and then further reduces these polysaccharides to mono-sugars (Fengel and Wegener, 1984). This type of application commonly utilizes either concentrated acid at a low temperature or dilute aid at a high temperature (Sun and Cheng, 2002). In general, concentrated acid hydrolysis is much more effective than dilute acid hydrolysis (Grahmann *et al*., 1985). Acid hydrolysis of cellulose occurs at faster rate and the acids used are cheaper than the enzyme; however, enzymatic hydrolysis is a cleaner and more selective process takes place at moderate pressure and temperatures (Camacho*et al*., 1996). It has been reported that glucose yield of 72-82% can be achieved from mixed wood chips using such concentrated acid hydrolysis process (Iranmahboob *et al*., 2002). However, concentrated acid hydrolysis has a major drawback in its use of highly concentrated acid that could cause serious environmental concerns (Sun and Cheng, 2002). Technologies for biomass to ethanol conversion are also under various stages of development, various bottle necks in such technologies include the pre-treatment of biomass, saccharification of pre-treated biomass and fermentation of the hexose and pentose sugars release by hydrolysis (Ghosh and Ghose, 2003). Therefore in present work, process of concentrated acid treatment followed by relatively dilute acid hydrolysis has been studied.

India is a county with a positive outlook towards renewable energy technologies and committed to the use of renewable sources to supplement its energy requirement. The country has a large share of cultivable land which had been a key factor in the socioeconomic development and is one among the few nations to have a separate ministry for renewable energy which address the development of biofuels along with other renewable energy sources. Cotton is one of the most abundant crops in India with annual residues generation is 18.9 million

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metric tons (Sukumaran *et al*., 2010). The objective of this research work was to study a two-step acid treatment of cotton stalk involving the effectiveness of different concentration of sulphuric acid for decrystallization followed by dilute acid hydrolysis.

MATERIALS AND METHODS

Physical pre-treatment of biomass

The cotton stalks were collected from nearby field at Government Institute of Science in Marathwada region. The stalk were shredded and bailed in the field and were debarked, chopped, over dried and ground to pass 1-2 mm sieve in laboratory. Dried sample were stored in sealed plastic bags at room temp until use.

Compositional analysis

The fractions of cotton stalk (cellulose, hemicellulose and lignin etc) were analyzed using standard NREL (National Renewable Energy Laboratory) procedure.

FTIR analysis

Fourier Transform Infrared Spectroscopic analysis of debarked cotton stalk was carried out to analysis different constituent of sample qualitatively by detecting functional group.

Acid treatment

The sets of different concentration of sulphuric acid (65%, 70%, 75%, and 80%) were prepared from conc. Sulphuric acid solution (Merk Sp. Gr 1.84). In order to obtained the optimal condition for decrystallization, the biomass is mixed with these four concentrations of acid separately to obtained homogeneous paste at fixed sample-acid ratio of 1:2(by weight), till the colour of the paste turned brown without resulting in to the severer oxidation by acid. Distilled water was added to the paste to obtain the different normality (1N, 2N, 3N) in different tubes in each set for hydrolysis. Sets were then treated with high pressurise steam for 30 min. at 121^{o}C (Somkid and Wuttichai, 2009) and finally this steam treated hydrolyzates was heated at 90^{o}C for four hours in water bath. During heat treatment samples were taken at regular interval of time for concentration of fermentable sugar and Glucose analysis.

Analytical method

After appropriate dilutions the solubilisation of fermentable sugar were determine by DNS method of Miller(1959) and specifically dextrose concentration was using glucose oxidase kit prepare by Ambica diagnostics ltd. based on Bregmeyer *et al*.(1974) method.

Statistical analysis

An analysis of variance (ANOVA) of fermentable sugar and glucose yield was performed for each individual experiments mention above using the statistical analysis system.

RESULTS AND DISCUSSION

Cotton stalk (*Gossypium. hirsutum*) is good source of lignocellulosic biomass. The major chemical constituent of biomass cellulose, hemicellulose and lignin. The stalk used in this study was debarked for the purpose of increasing concentration of cellulose and minimizing the percentage of lignin.

FTIR Analysis

Fourier Transform Infra Red spectroscopic analysis of the sample has been shown in Fig.1.The analysis was carried out to detect the constituents of sample qualitatively with the help of functional group present in it. It was recorded between 400cm^{-1} and 4000cm^{-1}by taking 45 scans per sample at resolution of 4cm^{-1}and analysis were carried out by mixing potassium bromide and sample with ratio of 1:10 (w/w). Spectral analysis of FTIR shows the absorption band at region from 3446 to 3066cm^{-1}corresponding to polysaccharide groups mostly present in holocellulose. The band at 1425cm^{-1}correspends to CH_2 groups and absorption at 2855cm^{-1} represents C-H group which acquire peak position in figure. Position of dominated bands in the figure indicates the major constituent of sample is cellulose.

Compositional analysis of cotton stalk

. The chemical composition of cotton stalk varies, depending on growing location, season, harvesting method, as well as analysis procedure (Agblevor *et al*., 2003).The Silverstein *et al*. got 41.80% holocellulose and 30.1% lignin by using native cotton stalks. Nearly same results were obtained by Ververis *et al*. (2004). In the present study debarked cotton stalk contains 57% holocellulose in which 40.60% was alpha cellulose and 17.24% was hemicellulose. The lignin content was 24.81%. Increase in the concentration of cellulose and decrease in the concentration of lignin of present study as compare to previous study was due to debarking. John Harkin and John Rowe (1971) reported that 40 to 55% lignin is present in bark of soft wood.

Effect of different dilutions on decrystallized biomass

The experiment was performed in two steps involving decrystallization by concentrated acid (65%, 70%, 75% and 80% H_2SO_4 separately) followed by dilution (Liao *et al*., 2006) up to 1, 2 and 3 N for hydrolysis separately. The diluted samples were analysed at regular interval of time for solubilisation of fermentable sugar and glucose, are presented in Table 1. On the basis of statistical data 75% H_2SO_4 (used for decrystallization) followed by dilution up to 1N with heat treatment for 4 hour may be considered as optimum value of sugar yield. Comparative analysis of different dilutions has shown in Fig. 2, 3, 4 and 5.

Effect of dilution of decrystallized biomass up to 1N

The concentration of fermentable sugar in steam treated hydrolyzates increased from 0.27 g/g of biomass (decrystallized by 65% H_2SO_4) to 0.39 g/g of biomass (decrystallized by 80% H_2SO_4) after one hour of heat treatment and this concentration were increased up to 0.32 g/g

Table 1. Fermentable sugar and Glucose yield by decrystallization with concentrated acid followed by dilution with different normalities

Decrys-allizati-on	Dilution of hydrolyzates	Heating Time							
		One hour		Two hour		Three hour		Four hour	
		F.S	Glucose	F.S	Glucose	F.S	Glucose	F.S	Glucose
	Dilution up to 1N	0.27	0.16	0.30	0.17	0.31	0.18	0.32	0.18
65%	Dilution up to 2N	0.15	0.09	0.17	0.09	0.18	0.10	0.18	0.10
H2SO4	Dilution up to 3N	0.11	0.06	0.12	0.07	0.13	0.07	0.13	0.07
	Dilution up to 1N	0.33	0.17	0.37	0.18	0.38	0.20	0.39	0.20
70%	Dilution up to 2N	0.18	0.09	0.20	0.10	0.21	0.11	0.22	0.11
H2SO4	Dilution up to 3N	0.13	0.06	0.14	0.07	0.15	0.08	0.15	0.08
	Dilution up to 1N	0.34	0.19	0.40	0.22	0.44	0.24	0.45	0.23
75%	Dilution up to 2N	0.21	0.10	0.23	0.11	0.24	0.13	0.24	0.13
H2SO4	Dilution up to 3N	0.16	0.07	0.17	0.07	0.17	0.07	0.17	0.08
	Dilution up to 1N	0.39	0.20	0.44	0.23	0.46	0.24	0.46	0.24
80%	Dilution up to 2N	0.20	0.11	0.24	0.12	0.25	0.13	0.25	0.13
H2SO4	Dilution up to 3N	0.15	0.07	0.16	0.07	0.17	0.08	0.17	0.08

F. S. Fermentable sugar

Table 2. SE and CD value of effect of normality and time after acid decrystallization obtain from Analysis of variance (ANOVA)

Source term	Degree of freedom	Decrystallization with 65% acid		Decrystallization with 70% acid		Decrystallization with 75% acid		Decrystallization with 80% acid	
		SEm±	CD	SEm±	CD	SEm±	CD	SEm±	CD
Normality of acid	2	0.0048	0.0141	0.0063	0.0186	0.0080	0.0236	0.0085	0.0247
Times in hour	3	0.0055	0.0163	0.0073	0.0215	0.0093	0.0273	0.0097	0.0285
Normality ×hour	6	0.0096	0.0283 (NS)	0.0127	0.0372 (NS)	0.0161	0.0473	0.0169	0.0494 (NS)
Error	22								
Total	35								

NS: Non Significant; SEM: Standard Error mean; CD: Coefficient of Differentiation

Table 3. SE and CD value of effect of normality and time after acid decrystallization obtain from Analysis of variance (ANOVA)

Source term	Degree Of freedom	Decrystallization with 65% acid		Decrystallization with 70% acid		Decrystallization with 75% acid		Decrystallization with 80% acid	
		SEm±	CD	SEm±	CD	SEm±	CD	SEm±	CD
Normality of acid	2	0.0069	0.0204	0.0063	0.0186	0.0082	0.0239	0.0081	0.0238
Times in hour	3	0.0080	0.0235	0.0073	0.0215	0.0095	0.0277	0.0094	0.0275
Normality ×hour	6	0.0139	0.0408 (NS)	0.0127	0.0372 (NS)	0.0164	0.0479	0.0163	0.0477 (NS)
Error	22								
Total	35								

NS: Non Significant; SEM: Standard Error mean; CD: Coefficient of Differentiation

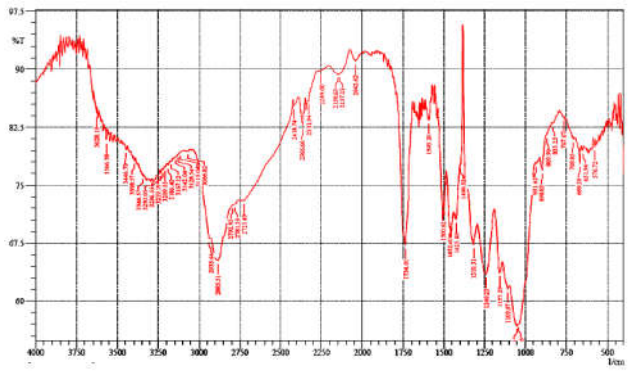
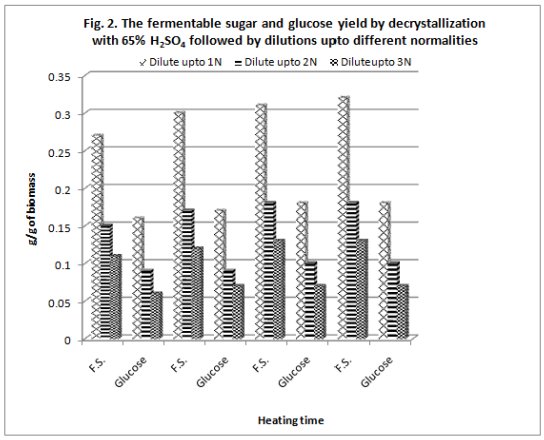
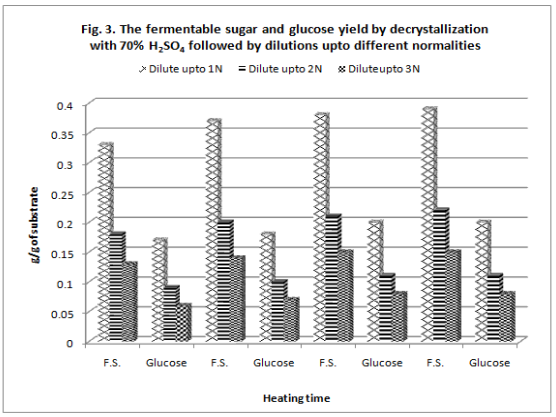
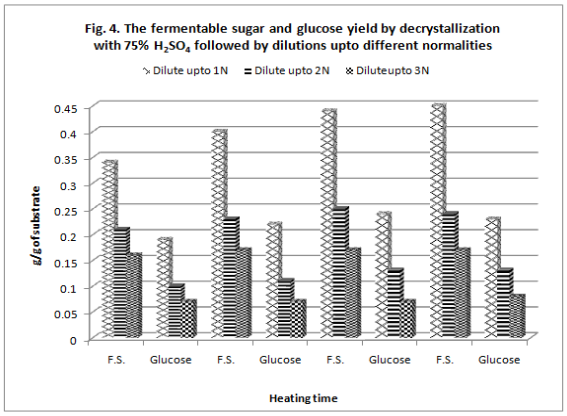


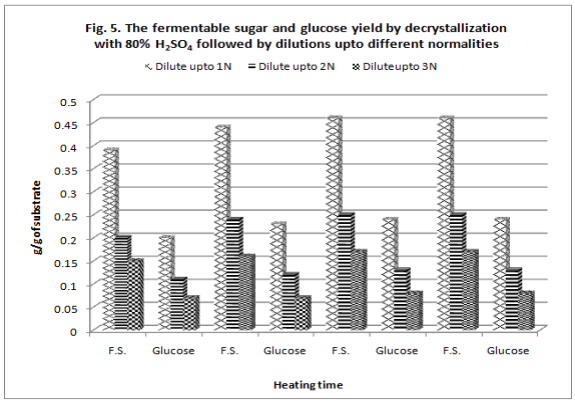
Fig. 1. FTIR analysis of native debarked cotton stalk



of biomass (decrystallized by 65% H_2SO_4) to 0.46 g/g of biomass (decrystallized by 80% H_2SO_4) during fourth hour of heat treatment showing in Table 1. Meanwhile the glucose concentrations increased from 0.16 g/g of biomass (decrystallized by 65% H_2SO_4) to 0.20 g/g of biomass (decrystallized by 80% H_2SO_4) after one hour of heating and were increased up to 0.18 g/g of biomass (decrystallized by 65% H_2SO_4) to 0.24 g/g of biomass (decrystallized by 80% H_2SO_4) during the fourth hour of heat treatment. The data demonstrated that, yield of fermentable sugar and glucose was increased slowly when the acid concentration was changed from 75% to 80% for decrystallization (concentration of fermentable sugar increase from 0.45 g/g of biomass to 0.46 g/g of biomass and of glucose was 0.23 g/g of biomass to 0.24 g/g of biomass during fourth hour of heat treatment, as shown in table 1) while this yield was drastically increase when the acid concentration was changed from 65% to70% for decrystallization (concentration of fermentable sugar increased from 0.32 g/g of biomass to 0.39 g/g of biomass and of







glucose was 0.18% to 0.20% during fourth hour of heat treatment) and at the concentration of 75% of acid, the yield was proportionally high (concentration of fermentable sugar and glucose was 0.44 g/g of biomass and 0.24 g/g of biomass respectively) during third hour of heat treatment. After four hours the yield was not significantly increased(data not shown).Nearly same kind of work has also been observed by performing the concentrated acid hydrolysis of mixed wood chips (Iranmahboob *et al*., 2002). The reason of proportionally slow increasing in concentration of fermentable sugar and glucose in the hydrolyzates of 80% decrystallized biomass might be results in the formation of furan compounds.

Effect of dilution of decrystallized biomass up to 2 N

The solubilisation of decrystallized biomass with the same strength of acids (65%, 70%, 75% and 80%) separately followed by dilution up to 2 N has been shown in table 1

The concentration of fermentable sugar ranged from 0.15 g/g of biomass (decrystallized by 65% H_2SO_4) to 0.20 g/g of biomass (decrystallized by 80% H_2SO_4) after one hour of heat treatment and this yield will increased up to 0.18 g/g of biomass (decrystallized by 65% H_2SO_4) to 0.25 g/g of biomass (decrystallized by 80% H_2SO_4) at fourth hour of heat treatment. Similarly the glucose concentration also ranged from 0.09 g/g of biomass (decrystallized by 65% H_2SO_4) to 0.11 g/g of biomass (decrystallized by 80% H_2SO_4) after one hour of heat treatment and this will increase up to 0.10 g/g of biomass (decrystallized by 65% H_2SO_4) to 0.13 g/g of biomass (decrystallized by 80% H_2SO_4) during third hour of heat treatment and it become constant thereafter. The comparative analysis of different hydrolyzates of 2 N strength shows that the concentration of fermentable sugar and glucose were proportionally higher at 75% H_2SO_4 then other concentration (the concentration of fermentable sugar and glucose were 0.21g/g of biomass and 0.1g/g of biomass respectively after one hour of heating and this was increase up to 0.24g/g of biomass and 0.13g/g of biomass of fermentable sugar and glucose respectively during the fourth hour of heat treatment). The same rate of increasing was observed in previous set which was diluted up to 1N. In both cases 75% H_2SO_4 is suitable for decrystallization of biomass and yield of fermentable sugar and glucose is much less as compare to previous set up where the decrystallized biomass was diluted up to 1N. This reduction in the yield may be result due to burden of acid concentration and quick conversion of releasing sugar in to furan compound.

Effect of dilution of decrystallized biomass up to 3N

The third set of experiment was prepared by decrystallization of biomass with concentrated acid followed by dilution up to 3N. In the hydrolyzates of 3N strength, the concentration of fermentable sugar varied in the range from 0.11 g/g of biomass to 0.15 g/g of biomass after one hour of heating and this concentration was increased up to0.13 g/g of biomass to 0.17 g/g of biomass and the same case has been observed in glucose yield, which was increased from 0.06 g/g of biomass to0.07 g/g of biomass after one hour of heating and no drastically change was observed by heating up to four hours.

Statistical analysis

The statistical analysis of fermentable sugar and glucose was carried out by factorial completely randomized design and results obtained are present in Table 2 and 3 respectively. The value of fermentable sugar and glucose indicates significant increase with increase in concentration of acid from 65% H_2SO_4 to 80% H_2SO_4 (used for decrystallization in first stage). However increase in dilution of hydrolyzates (in second stage) decreased the yield of fermentable sugar and specifically glucose, highest value at all the acid concentration used for dilution was recorded with 1N. Similarly statistical analysis indicates significant increase in fermentable sugar and glucose in the hydrolyzates up to four hour of heating time in all acid concentration. Scrutiny of the data further shows better value of these sugars with 80% acid concentration ×1N dilution ×4 hour heating time which was found at par with 75% H_2SO_4 (used for decrystallization). Normality in to time interaction results not reached to the level of statistical significance though 80%acid showed highest value of fermentable sugar

and glucose with same normality (1N) and same time of heating (4hour).

CONCLUSION

Increased in concentration of acid in second step of hydrolysis significantly decreases the yield of fermentable sugar and glucose which might be due to increase in side reactions (furans and other by product formation). The result of this study show that optimal conditions for acid hydrolysis of cotton stalk was decrystallization with 75% H_2SO_4 at fixed sample-acid ratio of 1:2 (by weight) followed by dilution up to 1N and steam treatment at 121^oC for 30 min. followed by heating up to four hours at 90^oC in water bath, gives higher yield. There for, optimising acid concentration for decrystallization followed by dilution and then heat treatment are key factor of hydrolysis. This study can serve as one step towards successful hydrolysis of cotton stalk. In addition more research efforts are require to optimising the procedure for increasing the yield of fermentable sugar and decreasing concentration of by products to make the process more feasible for bioethanol production.

REFERENCES

Agblevor, F.A., Batz, S., Trumbo, J.2003. Composition and ethanol production potentialof
Cotton gin residues. *Appl Biochem Biotechnol* 105:219-230.

Balat, M., Balat, H., Oz C.,2008.Progress in bioethanol processing.*Prog Energy Combust Sci* 34:551-573.

Binod, P., Sindhu, R., Singhania, R.R.,Vikram, S, Devi ., Nagalakshmi, S, Kurien, N, Sukumaran, RK, Pandey, A.2010. Bioethanol production from rice straw: An overview. *BioresourTechnol* 101:4764-4774.

Bergmeyer, H.U. Bernt, E. In Determination with Glucose Oxidase and Peroxidase*;* Bergmeyer, H. U., Ed.; *Methods of. Enzymatic Analysis,* 2nd Ed.; 1974: 1205–1212.

Camacho, F, Gonzalez-Tello, P., Jurado, E., Robles, A.1996.Microcrystalline-cellulose
hydrolysis with concentrated sulphuric acid.*J Chem Tech Bioethanol* 67:350-356.

Choi, C.H., Mathews, A.P.1996. Two-step acid hydrolysis process kinetics in the saccharification of low-grade biomass: Experimental studies on the formation and degradation of sugars. *BioresourTechnol* 58:101-106.

Fengel, D, Wegener, G.1984.*Wood Chemistry*.Ultrastructure, Reactions. Walter De Gruyter, Berlin, Germany:.Ghosh, P., Ghose, T.K.2003.Bioethanol in India : Recent Past and Emerging Future. *AdvBiochemEngBiotechnol* 85:1-27.

Grahmann, K., Torget, R., Himmel, M.1985 Optimisation of dilute acid pre-treatment of biomass.*Biotechnology and Bioengineering Symposium* 15:59-80.

Iranmahboob, J, Nadim, F., Monemi, S.2002.Optimising acid hydrolysis: a critical step for production of ethanol from mixed wood chips.*Biomass and Bioenergy* 22:401-404.

John, M. Harkin, John, W. Rowe.1971 Bark and its possible uses. U.S. department of agriculture. . *Forest product laboratory*. Madison, Wis..

Liao, W., Liu, Y., Liu, C., Wen, Z., Chen, S.2006.Acid hydrolysis of fibers from dairy manure.*BioresourTechnol*97:1687-1695.

Miller, G.L.1959.Use of Dinitrosalicylic acid reagent for determination of reducing sugar.*Anal Chem* 426-429.

Siqueria, P.F., Drap, S.G,, Carvalho, J.C,, Saturm, W., Roddriguez-Lewn, J.A.,Tholozen, J.L. 2008. Production of bioethanol from soyabean molasses by saccharomyces cereviaiae at laboratory, pilot and industrial scales. *BioresourTechnol* 99: 8156-8163.

Silverstein, R.A., Sharma – Shivappa, R.R, Boyette, M.D, Osborne, J..2007. A Comparision of chemical pretreatment methods for improving saccharification of cotton stalks. *BioresourTechnol* 98: 3000-3011.

Somkid, D., Wuttichai, K.2009.Comparisons of hydrolysis conditions to recover reduching sugar from various lignocellulosic materials. *chiang Mu J Sci* 36(3):384-394.

Sukumaran, R.K., Surender, V.J., Sindhu. R., Binod,. P, Janu. K.U., Sajna, K.V., Rajasree, P., Pandey, A.2010.Lignocellulosic ethanol in India: Prospects, challenges and feedstock availability. *BioresourTechnol* 101:4826-4833.

Sun, Y., Cheng, J.2002. Hydrolysis of lignocellulosic material for ethanol production: A review. *BioresourTechnol* 83:1-11.

Varveris, C., Georghiou, K., Christodoulakis, N., Santas, P., Santas, R.2004.Fiber dimensions, lignin and cellulose contents of various plant materials and their suitability of paper production. *Ind crop Prod*19:245-54.

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