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Bioethanol Production and Optimization by Response Surface Methodology from Corn Cobs by Alkali Pretreated

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Ethanol is an important renewable biofuel which can be used in pure or blended form. Corncob is a residual waste from corn processing unit, is a cheap and abundant raw material which can be used for ethanol production. Due to the high content of cellulose corncob needed to be pretreatment in order to convert corncob to reducing sugar. Corncob was pretreatment in the alkali condition and hydrolysed with cellulases extracted from *Aspergillus niger*. A central composite design was adopted to determine the optimum levels of these factors. Sodium hydroxide solution by varying the factors such as sonication time (3-5 hrs), Temperature (40-80 C), and NaOH concentration (3-6 %). The product of hydrolysis was fermented using the bakers yeast to ethanol. The GCMS Analysis proves that the corncob is an efficient source for the production of ethanol with 96.214% pure.

Keywords: Ethanol, corncobs, sodium hydroxide, Response surface methodology, sonication.

Due to the diminishing deposits of fossil fuel as well as global warming and environmental problems, development and discovery of environmentally benign and renewable energy fuels has become an important issue. Bioethanol has become an alternative source of energy due to its raw material being in abundance and its potential in a large scale production process. Ethanol contains oxygen and clean octane, so combustion of ethanol is clean and poses no negative environmental effect and reduces the emissions of green house gas. Bioethanol can be produced from various feed stock such as refined sugar, sugarcane juice, sugar beet juice, cane or beet molasses. Production of ethanol from non-edible feedstock reduces the cost for production and avoids competition in food to fuel crisis[3]. Corn cob is a byproduct of corn plant, which is burnt in the field or thrown as waste, can be used for this purpose[4]. To overcome lignin barrier, pretreatment is required to alter the physical features. For the production of ethanol from agricultural residue alkali (bases) pretreatment found to be effective and low-cost[5]. Among bases, NaOH solution is an effective method for the cleavage of lignin barrier[6, 7]. The objective of this study is to (1) utilization of cheap source, commonly available agricultural residue corn cob for the production of ethanol (2) optimization of process parameter for the pretreatment of corncobs using response surface methodology and (2) use crude cellulase enzyme obtained from *Aspergillus niger* for the hydrolysis of treated corncobs.

MATERIALS AND METHODS

Corn cobs were collected from nearby shops and local markets. Cobs were chopped into small pieces, grinded and were dried in hot air oven at 45 C overnight. Dried Cob powder was screened to obtain the average particles of size 0.500 mm (30 BSS) and it is stored in air tight containers for further use and to avoid microbial degradation.

Compositional analysis

The composition of the Corn cob was AOAC method [9, 10, 11 12] to evaluate the total solid, lignin, Cellulose, galactose, mannose, moisture, ash

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and protein test. From the analysis the biomass was composed 92.08 % of total solids, 15.06% of lignin, 66 % of cellulose, 2.6% galactose, 3.7% mannose, 13.2% protein, 2.15% ash, 16.5% of moisture.

Pretreatment

5 g of screened corn cobs were soaked in 100 ml of sodium hydroxide solution in 250 ml conical flask and maintained at constant temperature under sonication. The treated biomass was filtered then washed with distilled water for detoxification and neutralization. Pretreated biomass is continuously washed until the solution turns neutral.

Response surface methodology

Response surface methodology (RSM) was used to study the effect of time of sonication, temperature and amount of sodium hydroxide on amount of reducing sugar produced[13]. The pretreatment conditions tested were temperatures of 40-80 °C, reaction times of 3–5 h under sonication, and NaOH concentrations of 3-6% (w/w) at a fixed solid-to-liquid ratio, where the experimental conditions of 20 combinations of the three variables were designed.

Hydrolysis

The pretreated biomass was hydrolyzed using Cellulase enzyme extracted from fungi *Aspergillus niger by* growing them in mineral salt medium containing NaOH pre-treated corn cobs.

Microorganism

The fungi *Aspergillus niger* (MTCC.No-9652) was obtained from MTCC, Chandigarh and was cultured in a glycerol stock to preserve it. The organisms were maintained on potato dextrose agar slants at 4 °C till required.

Inoculum preparation

The inoculum was prepared by growing the organism in 250 ml Erlenmeyer flask with 100 ml of Potato Dextrose broth. The medium was inoculated with inoculum from potato dextrose agar slants and incubated at room temperature for 5 days.

Submerged fermentation (SMF)

The composition of the medium contained the following g/l of distilled water: Corn cob, 20; Na_2HPO_4, 5; $MgSO_4$, 2; K_2HPO_4, 2; $CaCL_2$, 2; protease peptone, 7.5; $FeSO_4$, 5; $MnSO_4$, 1.6; $ZnSO_4$, 1.4. The medium (100 ml) in 250 ml Erlenmeyer flasks was sterilized by autoclaving at 121 C for 15 min. Sterilized flask was inoculated with 1 ml of the above said inoculum. The culture was tightly plugged with cotton and incubated on a rotator shaker (150 rpm) at 30 C for 5 d.

Enzyme extraction

At the end of fermentation the culture broth from submerged fermentation was centrifuged at 6000 rpm for 20 min and the supernatant was used as a source of extracellular enzyme [14].

Enzymatic hydrolysis

The samples were digested by adding cellulase enzyme at 50°C and pH 4.8 (adjusted using 0.1 M sodium acetate buffer) in a shaker incubator at 150 rpm for 72 h. Samples were taken periodically and analyzed for reducing sugars concentration.

Fermentation

1g of dry Baker's yeast was added to the hydrolysed sample. The experiment was carried out under anaerobic condition by keeping the flask in shaker incubator at 150rpm for 48h at room temperature. The samples were withdrawn and centrifuged at 7000rpm for 20min. The supernatant were distilled twice for collecting pure ethanol [16].

Analytical test

Crude cellulase activity was determined by the CMC method of the International Union of Pure and Applied Chemistry [17]. Presence of ethanol in the final product was detected with gas chromatography. Gas chromatography- mass spectrometry combines the fine separating power of gas chromatography with the powerful detection capability of Mass spectrometry. It is particularly suitable for analyzing volatile compounds and also compounds having low relative molecular mass. The GC-MS setup was a Perkin Elmer (Clarus 500). The column was a capillary column Elite-5MS (5% Phenyl 95% dimethylpolysiloxan).

RESULTS AND DISCUSSION

Effect of varying pretreatment parameters on biomass

Pretreatment was performed by varying three important process variables such as weight percentage of sodium hydroxide, temperature and sonication time to achieve the maximal yield of reducing sugar as shown in Table 1. Taylor equation is the heart of RSM technique. It is of the form:

Percentage yield of reducing sugar = 31.7942 –

$0.6418\,T - 10.8752\,\theta - 1.82337\,W + 0.00757\,T^2 + 0.593642\,\theta^2 + 0.09091\,W^2 + 0.0870625\,T\theta - 0.005875\,TW + 0.715833\,\theta W$

In the above equation T, θ, W represents temperature, time of sonication and sodium hydroxide weight percentage. Percentage recovery indicates the yield of reducing sugar. The predicted model was correlated to coefficients of linear, quadratic and interaction effects. The correlation coefficients for each model are shown in Table 2.

The significance of the variables was determined by the probability values (Table 3). All the factors and their square interactions ($P < 0.05$) except square term of sodium hydroxide and

Table 1. Experimental results based on central composite design

Std Order	RunOrder	PtType	Blocks	Temp (T)	Time (θ)	NaOH(w%) (W)	Exp	Pre
1	1	1	1	40	3	3	2.10	2.370
2	2	1	1	80	3	3	22.45	22.91
3	3	1	1	40	5	3	2.33	1.49
4	4	1	1	80	5	3	29.00	29.47
5	5	1	1	40	3	6	5.11	5.211
6	6	1	1	80	3	6	24.11	24.47
7	7	1	1	40	5	6	8.99	8.51
8	8	1	1	80	5	6	35.60	35.19
9	9	-1	1	26.36	4	4.5	0.66	1.10
10	10	-1	1	93.63	4	4.5	41.22	41.55
11	11	-1	1	60	2.318	4.5	11.22	10.15
12	12	-1	1	60	5.681	4.5	16.88	17.95
13	13	-1	1	60	4	1.977	9.11	9.15
14	14	-1	1	60	4	7.022	16.79	16.75
15	15	0	1	60	4	4.5	12.43	12.37
16	16	0	1	60	4	4.5	13.22	12.37
17	17	0	1	60	4	4.5	12.42	12.37
18	18	0	1	60	4	4.5	10.66	12.37
19	19	0	1	60	4	4.5	12.77	12.37
20	20	0	1	60	4	4.5	12.77	12.37

Table 2. Analysis of Variance for pretreatment

Source	DF	Seq SS	Adj SS	Adj MS
Regression	9	2204.91	2204.91	244.99
Linear	3	2037.43	2037.43	679.14
Temperature (T)	1	1894.33	1894.33	1894.33
Time of sonication (è)	1	73.44	73.44	73.44
NaOH (wt%) (W)	1	69.66	69.66	69.66
Square	3	133.74	133.74	44.58
Temperature*Temperature	1	128.36	132.28	132.28
Time of sonication*Time of sonication	1	4.78	5.08	5.08
NaOH (wt%)*NaOH (wt%)	1	0.60	0.60	0.60
Interaction	3	33.73	33.73	11.24
Temperature*Time of sonication	1	24.26	24.26	24.26
Temperature*NaOH (wt%)	1	0.25	0.25	0.25
Time of sonication*NaOH (wt%)	1	9.22	9.22	9.22
Residual Error	10	8.86	8.86	0.89
Lack-of-Fit	5	4.89	4.89	0.98
Pure Error	5	3.97	3.97	0.79
Total	19	2213.76		

Table 3. Estimated Regression Coefficients for pretreatment

Term	Coef	SE Coef	T	P
Constant	12.3782	0.3839	32.247	0.000
Temperature (T)	11.7775	0.2547	46.245	0.000
Time of sonication (θ)	2.3189	0.2547	9.105	0.000
NaOH (wt%) (W)	2.2585	0.2547	8.868	0.000
Temperature*Temperature	3.0296	0.2479	12.22	0.000
Time of sonication*Time of sonication	0.5936	0.2479	2.394	0.038
NaOH (wt%)*NaOH (wt%)	0.2046	0.2479	0.825	0.429
Temperature*Time of sonication	1.7412	0.3328	5.233	0.000
Temperature*NaOH (wt%)	-0.1762	0.3328	-0.53	0.608
Time of sonication*NaOH (wt%)	1.0738	0.3328	3.227	0.009

S = 0.941168 PRESS = 43.1577
R-Sq = 99.60% R-Sq(pred) = 98.05% R-Sq(adj) = 99.24%

Table 4. GCMS analysis

Compound	Molecular weight	% Weight
Ethyl alcohol	46	95.12
Acetic acid	60	1.109
1-Propanol, 2-methyl-	74	1.9630
Butanoic acid, 2-methyl-	102	0.1402

interaction terms of temperature and sodium hydroxide weight percentage content were statistically significant at the 95% confidence level and influence the percentage yield of reducing sugar.

Effect of time and temperature on yield of reducing sugar

When dilute NaOH concentration has been used, temperature and residence time must be increased in order to enhance the digestion of lignocelluloses [19]. In this experiment, temperature has played a vital role in reducing sugar yield. High yield of 41.22 % of reducing sugar was obtained at 93.63 C, 4 h, with 4.5 % of NaOH concentration. At elevated temperature, higher the diffusion of water molecule through the corncobs and transportation of alkali for the pretreatment. Figure 1 depicts that, at constant NaOH concentration, reducing sugar percentage increase as the temperature increases. High reducing percentage was observed at high temperature and moderate residence time[20].

Effect of NAOH and temperature on yield of reducing sugar

Similar type of surface plot with ups and downs has been observed at Figure. 2 depicting effect of NaOH and temperature on reducing sugar

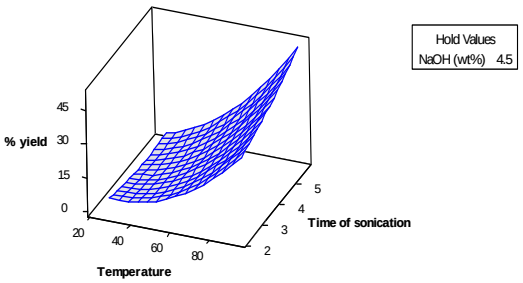

Fig. 1. Surface plot for Reducing sugar Vs Time, Temperature at constant concentration of 2% of NaOH

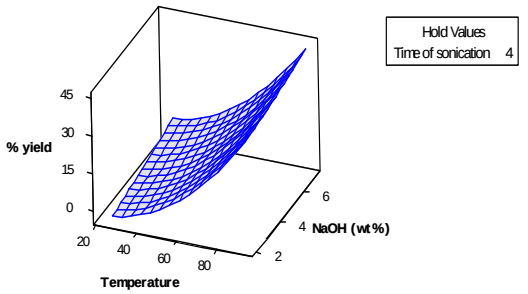

Fig. 2. Surface plot for Reducing sugar Vs NaOH, Temperature

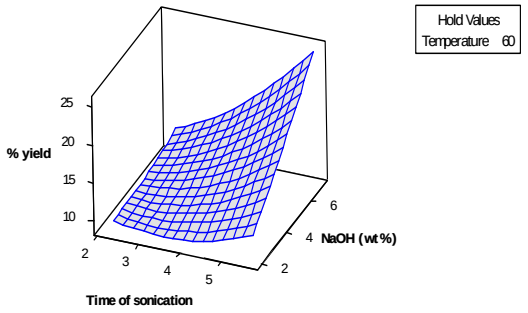

Fig. 3. Surface plot for Reducing sugar Vs Time, NaOH

yield. These ups and downs are due to variation in NaOH concentration, since only 2% of NaOH concentration was kept constant at the Figure. 2. Two main ups were observed near 90 C and 2% NaOH. These show that, high amount of reducing sugar has been obtained at 2% NaOH at 90 C [21].

Effect of time and NAOH on reducing sugar yield

A surface plot has been designed for reducing sugar vs NaOH and time. In Figure 3, horizontal surface plot has been observed which shows moderate reducing sugar yield in the absence of high temperature. A lite elevation is observed at a concentration of 3% NaOH and residence time of 12 h which depicts, at lower temperature NaOH concentration and residence time must be increased to enhance the biomass digestion [22].

GCMS analysis

GCMS analysis (Table 4) result reveals that ethyl alcohol (96.214%) (Water free) is the major constituent of distilled product. of was obtained. Byproducts such as acetic acid, 1- pentanol, propanoic acid were produced along with ethanol in negligible quantity.

CONCLUSION

Utilization of waste corn cob an agricultural left out can be used as a best source for production of bioethanol efficiently. The present study proves that; treatment of dilute sodium hydroxide solution under elevated temperature and moderate residence time under sonication results in higher yield of reducing sugar.

REFERENCES

1. Galbe, M. M.,. A review of the production of ethanol from softwood, *Appl. Microbiol. Biotechnol.* 2002; 618–628. doi:10.1007/s00253-002-1058-9.
2. Zhang, W., Zhang, J., and Cui, H., The Isolation and Performance Studies of an Alginate Degrading and Ethanol Producing Strain. *Chem. Biochem. Eng. Q.* 2014; **28**: 391–398.
3. El-zawawy, W. K., Ibrahim, M. M., Abdel-fattah, Y. R., Soliman, N. A., & Mahmoud, M. M., Acid and enzyme hydrolysis to convert pretreated lignocellulosic materials into glucose for ethanol production. *Carbohydrate Polymers*, 2011; **84**: 865–871. doi:10.1016/j.carbpol.2010.12.022.
4. Wang, G. S., Lee, J., Zhu, J. Y., & Jeffries, T. W., Dilute Acid Pretreatment of Corncob for Efficient Sugar Production, *Appl. Biochem. Biotechnol* 2011; 658-668.doi:10.1007/s12010-010-9071-4.
5. Xu, J., Cheng, J. J., Sharma-shivappa, R. R., Burns, J. C., Carolina, N., Science, C., & Box, C. Sodium Hydroxide Pretreatment of Switchgrass for Ethanol Production, *BioResources*. 2010; **9**: 2113–2119. doi:10.1021/ef9014718.
6. Sun, Y., & Cheng, J., Hydrolysis of lignocellulosic materials for ethanol production/ : a review. Bioresource. *Technol.* 2002; **83**: 1–11.
7. Sukumaran, R. K., Singhania, R. R., & Pandey, A.. Microbial cellulases Production , applications and challenges. *Renew. energy.* 2005; **64**: 832–844.
8. Kumar, S., Sharma, H. K., & Sarkar, B. C., Effect of Substrate and Fermentation Conditions on Pectinase and Cellulase Production by Aspergillus niger NCIM 548 in Submerged and Solid State Fermentation (SSF), *Food. Sci. Biotechnol.* 2011; **20**: 1289–1298.
9. Singh, A., & Bishnoi, N. R.. Enzymatic hydrolysis optimization of microwave alkali pretreated wheat straw and ethanol production by yeast. *Bioresource. Technol,* 2012; **108**: 94–101. Doi:10.1016/j.biortech.2011.12.084
10. Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D., Templeton, D.W. Compositional Analysis of Lignocellulosic Feedstocks Review and Description of Methods. *J. Agric. Food. Chem.* 2010; **58**: 9043-9053.
11. Templeton, D.W., Scarlata, C.J., Sluiter, J.B., Wolfrum, E.J., Compositional Analysis of Lignocellulosic Feedstocks. 2. Method Uncertainties. *J. Agric. Food Chem*. 2010; **58**: 9054-9062..
12. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D *et al.*, Determination of structural carbohydrates and lignin in biomass. National Renewable Energy Laboratory, Golden CO. Technical Report NREL/TP-510-42618, 2008.
13. Singh, A., and Bishnoi, N.R ., Optimization of enzymatic hydrolysis of pretreated rice straw and ethanol production. *Appl. Microbiol. Biotechnol.* 2012; **93**: 1785-1793.
14. Gomathi, D., Muthulakshmi, C., Guru Kumar, D., Ravikumar, G., Kalaiselvi, M., C Uma, Submerged fermentation of wheat bran by *Aspergillus flavus* for production and characterization of carboxy methyl cellulose. *Asian. Pac. J. Trop. Biomed.* 2012; 67-73.

15. Chittibabu, S., Rajendran, K., & Santhanmuthu, M. Optimization of microwave assisted alkali pretreatment and enzymatic hydrolysis of Banana pseudostem for bioethanol production, 2011; **6**: 67–71.
16. Luiza Jecu, Solid state fermentation of agricultural wastes for endoglucanase production, Ind. Crops. Prod. 2000; **11**: 1–5.
17. Ghose, T.K., Measurement of cellulase activities, 1987; **59**: 257-268.
18. Borreti G, Garofano L, Montecucchi P, Spalla C. Cellulase production with Penicillium iriense. *Arch Microbiol*. 1972; **92**: 189-200.
19. Ye Sun, Jiayang Cheng ,Hydrolysis of lignocellulosic materials for ethanol production: a review, *Bioresource .Technol.* 2002; **83**: 1–11.
20. Fang, H., Zhao, C., & Song, X., Bioresource Technology Optimization of enzymatic hydrolysis of steam-exploded corn stover by two approaches/ : Response surface methodology or using cellulase from mixed cultures of Trichoderma reesei RUT-C30 and *Aspergillus niger* NL02. *Bioresource .Technol*, 2010; **101**: 4111–4119. doi:10.1016/j.biortech.2010.01.078.
21. Goh, C. S., Tan, K. T., Lee, K. T., & Bhatia, S., Bioresource Technology Bio-ethanol from lignocellulose/ : Status , perspectives and challenges in Malaysia. *Bioresource. Technol*, 2010; **101**: 4834–4841. doi:10.1016/j.biortech.2009.08.080.
22. Kyong, J., Seop, J., Woo, M., Jin, H., Choi, I., Hyun, T., & Heon, K., Bioresource Technology Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes. *Bioresource. Technol*, 2009; **100**: 4374–4380. doi:10.1016/j.biortech.2009.04.026.