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Effect of Various Treatments on the Production of Bioethanol from Wheat Bran

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Abstract **- Wheat bran is the largest by-product produced during roller wheat milling. Attempts were made to use wheat bran for the production bio-alcohol was of partial success. Production of ethanol from hexose sugar is in practice worldwide. Present study deals with the production of alcohol from pentose sugar which is a sole constituent of wheat bran. Physical treatment like Particle size reduction of wheat bran along with chemical and enzymatic treatments, were studied for the production of bio-alcohol production. Wheat bran of reduced particle size was treated with moist heat at 80^{0}C, coupled with acid treatment produced total sugar with fermentable range. On fermentation with *Candida tropicalis* MTCC230 a maximum of 1.21 g/g of bio-alcohol was produced. Which relatively higher than earlier study. Viscosity was subordinate for 1% acid treated wheat bran due to breakdown of polysaccharides into simple sugar which is accountable for viscosity. Limited fermentation is seen in sample of wheat bran extract treated with higher concentration of acid, when subjected to fermentation with *Candida tropicalis* MTCC230 resulting in low yield of bio-alcohol. This is attributed to low reducing sugar and fermentation inhibition by acid. Crude fibre content is high in pretreated bran when compare to raw wheat bran. Reological parameters indicated that the fermentation of acid and heat treated bran extract exhibited shear thinning behaviour of non-Newtonian fluids.**

Keywords : - Bioethanol, Plate mill, overtails, flow behaviour index (Power law index) and consistency index.

INTRODUCTION

While energy demand is growing rapidly, non-renewable fossil fuel reserves are limited which has compelled scientists across the world to explore for renewable source of energy from bio wastes and lignocelluloses. Although future bio-fuel ethanol will certainly require cellulosic technology, corn starch is still the major substrate widely used (Farrell et al., 2006). Use of bioethanol as a biofuel is both renewable and environment friendly (Vijayalaxmi et al. 2013; Torres et al. 2013). Although sugarcane and corn are the dominant feedstocks today (Wheals et al. 1999), projected fuel demands indicate that ethanol production from other starch-rich grains, food processing by-products, agricultural and forest residues, and agricultural energy crops will be required in the future.

Bio-ethanol production needs an economical approach to address the fuel needs and also in utilization of residual wheat bran that goes as cattle feed. The present study is focused on the utilization of wheat bran for the production of biofuel.

Wheat is the major food crop grown throughout the world and amongst the various wheat milling by-products, wheat bran is the one produced in larger quantities. Recently, various researchers have utilized wheat bran for different purposes. Various studies on ethanol conversion systems from wheat products have been conducted using raw wheat flour or wheat grains or from other wheat products, but not on wheat bran as such. Industrial wheat bran usually accounts for 14-15% of the grain and comprises the outer coverings and the ruments of the starchy endosperm (Javed et al. 2011).

Wheat bran is rich in carbohydrates (60%) of which 34% is starch, protein (12%), fat (0.5%), minerals (2%), bioactive compounds and vitamins (Slavin 2003; Palmarola-Adrados et al. 2005). Precise composition of macro and micro nutrients may vary from cultivar to cultivar. Wheat bran is commonly used as cattle feed and also as a source of dietary fibre, an ingredient for functional foods.

Lots of work has been carried out to produce bioethanol utilizing different lignocellulosic material, including combination of materials. But limited work has been carried out on wheat bran. *Candida tropicalis* is known to produce ethanol from starch, although at a low rate, due to its production of a glucoamylase (Nakamura, 1970). But the ethanol production in pretreated bran is high (Moiser et al., 2005; Chandel et al., 2007a,b). In the present work, studies have been carried out concentrating mainly on different hydrolysis process to yield sugars which aids in fermentation for the production of alcohol.

MATERIALS AND METHODS

Material procurement: The wheat bran which was used as substrate in the study was collected from Sri Basaveshwara Roller Flour Mill, KRS Road, Hootagalli Industrial area, Mysore.

Commercial enzymes (xylanse, hemicellulase and cellulase) were used in the study.

Size reduction of wheat bran: Buhler Plate mill was used in the study for the size reduction of coarse wheat bran (flakes). Milled wheat bran was classified based on size using Buhler's lab sifter. Sieves used were 6xx (212

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microns) & 10xx (129 microns) for studying effect of particle size. After grinding Bran flake in the plate mill, 50 grams of ground stock was sieved for 5 minutes and then all the overtails and throughs were calculated to express in percentage.

For all experiments wheat bran was maintained with 2.5 time its water holding capacity. All experiments were carried out in triplicates and averages of the same are reported.

Enzyme treatment: Enzymes (0.5% w/w) were added to the moistened wheat bran and incubated at 50^0C for 2 hours. The extracts were analyzed for sugar concentrations and rheological properties.

Enzyme treatment with thermal pretreatment: The moistened bran was pretreated by incubating for 1 hour at 80^0C. Enzyme (0.5% w/w) was added and incubated at 50^0C for 2 hours.

Enzyme treatment with acid pretreatment: The moistened wheat bran was pretreated with Sulphuric acid (0.5 and 1%) at 60^0C and 80^0C for 1 hour respectively, followed by enzyme hydrolysis after adjusting to the enzymes optimum pH for 2 hours.

Estimation of total sugar: Method based on (Scott and Melvin 1953) was performed to estimate the total sugars. Different aliquots of the samples were adjusted to 1ml with distilled water and treated with 5% of 1 ml phenol and 5 ml concentrated sulphuric acid. The test tubes were incubated at ambient temperature for 30 minutes. The colour developed was read at 490 nm against the reagent blank. The concentration of total sugar in the sample was calculated using the standard graph.

Estimation of reducing sugar: Method based on that of (Miller 1959) was performed to estimate the reducing sugars. Different aliquots of test solution were made upto 3 ml with distilled water. 3 ml of Dinitro salicyclic acid (DNS) reagent was added to all the tubes. The tubes were heated in boiling water bath at 95^0C for 10 minutes. Mixture was cooled and then 1 ml of 40% Rochelle salt was added to all the test tubes. Colour developed was read at 540 nm against the reagent blank. Concentration of reducing sugars in the samples was calculated using the standard graph.

Rheology measurement: The rheological estimations were carried out using a rheometer of cup and bob arrangement using a universal stress rheometer SR5 (Waters make). A cup of diameter 30 mm and a bob of diameter 28.8 mm and 50 mm length were used. A gap of 5 mm was fixed. Measured volume of sample was added in the cup and bob gap set. The temperature was set to 30^0C. A simple shear rate- viscosity and shear rate- stress data were obtained. The parameter like flow behaviour index (Power law index) and consistency index were calculated using Power law model ($\mu=K\gamma n-1$) using experimental viscosity versus shear (shear rate – stress/ shear stress) data.

Fermentation studies: Xylose fermenting *Candida tropicalis* MTCC230 was procured from MTCC, Chandigarh. Fermentation studies using wheat bran extract was carried out with inoculum grown on xylose medium under aerobic condition for 48hr. 5% of the culture (4.8x106CFU/ml) was used for the study. The fermentation was carried out at 30±20C at pH 5. Samples were withdrawn and analyzed for alcohol production after 48hr and 72hr.

Estimation of alcohol percentage: Method based on (Caputi et al. 1968) was performed to estimate the alcohol percentage. Sample (2 ml) was taken in 50 ml distilled water in a distillation flask and was distilled at 65^0C. 15 ml of the distillate was collected in a conical flask and chromic acid (25 ml) was added. Volume was made up to 50 ml with distilled water. The conical flasks were incubated at 60^0C on a water bath for 30 minutes. The solutions were brought to room temperature and the optical density was read at 600 nm spectrophotometrically. The alcohol percentages of the samples were calculated using the standard graph produced using absolute alcohol.

Gas chromatographic determination of alcohol: The alcohols present in the samples were determined using the gas chromatography Shimadzu GC 6A. A paropak-Q column at temperature of 180^0C was used with nitrogen as the carrier gas with flow rate 40 ml/min. The injection and departure temperatures were 220^0C and 230^0C. 0.2 µl of alcohol standards and sample distillate were injected into column. Peaks were identified by comparing the retention time of alcohol standards.

Colour measurement: The values of surface colour of raw and residual wheat bran were measured in terms of lightness (L) and colour (+a:red; -a:green; +b:yellow; -b:blue) using Hunter Lab Colour measuring system (Colour Measuring Labscan XE, system, USA). A standard white tile of barium sulphate (100% reflectance) was used as a perfect white object for calibration of the instrument with the illuminate. Samples were placed in the sample holder and reflectance was auto recorded for the wavelength ranging from 360 nm to 800 nm.

Fiber content of residue: AACC (American Association of Cereal Chemists) AACC (2006) method was followed to analyse fiber content of the residue bran after hydrolysis. Both crude and dietary fibers were analysed.

Crude fiber analysis: Sample (2 g) was refluxed with 200 ml of sulphuric acid for 30 min. The contents were filtered through linen in fluted funnel and washed with boiling water until washings are no longer acid. NaOH (200 ml) was added to this filtrate and further refluxed for 30 min. followed by filtering it through crucible. The crucible was dried in oven at 130°C till it attained a constant weight. After cooling the crucible in desiccators, it was weighed. The contents of crucible were ignited in muffle furnace at 300^0C for 20 min. Followed by weighing the cooled crucible in desiccators, the loss in weight was taken as crude fibre.

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Dietary fiber analysis: Sample (1 g) along with 50 ml sodium phosphate buffer and teramyl (50 µl) was incubated at 95°C ±10°C for 30 min. the mixture was cooled and pH was adjusted to 7.5 followed by incubation for 30 min at 30°C with 25 µl protease. Further the sample was incubated with amyloglucosidase for 30 min adjusting the pH to 4.6 at 60°C. This was filtered in a crucible; the insoluble residue was washed thrice with 95% alcohol and acetone. The filtrate was collected and alcohol was evaporated. Both alcohol soluble and insoluble residues were dried in oven at 130°C to constant weight, followed by igniting crucibles in muffle. Loss in weight of alcohol soluble residues was taken as soluble and insoluble residues as insoluble dietary fibre.

RESULTS AND DISCUSSION

Feasibility of enzyme treatment: To know the feasibility of wheat bran as substrate for the biofuel production different studies were conducted based on the pretreatment using the xylanase, cellulase and hemicellulase enzymes.

The total sugar concentration (results not included in this paper) yielded from the hydrolysis of raw wheat bran was not encouraging to carry out fermentation. Hence, the hydrolysis study was continued with the size reduced wheat bran (212µ size). The size reduced bran yielded better sugar concentrations compared to the raw wheat bran. This may be due to the increase in surface area for interactions.

Among the enzymes used for hydrolysis, xylanase exhibited better result compared to other two enzymes with respect to total sugar and reducing sugar concentrations with 54.72 mg/g and 49 mg/g respectively (Table 1).

Effect of treatment on the Viscosity: The viscosity of the hydrolysate extracted can be related to the sugar concentration. Thicker the extract higher will be viscosity. Viscosity variation was marginal with respect to the sugar concentration compared to the different hydrolysates as shown in the Table.1 7.51 mPa-S for 54.7 mg/g total sugar concentration and 8.07 mPa-S for 42.9 mg/g. With 37 mg/g total sugar concentration the viscosity of the hydrolysates was 4.52 mPa-S. The extraction efficiency of substrate obtained had marginal difference for different enzymes as shown in Table 1. The extraction efficiency of the control hydrolysis was low with 53.8% as shown in (Table 1).

A group of enzymes are responsible for the hydrolysis of xylan (Girio et al. 2010). Earlier working on baggase we had proved that a combination of enzymes worked better (Vijayalaxmi et al. 2013). In the present experiment at 0.5% concentration of xylanase, better results were obtained with respect to sugar concentration and extraction efficiency (58%). Further hydrolysis study was continued with the 0.5% enzyme concentration.

Thermal treatment: Thermal pretreatment resulted in the release of 108.1mg/g and reducing sugar concentration was 8.3mg/g at 0.5% enzyme concentration as shown in (Table1). The yield of sugar concentration improved on pretreatment.

Effect of acid and thermal pretreatment: Acid impregnation with the sulphuric acid at 0.5% and 1% concentrations and thermal pretreatment at 60^0C and 80^0C followed by enzyme hydrolysis (pH-4.5) were carried. Results showed that at 1% acid concentration followed by thermal pretreatment at 800C was better compared to that at 60^0C. The hydrolysis was carried out without any enzyme addition. The best extraction volume obtained was at 1% acid concentration with 87.3% extraction efficiency. At 1% acid concentration and 80^0C, the viscosity was 7.62mPa-S (Table 2). Though the total sugar concentration was high with 332.9 mg/g the viscosity was low with 7.62 mPa-S. This may be because of the acid pretreatment given to the polysaccharide, which, would have degraded them into simple sugars, accounting for total sugar concentration and not in the thickening of the extracts (Somashekar and Anu Appaiah 2012). But in other cases the sugar concentrations had accounted for the viscosity. Further experiments with acid pretreatment followed by enzyme hydrolysis, were high with 1% acid concentration and 80^0C with efficiency of 94.4% (Table 1).

Table 1: Effect of enzyme hydrolysis on the hydrolysis of Wheat bran

Treatment (%)	Extraction efficiency (%)	TS (mg/g)	RS (mg/g)	Viscosity (mPa-S)
Control	53.8	23.43	0.36	3.56
C (0.5)	58.6	42.92	3.37	8.07
HC (0.5)	57.8	37.08	3.42	4.52
X (0.25)	53.0	44.69	3.49	12.28
X (0.5)	60.0	54.72	4.94	7.51
X (0.75)	51	50.80	2.36	17.51
X (1)	56	46.87	3.65	22.01
WB +T+X (0.5%)	74.0	108.1	8.28	18.03
WB+T	67.7	89.46	2.1	12.46
1% acid,	87.3	332.94	13.14	7.62
T-80				
1% acid,	94.4	249.2	9.51	40.15
T-80 + X (0.5%)				

Legend: X-Xylanase, C-Cellulase, HC-Hemicellulase, TS- Total sugar, RS-Reducing Sugar, Control-no enzyme addition, T- Thermal treatment.

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Table 2: Rheological parameters of fermentation broth of size reduced wheat bran

Time (hr)	K (Pa-S)	n	N	R^2	TS (mg/g)	RS (mg/g)	Growth (660nm)
0	0.00262		0.0894	0.9624	37.8	3.60	1.308
24	0.00295		0.2405	0.9925	24.0	2.20	5.355
48	0.00389		0.6351	0.9737	15.9	1.09	6.391
72	0.00527		0.3180	0.7948	9.8	0.98	6.399

Legend: TS- Total sugar, RS-Reducing Sugar, K-Consistency index, n-Power law index, R^2- Correlation Co-efficient.

Hydrolysis was carried out with acid pretreatment in which acid acts as the catalyst rendering the cellulose fraction more amenable for further enzymatic treatment. Treatment at higher concentrations of acid and temperature hydrolysis with enzymes indicated poor results due to charring of enzymes by extreme reaction conditions.

The acid pretreatment followed by enzyme hydrolysis has shown higher efficiency compared to only enzyme hydrolysis with respect to the sugar concentration. This shows that strong catalyst like acid has to be used for delignification and degradation of hemicelluloses.

Fermentation studies of the hydrolysate: The hydrolysate obtained after acid & thermal and acid with thermal & enzyme treatment were used for fermentation studies. Utilization of sugars was exponential during the first 48hrs of growth (Table 3). This was due to the increase in growth of the organisms. After 48hrs, both growth and sugar utilization decreased. Alcohol production started after 48hrs of growth. Both pentoses and hexoses and broad range of substances as reaction by-products from sugar and lignin degradation. Many of these substances have an inhibitory effect on the microorganism in subsequent fermentation steps (Balat and Balat, 2009; Chandel et al., 2007c). At the end of the fermentation total alcohol production was low (1.21%). Earlier working with xylose sugars, (Abbi et al. 1996) had achieved low alcohol production (0.40%). The present yield is higher than the earlier result.

Flow behavior of the fermented broth: Fermenting broths showed shear thinning behaviour of Non- Newtonian fluid. These results are similar to the results of (Goudar et al. 1999) who have worked on influence of microbial concentration on the rheology of Non-Newtonian fermentation broths. The present study results showed that the viscosity of the broth gradually decreases with the sugar consumption. It can be related to the growth of the fermenting yeast, which leveled-off indicating the onset of endogenous respiration. The results fit best to the Power law model. The correlation of broth after 72 hr of fermentation deviated compared to 0 hr and 24 hr correlations. Acetic acid or other weak acids which are generated due to the hydrolysis of the acetyl groups or other linkage present in hemicellulosic backbone brings down the R2 values (Chandel et al. 2011). The 72 hr extract correlation deviation may be because of the growth rate which got leveled up during this period (Table 3).

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Table 3: Fermentation of acid and thermally pretreated (A),
acid and thermally pretreated followed by enzyme treated (B) wheat bran hydrolysate by Candida tropicalis

Fermentation Period (hr)	Growth (660nm)	pH	Total sugar (mg/g)	Reducing sugar (mg/g)	Viscosity (mPa-S)	Alcohol (g/g)
A 0	0.888	5.60	332.9	13.14	15.40	-
A 72	5.670	5.29	99.9	4.75	3.65	0.97
B 0	1.308	5.63	249.2	9.51	17.98	-
B 72	6.399	5.82	64.6	2.58	3.14	1.21

The correlation between the consistency index and substrate utilization was found to be acceptable (Fig 1 & Fig 2) for the fermenting broths. In Fig 2 the correlation between consistency index and reducing sugar utilization (0.655) had deviated comparatively when compared to total sugar concentration. In addition to the sugars, several by-products are formed or released in the hydrolysis process (Larsson et al.1999). This may be attributed to the low reducing sugar concentration and the fermentation inhibitors. Which are expected in case of acid pretreatment, indicating the lag phase of growth rate and exhaustion of simple sugars (Fig 1). In case of acid and thermally pretreated followed by enzyme treated wheat bran extract, the correlation was (0.707). Deviation may be due to the growth levelling at this period. The correlation between total sugar concentration and consistency index was more convincing comparatively to the reducing sugar concentration in both the cases with correlation co-efficient 0.813 & 0.861 respectively. This may be due to the substrate utilized for the growth of Candida tropicalis and partly by the production of alcohol.

(Jefferies 2006) suggested that excessive heating can bind amino acids and protein to other compounds, such as fiber, effectively reducing the digestibility of amino acids in the animal. The data indicate that there was inhibition to the enzymes (hydrolysis studies). This inhibitory effect may be due to feedback by the products formed during hydrolysis, or due to the breakdown of the more easily hydrolyzed substrate leaving the more difficult to digest substrates as residual sugars.

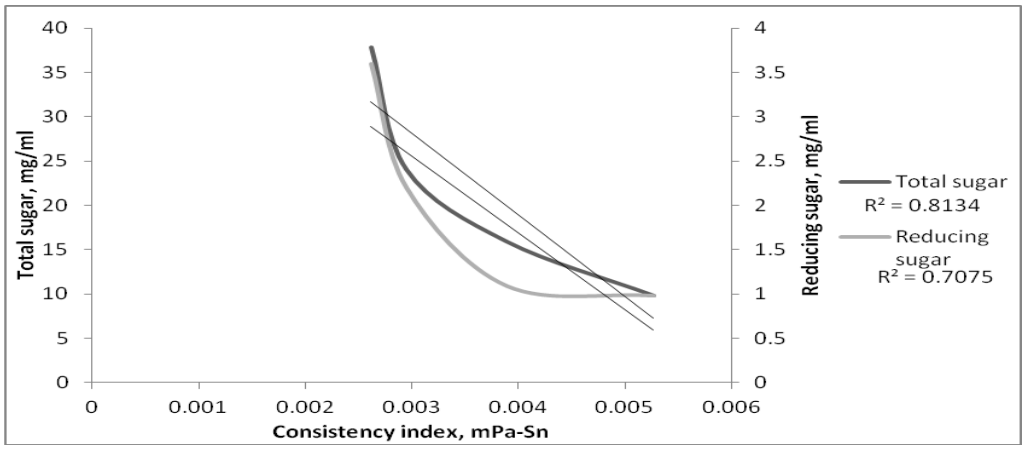

Fig 1: Correlation between consistency index and substrate utilisation of size reduced wheat bran fermentation broth.

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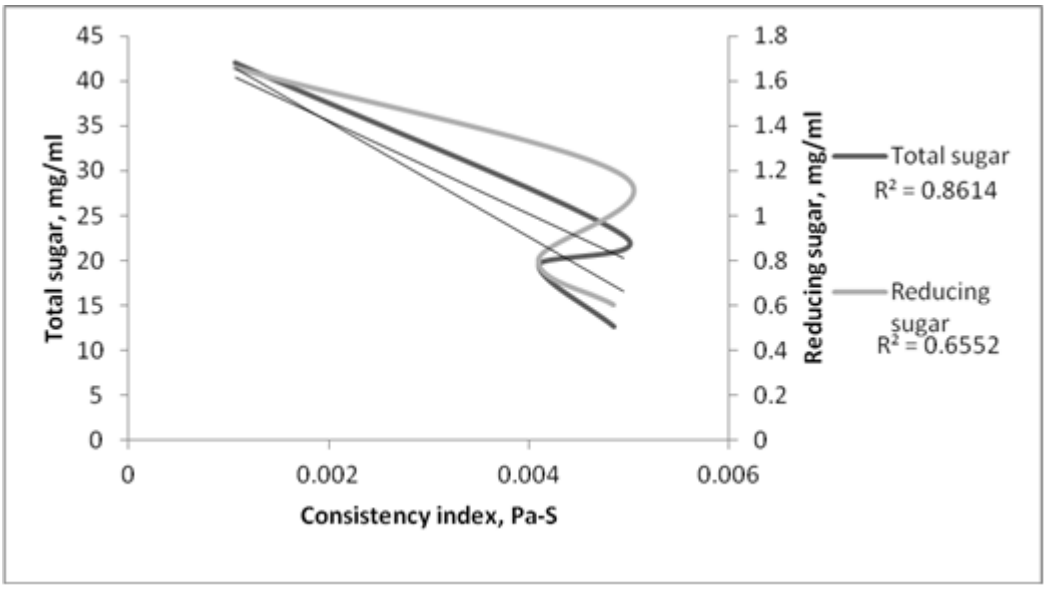

Fig 2: Correlation between consistency index and substrate utilisation of acid pretreated wheat bran fermentation broth.

Effect of pretreatment on the colour of the hydrolysate: There was considerable variation between the bran hydrolysate samples regarding colour. The raw bran sample has generally exhibited greater lightness compared to other samples, which are having greater values for +a and +b. The acid and thermally treated residue sample tilted more towards red in colour which followed closely by the enzyme treated after pretreated bran residue. The raw sample was lighter and less yellow compared to the other two samples which are too close in terms of yellow colour as shown in (Table 4.) It is known that the separation of the peripheral tissues from the endosperm during milling depends on the elasticity of the different tissues and their adherence to each other. The smaller particle size of the bran samples probably depends on the peripheral tissues and stronger adherence to the endosperm. The adherence of bran to the endosperm affects the milling properties of wheat (Jefferies 2006). This may result in the presence of trace endosperm (starchy material) concentration on the surface of the bran after milling which has resulted in the higher lightness value of the raw bran with 62.86 compared to the other two lightness values 55.82 and 53.06 of acid and thermally treated and acid with thermal and enzyme treated residues.

Table 4: Results of colour measurement of the bran residues

Samples	L*	a*	b*
Raw	62.86	6.28	23.2
Acid pretreated hydrolysis residue	55.82	8.10	27
Steam explosion residue	53.06	7.82	27.44

Legend: L*= Lightness; a*negative=green, positive=red;
b*negative=blue, positive

Content of fibre in raw and treated wheat bran: The present study showed that the fibre fractions, such as crude and dietary fibre of treated residue were significantly higher compared to the raw bran (Table 5). Results of the present study can be compared with (Bindelle et al. 2007) who have worked on the wheat bran residues of hydrolysis. The residue after treatment can be used as cattle feed as the fiber content is high.

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Table5: Results of fiber contents of the bran residues

Sample	Dietery fiber (%)		Crude fiber (%)
	Insoluble	Soluble	
Raw	50.5	2.4	47.9
Acid pretreated hydrolysis residue	59.8	0.8	48.7
Steam explosion residue	60.6	0.5	48.7

CONCLUSION

Our results establish that the pretreatment of wheat bran is sufficient to procure acceptable amount of sugar accomplishing a carbon requirement for bio-alcohol production. Though in pentose sugar fermentation the total alcohol produced is low and requires a detailed study to investigate the fermentation abilities of the selected organisms. Saccharification step adopted in present study is cost effective, since in the present technology complete liquefaction of the biomass is not possible. This raises questions on the management of residues generated. Combination of treatments as adopted can reduce the release of inhibitor molecules, however the total sugars released may not be enough to achieve commercially feasible model. The residue after treatment can be used as cattle feed as the fiber content is high.

REFERENCES

[1] Abbi, M., Kuhad, R.C., and Singh, A. (1996). Fermentation of xylose and rice straw hydrolysate to ethanol by Candida shehatae NCL-3501.J. Ind. Microbiol. Vol 17, pp 20-23.
[2] AACC (2006) (Method 32-05.01) for Total Dietary Fiber 9 edition. AACC International Press, USA.
[3] Balat, M and Balat, H. (2009). Recent trends in global production and utilization of bio-ethanol fuel. Applied Energy, 86, 2273–2282.
[4] Bindelle, J., Buldgen, A., Boudry, C. and Leterme, P. (2007). Animal Feed Science and Technology, 132(2), 111-122.
[5] Capauti, A., Ueda, J. M., and Brown, T. (1968). Spectrophotometric determination of chromic complex formed during oxidation of alcohol. AM. J. Enol. Viticulture, Vol 19, pp 160-165.
[6] Chandel A. K, Kapoor, R. K., Narasu ML, Viswadevan V, Kumaran. S. S. G., Ravinder, R., Rao, L. V., Tripathi, K. K., Lal, B., Kuhad, R. C., (2007b). Economic evaluation and environmental benefits of biofuel: an Indian perspective. Int. J. Global Energy Issues, 28: 357-381.
[7] Chandel A. K, Kapoor R. K, Singh, A. K, Kuhad, R. C (2007c). Detoxification of sugarcane bagasse hydrolysate improves ethanol production by Candida shehatae NCIM 3501. Biores. Technol., 98: 1947-1950.
[8] Chandel, A. K., Kapoor, R. K., Singh, A., and Kuhad, R. C. (2007). Detoxification of sugarcane bagasse hydrolysate improves ethanol production by Candida shehatae NCIM 3501. Biores. Technol., Vol 98, pp1947–1950.
[9] Farrell, A. E., Plevin, R.J., Turner, B.T., Jones, A.D., O'Hare, M., Kammen, D.M., 2006. Ethanol can contribute to energy and environmental goals. Science 311, 506–508.
[10] Girio, F. M., Fonseca, C., Carvalheiro, F., Duarte, L.C., Marques, S., & Bongel-Lukasik, R. (2010). Hemicelluloses for fuel ethanol: A review. Biores. Technol., Vol 101, pp 4775-4800.
[11] Goudar, C. T., Strevett, K. A., & Shah, S. N. (1999). Influence of microbial concentration on the rheology of non-Newtonian fermentation broths. App Microbiol Biotechnology, Vol. 51, pp. 310-315.
[12] Javed, M. M., Zahoor, S., Shafaat, S., Mehmooda, I., Gul, A., Rasheed, H., Bukhari, A. I., Aftab, M. N., & Haq, I. (2012). Wheat bran as a browngold: Nutritious value and its biotechnological applications. African J. Microbiol. Res., Vol 6, pp. 724-733.
[13] Jeffries, T. W. (2006). Engineering yeasts for xylose metabolism. Curr. Opinion Biotechnol., Vol 17, PP 320–326.
[14] Larsson, S., Palmqvist, E., Hahn-Hagerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., Nilvebrant N.O., The generation of fermentation inhibitors during dilute acid hydrolysis of softwood Enzyme Microb. Technol., 24 (1999), pp. 151–159
[15] Miller, G. L. (1959). Use of Dinitrosalicyclic acid reagent for determination of reducing sugar. Anal. Chem., Vol 31, pp. 426-428.
[16] Moiser, N., Wyman, C., Dale, B., Elander, R., Lee, YY., Holtzapple, M., Ladisch, M.(2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Biores. Technol., 96: 673-686.
[17] Nakamura, L. K., 1970. InXuence of the acceptor during transglucosylation by transglucosylamylase of Candida tropicalis. Can. J. Biochem. 48, 1260–1267.
[18] Palmarola-adrados, Beatriz., Choteborska, pavla., Galbe, Mats and zacchi, Guido. (2005) Ethanol production from non-starch carbohydrates of wheat bran. Bioresource Technology, vol. 96, no. 7, p. 843-850.
[19] Scott, J. A., and Mevlin, E. H. (1953). Determination of dextrane with anthrone. Anal. Chem., Vol. 25, pp 1656.
[20] Slavin, J. (2003). Why whole grains are protective: biological mechanisms. Proceeding of Nutritional Society, Vol 62, pp. 129-134.
[21] Somashekar, K. L., and Anu Appaiah, K. A. (2013). Coffee cherry husk- A feed stock for alcohol production. Int. J. Environment and Waste Management, Vol 11, pp 410-419.
[22] Torres A. F. Van der Weijde T Dolstra O. Visser R G. F & Trindade L. M. (2013) Effect of maize biomass composition on the optimization of dilute-acid pretreatments and enzymatic saccharification. Bioenergy Res. Vol 6, pp. 1038–1051.
[23] Vijayalaxmi, S., Anu Appaiah, K. A., Jayalakshmi, S . K., Mulimani, V. H., and Sreeramulu, K. (2013) Production of bioethanol from fermented sugars of sugarcane bagasse produced by lignocellulolytic enzymes of Exiguobacterium sp. VSG-1.App. Biochem. Biotechnol. Vol.171 pp 246–260
[24] Wheals, A. E., Basso, L.C., Alves, D.M.G., Amorim H.V., (1999) Fuel ethanol after 25 years Trends Biotechnol, pp. 482–487.