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Bio-ethanol Production from Rice Husk Using Simultaneous Saccharification and Fermentation and Optimization of Pretreatment Methods

Rawinder Kaur, Himanshu Singh*

Department of Biotechnology, School of Bioengineering and Biosciences,
Lovely Professional University, Punjab-144411, India

ABSTRACT

This study was done to find out the optimized pretreatment for the production of bioethanol from rice husk and to obtain the maximum yield of ethanol by the process of Simultaneous Saccharification and Fermentation. The rice husk homogenized samples were pretreated with 1%, 1.5% and 2% HCl and with 1%, 2% and 3% NaOH solution. The pretreated samples were used for Simultaneous Saccharification and Fermentation (SSF) at 28 ± 2°C at 120 rpm. It has been found through analysis of 3,5-Dinitrosalicylic Acid (DNSA), FTIR and GC that 2% HCl pretreated sample and 3% NaOH pretreated samples resulted into maximum ethanol yield of 6.34% and 5.89% respectively.

Keywords: Pretreatment, Lignocellulose, Rice husk, Saccharification, Fermentation, Bioethanol

INTRODUCTION

The main focus for the bioethanol production is the agricultural wastes these days due to the food and feed competition that resulted in the global scarcity of food in previous few years [1]. The use of the agricultural residual wastes is the cost effective way for the production of ethanol. In this review our main concern is to study the bioethanol production from the residual wastes of rice crop. As in many regions burning is the main practice preferred by the farmers to decompose this waste or the other way is that they use these types of agro-waste as fodder for cattle. Therefore to use these waste products in production of bioethanol is of more economic use and is environmental favorable as the burning of these waste produces a lot of gases harmful to the environment. Rice is the third most important grain crop around the world. As per FAO statistics, world annual rice production in 2007 was about 650 million tons. Accordingly it was estimated that about 650-975 million tons of rice straw produced every year all around world [2]. Waste utilization and cost reduction in industrial processing by rice husk as a valued materials. Large part of these rice residual wastes is made up of complex carbohydrates like cellulose and hemicelluloses. These cellulose and hemicelluloses can be converted into sugars and ethanol fermenting microorganisms can utilize these sugars to convert it into ethanol. Chemical composition for rice straw consists of cellulose (32-47%), hemicellulose (19-27%) and lignin (5-24%) [2]. Composition of rice husk is that it contains 75-90% of organic matter such as lignin, cellulose and hemicellulose [3]. Rice husk generally contains approx. 29.3% hemicellulose and approx. 34.4% cellulose which can be degraded to get reducing sugars [4]. Rice straw is one of the abundant lignocellulosic materials which is easily found anywhere around the world. Its annual production is about 73 million tons globally. This much of the residue is able to produce a huge amount of bioethanol per year. The worldwide annual rice husk output is about 80 million tons with an annual energy potential of 1.2×10^9 GJ, corresponding to a heating value of 15 MJ/kg [5]. The technology of using rice husk is usually in trend in Asia whereas the rice straw is rarely used as the renewable resource in industries. The reason for this is that rice husk is easily available at the rice mills at any time throughout the year whereas the availability of rice straw is limited to harvest time. Rice husk constitutes about one fifth of the annual gross rice production of the world [6].

The production of ethanol from lignocellulosic waste comes under the second generation biofuel production. It is an alternative to the first generation biofuels which are produced directly from the food crops such as sugarcane, potatoes, corn etc. and emerges into the food and fodder concerns [7]. This residual waste contains cellulose, hemicelluloses and lignin. Lignin is the outer most layers which need to remove. Several pretreatment techniques are required to remove this hard outer covering. The main concern of the study is for the optimization of various techniques used for the bioethanol production in order to reduce the production cost. The research is not only concerned with economic reasons but also take into consideration the ecological aspects. The alternative and cheaper sources of the ethanol production were studied in this study. It has been researched that the LCM as the cheapest source is the most beneficial but the major challenge is the expensive technology used for its pretreatment [8]. Pretreatment strategies help to increase the accessibility of enzymes to the cellulose to convert it into sugars [9].

Then hydrolysis of cellulose and hemicellulose is done to produce the fermentable sugars such as glucose, xylose, arabinose, galactose, mannose etc. In a study it has been found that alkali pretreatment of rice husk resulted into proper delignification of lignocelluloses and then its fungal treatment with *Trichoderma reesei* resulted into highest conversion into sugars. Highest ethanol yields (250 mg/g) were obtained after 6 days of fermentation with *S. cerevisiae* [10]. Simultaneous Saccharification and Fermentation (SSF) is one of the fermentation technology adopted as it lessens the costs and resulted into higher ethanol production as it minimizes the product inhibition compared to Separate Hydrolysis and Fermentation (SHF) [11] and requires shorter residence time and low enzyme loading and is cheap [12]. The major challenge for this technique is the difference in the optimized conditions for hydrolyzing and fermentation microorganisms.

MATERIALS AND METHODS

Materials

Rice husk was collected from the farms of Hoshiarpur near Verka Milk Plant, during the harvesting season of the paddy crop. Sulphuric acid (H_2SO_4) was used for the acidic pretreatment. Citric acid, sodium hydroxide, 3,5-dinitrosalicylic acid, potassium tartarate, glucose were used to perform the DNS analysis to determine the concentration of reducing sugars. Potato Dextrose Broth (Himedia) was used to culture *T. reesei*. Urea, Sodium Sulphate ($(NH_4)SO_4$), Sodium Nitrate ($NaNO_3$), Dipotassium phosphate (K_2HPO_4), Magnesium Sulphate Hepahydrate ($MgSO_4.7H_2O$), Calcium Chloride ($CaCl_2$), Manganese Sulphate Hepahydrate ($MnSO_4.7H_2O$), Zinc Sulphate Hepahydrate ($ZnSO_4.7H_2O$), Peptone, Yeast Extract (Himedia), dextrose were used to prepare the media for SSF. *Trichoderma reesei* (MTCC 164) and *S. cerevisiae* (MTCC 464) were the strains that have been used. Spectrophotometer readings for DNSA analysis has been taken with the help of Elico SL 210 UV VIS Spectrophotometer at 540 nm. FTIR analysis was done on Shimadzu FTIR 8400 S Spectrophotometer. The samples were sent to Herbal Health Research Consortium, Amritsar for GC analysis.

Methods

Physical (mechanical) pretreatment

After the hand picking of the raw material to clean the sample, it was used to grind in the grinding machine. The homogenized small size particles were obtained after grinding.

Acidic pretreatment

Acidic Pretreatment is done at three different concentrations of H_2SO_4 were 1%, 1.5% and 2% [13,14].

Basic pretreatment

Basic treatment was also done at three different concentrations were 1%, 2% and 3% [15]. All the above flasks were the autoclaved at 121°C at 15 psi. After treatment, the samples were filtered out with the help of muslin cloth. The samples were then washed out gently, first with the tap water and then with the distilled water. The samples were air dried and then stored in the refrigerator at 4°C for further use. The solution which came as the filtrate was also preserved for the DNSA analysis to determine the concentration of reducing sugars present in that sample. All the experimentations had been performed in triplets to get the more accurate results.

Simultaneous SSF

Preparation of Basal Media: 1.2 g $NaNO_3$, 1.4 g $(NH_4)SO_4$, 3.0 g KH_2PO_4, 6.0 g K_2HPO_4, 0.2 g $MgSO4.H_2O$, 0.05 g $CaCl_2$, 0.01 g $MnSO_4.7H_2O$, 0.001 g $ZnSO_4.7H_2O$, 1.4 g Urea, 1% Yeast extract, 2% peptone were added in 500 ml of distilled water and make up the volume to 1000 ml [16] and pH of the media was adjusted to 5.5-6.0. The media was then autoclaved at 121°C and 15 psi for 15 min. 5% dextrose was added after the autoclaving of media [17].

100 ml of this media was then poured in each of 250 ml of flask containing pretreated rice husk samples. 100 µl of *T. reesei* was then inoculated in each of these flasks under sterile conditions and then incubated at 28 ± 2°C on the rotary shaker at 120 rpm for 48 h. The sampling from these flasks was done for DNSA analysis for estimation of sugar contents. After 72 h, *S. cerevisiae* was inoculated in the same flasks for the process of fermentation. The samples were then allowed to incubate for next 72 h on the rotary shaker at 120 rpm at 28°C [18]. Sampling was done after every 48 h for DNSA analysis for reducing sugar estimation.

Filtration and distillation process

Samples were then filtered by using muslin cloth to separate the solid substrate from liquid and then distillation was done at 78.37°C to get the ethanol samples for GC analysis. The solid substrate left was washed with distilled water and then air dried for the FTIR analysis.

Analytical methods

Different analytical methods were used to determine whether the degradation of sample has done and further for the qualitative changes in the sample. The estimation of ethanol yield was done by gas chromatography.

Dinitrosalicylic acid test

Freshly prepared DNS solution is required for DNS testing and need to be stored in the brown bottle to protect it from light. 5 g of DNS was added in 250 ml of distilled water at 80°C. When solution reaches at the room temperature, add 100 ml of 2 N NaOH and 150 g of potassium sodium tartarate-4-hydrate. Volume make up to 500 ml by added distilled water [19].

Lignin estimation test

The weight of untreated sample of powdered sample was taken and then weight of samples was measured after pretreatment of acid and alkali. The samples were washed with the distilled water and then dried completely to measure the weight of the samples.

$$\text{Lignin \%} = \frac{\text{Lignin wt.}}{\text{Substrate wt.}} \times 100$$

FTIR analysis

The samples in which maximum reducing sugar were obtained and maximum lignin content removal was found, FTIR analysis of those samples were done to determine the effect of pretreatment on various bonding present in the sample compared to the untreated samples [20,21]. FTIR was also done for the samples obtained after distillation in which maximum bioethanol production was expected according to the DNS test analysis in order to check if peaks representing ethanol bonding were present or not.

GC analysis

The samples for which peaks showing the presence of ethanol were good in FTIR analysis were used to perform gas chromatography. GC analysis has done for volatile samples in order to determine the concentration of ethanol in the sample obtained after distillation.

RESULTS AND DISCUSSION

DNSA analysis after pretreatment

The estimation of reducing sugars after pretreatment with acid and base was done with the help of DNSA analysis (Figures 1 and 2).

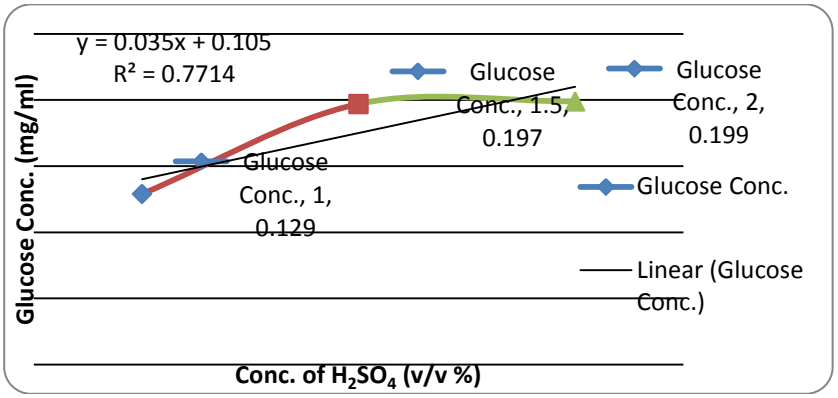

Figure 1: Estimation of reducing sugars after H_2SO_4 pretreatment

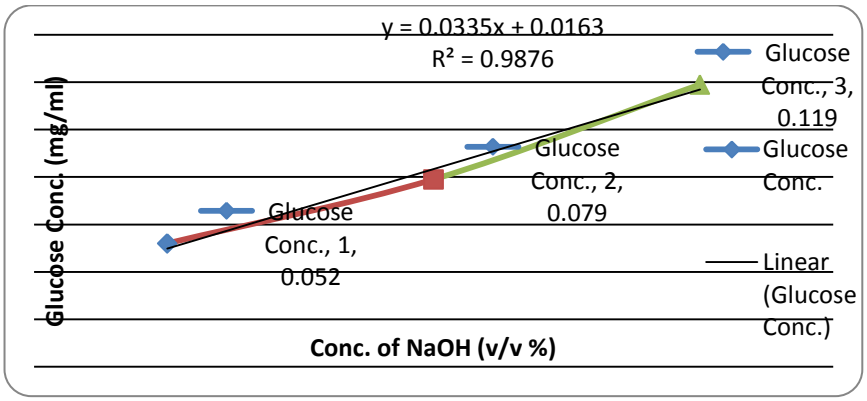

Figure 2: Estimation of reducing sugars after NaOH pretreatment

DNSA analysis during SSF

Samples were taken out after each 48 h during SSF. It has been observed that the reducing sugar level increased first and then started decreasing with time. The maximum sugar content utilized during the process was found in 2% H_2SO_4 and 3% NaOH pretreated samples.

Lignin content estimation

The removal of lignin after acidic and basic pretreatment was estimated. It has been found that the maximum lignin content removal was done in 2% H_2SO_4 and 3% NaOH. From this lignin removal, it has been determined that H_2SO_4 is more effective in removal of lignin compared to the NaOH.

$$\text{Lignin removal \% (2\% } H_2SO_4) = \frac{5-2.58\ (g)}{5\ (g)} \times 100 = 48.4\%$$

$$\text{Lignin removal \% (3\% NaOH)} = \frac{5-3.79\ (g)}{5\ (g)} \times 100 = 24.2\%$$

FTIR results after pretreatment

The effect of acid and base has determined compared to the untreated sample (Figures 3-5).

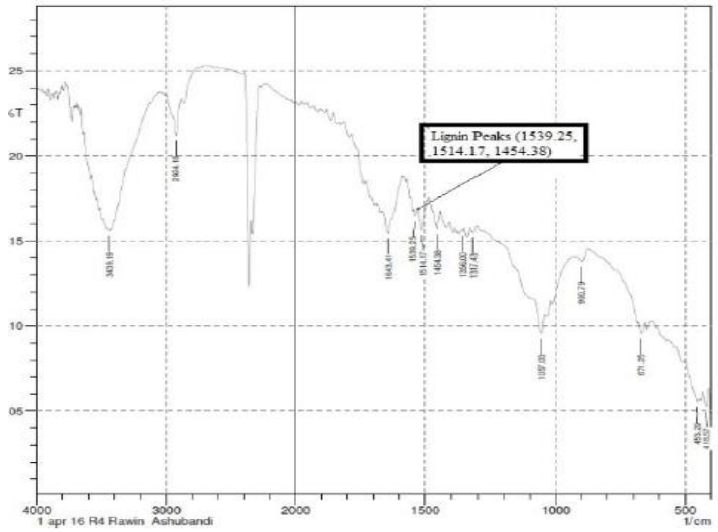

Figure 3: FTIR analysis for untreated rice husk sample

Lignin remains there in the structure with the help of various chemical bonds such as ester bonds, phenyl glycosidic bonds, acetal linkages. The band width of 1520 cm^{-1} and 1441 cm^{-1} signifies the rage of aromatic rings by which lignin has bound. From the spectrum, it can be analyzed that various peaks lies within this range as it is untreated or control sample.

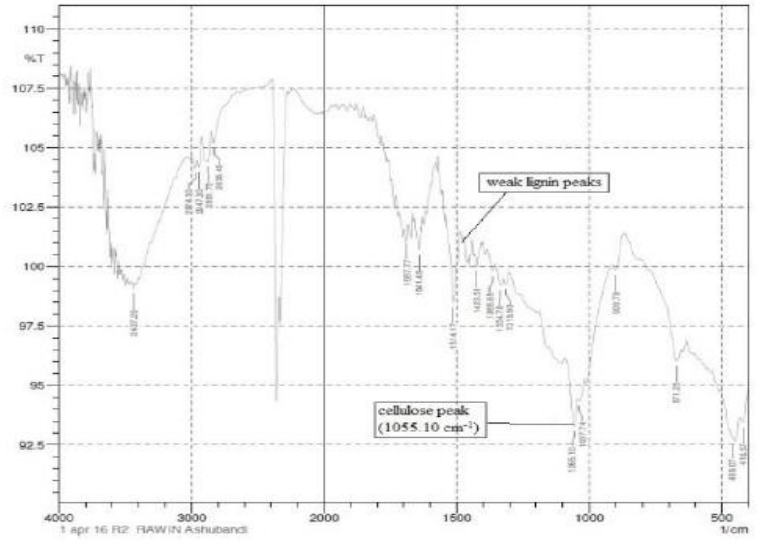

Figure 4: FTIR analysis for acidic pretreated (2% H_2SO_4) sample

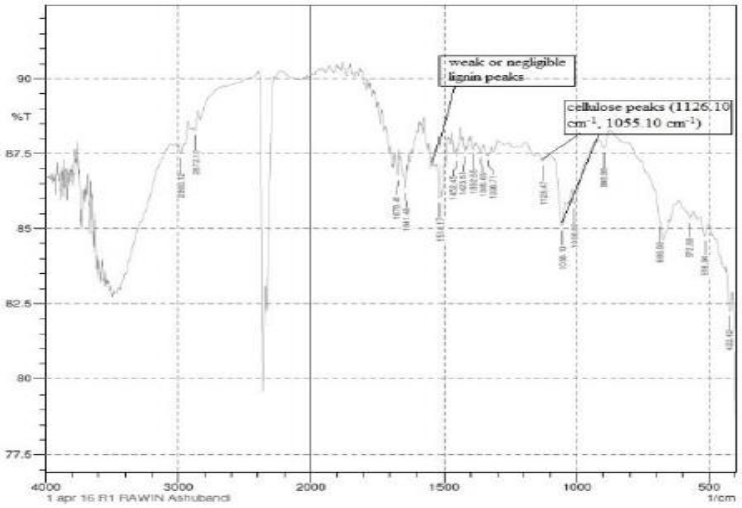

Figure 5: FTIR analysis for alkali pretreated (3% NaOH) sample

From these figures, it can be analyzed that the peaks became sharper and clear after the pretreatment with acid and base which indicates that the substrate became more pure after treatment. The lignin peaks also represented weaker compared to the untreated samples.

FTIR results of ethanol sample obtained after distillation

The stretch peak at 3743.96 cm^{-1} represents the stretching of OH group which has reduced after the acidic and further by enzymatic treatment. These results represents that partial degradation of cellulose has been done. The C-H stretch at 2982 cm^{-1} and 2881.75 cm^{-1} represents that various esters have also been disrupted. Similar results have been found in a study done by researchers (Figures 6 and 7).

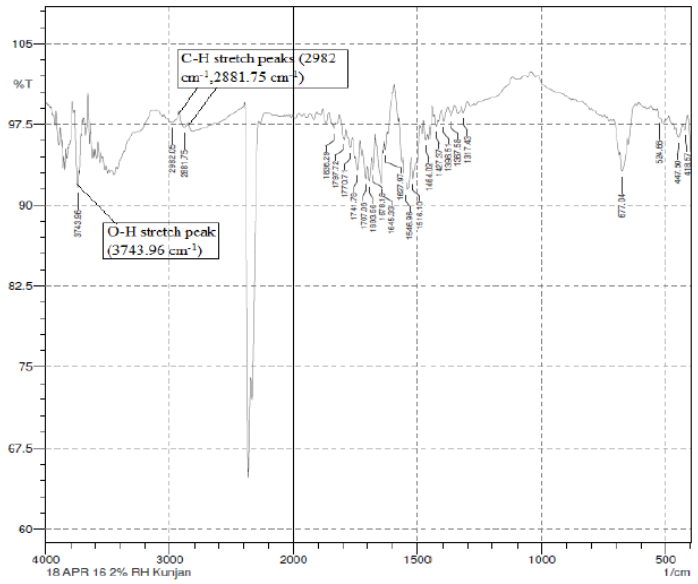

Figure 6: FTIR analysis of acidic pretreated sample after distillation of ethanol

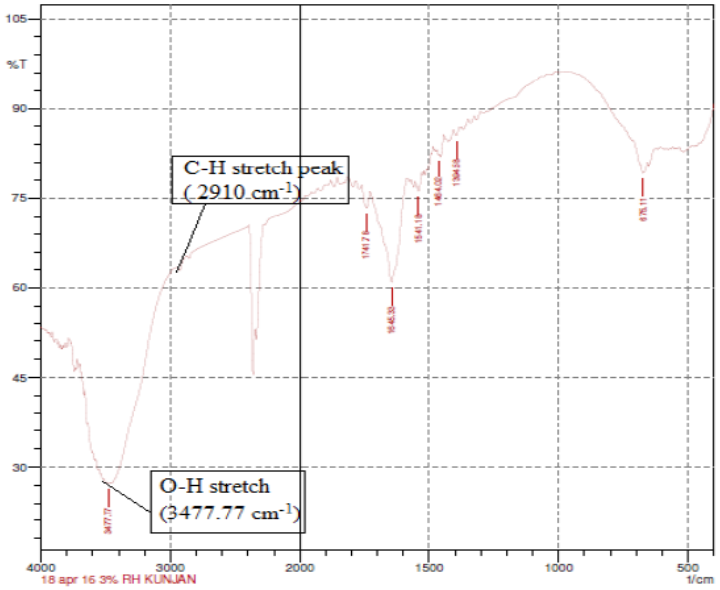

Figure 7: FTIR Analysis of alkali pretreated sample after distillation of ethanol

The O-H stretch at 3477.77 cm^{-1} shows reduction in cellulose linkages and peak at 2910 cm^{-1} represents stretching of C-O and C-H linkages. These type of reductions in various chemical bonds ensures the exposure of enzymes for higher yield of ethanol [22].

GC analysis of ethanol samples obtained after distillation

From the results of GC, it can be seen that the maximum ethanol content has found in the sample treated with sulphuric acid that is 6.34% than the sample treated with alkali solution where ethanol content is 5.89%. The result has been analysis by comparing the area of the sample peak to the peak of the standard used (Figures 8 and 9; Table 1) [23].

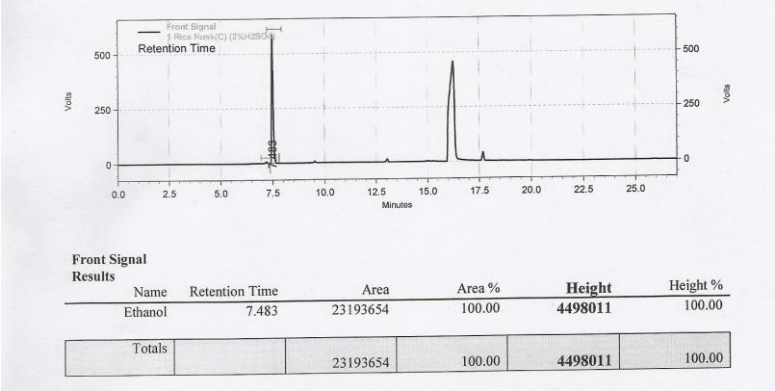

Figure 8: GC graph representing peak of ethanol for 2% H_2SO_4 pretreated rice husk

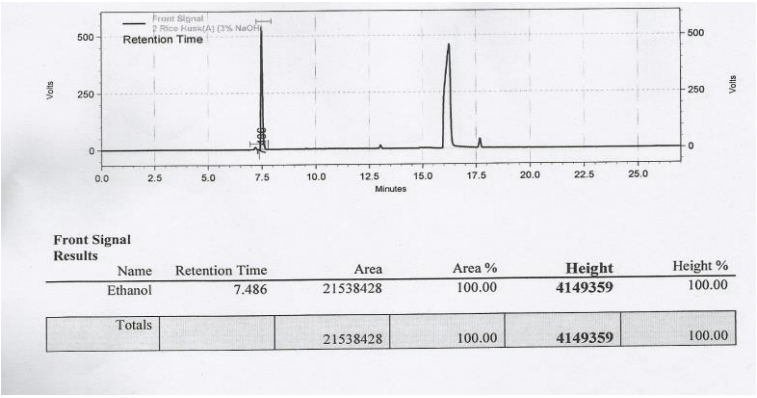

Figure 9: GC graph representing peak of ethanol for 3% NaOH pretreated rice husk

Table 1: Analysis of GC representing ethanol concentration

	Purity	Area	% Ethanol content
Standard	99.9	13253994	-
Sample 1 (2% H_2SO_4)	-	23193654	6.34
Sample 2 (3% NaOH)	-	21538428	5.89

CONCLUSION

This project was started to find out the optimized pretreatment method, as the pretreatment is the major challenge for the production of second generation fuels due to the layer of lignin which needs to be degraded for the exposure of cellulose to the enzymes and hence for the high yield of ethanol. From this study, it has been concluded that 2% H_2SO_4 and 3% NaOH are most effective concentrations for pretreatment of rice husk.

From the results it can be concluded that acidic pretreatment is better compared to the alkaline pretreatment as lignin content removal is found better in acidic pretreated sample compared to the alkaline pretreated. After FTIR and GC analysis, done on the basis of DNSA analysis and lignin estimation, it has been confirmed that acidic treated sample with 2% H_2SO_4 has major effect on the bonding of various groups and maximum ethanol content has also been found in 2% H_2SO_4 treated sample compared to the alkaline treated samples of rice husk.

The ethanol yield obtained after SSF is maximum at 2% H_2SO_4 in acidic pretreated samples and 3% NaOH in alkali pretreated samples. But acidic pretreatment is found better as it is more effective at lower concentration compared to the alkaline treated samples. Hence, acidic pretreatment at particular concentration can be considered as the optimized and economical pretreatment for SSF as it resulted into highest yield of ethanol.

Bioethanol produced by lignocellulosic waste such as rice husk can be most economical and efficient if produced under optimized conditions and strategies. SSF is also an effective way of production of bioethanol as it lowers the cost of various equipment required during the process.

REFERENCES

[1] A.B. Rabah, S.B. Oyeleke S.B. Manga, L.H. Gusao, *IJESD.*, **2014**, 5.
[2] P. Binod, R. Sindhu, R.R. Singhania, S. Vikram, L. Devi, S. Nagalakhshmi, N. kurein, R.K. Sukamaran, A. Pandey, *Bioresour. Technol*., **2009**, 101, 4767-4774.
[3] S. Kumar, P. Sangwan, R. Dhankhar, V. Mor, S. Bidra, *Res. J. Chem. Env. Sci*., **2013**, 1, 126-129.
[4] C.S. Kumar, R. Mythily, S. Chandraju, *J. Chem. Pharm. Res.*, **2012**, 4, 3588-3591.
[5] X.Z. Yuan, Y. Liu, H.J. Huang, H. Wang, G.M. Zeng, X.L. Wang, *Fuel. Process. Technol.*, **2013**, 112, 93-99.
[6] A. Abba, S. Ansumal, *Bioenerg. Res*., **2010**, 3, 328-334.

[7] B.K. Ahring, M. Alapuranen, A. Berlin, R. Bura, R.P. Chandra, J. Cherry, J.P. VanDijken, M. Galbe, *Biofuel. Adv. Biochem. Eng*., **2007**, 3.
[8] S. Prasad, A. Singh, H.C. Joshi, *Resour. Conserv. Recycl*., **2006**, 50, 1-39.
[9] A. Singh, S. Tuteja, N. Singh, N.R. Bishnoi, *Bioresour. Technol*., **2011**, 102, 1773-1782.
[10] A.J. Srivastava, P. Agrawal, A. Rahiman, *IJIRSET*., **2014**, 3, 10187-10194.
[11] K. Karimi, G. Emtiazi, M.J. Taherzadeh, *Enzyme Microb. Technol*., **2006**, 40, 138-144.
[12] E.T. Pejo, J.M. Oliva, M. Ballesteros, *J. Sci. Ind. Res*., **2008**, 67, 874-884.
[13] Z. Anwar, M. Gulfraz, M.J. Asad, M. Imran, Z. Akram, S. Mehmood, A. Rehman, P. Anwar, A. Sadiq, *Afr. J. Biotechnol.*, **2012**, 11, 992-998.
[14] M. Nikzad, K. Movagharnejad, G.D. Najafpour, F. Talebnia, *Int. J. Eng.*, **2012**, 26, 455-464.
[15] A. Das, T. Paul, S.K. Halder, A. Jana, C. Maity, P.K. Mohapatra, B.R. Pati, K.C. Mondal, *Biores. Technol.*, **2013**, 128, 290-296.
[16] M. Mandels, F.W. Parrish, E.T. Reese, *J. Bacteriol*., **1962**, 83, 400-408.
[17] N. Sharma, K.L. Kalra, H.S. Oberoi, S. Bansal, *Indian J. Microbiol*., **2007**, 47, 310-316.
[18] M. Hatami, H. Younesi, N. Bahramifar, *J. Appl. Res. Wat. Wast*., **2015**, 3, 103-107.
[19] G.L. Miller, *Anal. Chem*., **1959**, 31, 426-428.
[20] S.B. Kim, S.J. Lee, J.H. Lee, Y.R. Jung, L.P. Thapa, J.S. Kim, Y. Um, C. Park, S.W. Kim, *Biotechnol. Biofuels*., **2013**, 6, 109.
[21] Y. He, Y. Pang, Y. Liu, X. Li, K. Wang, *Energy Fuels*., **2008**, 22, 2775-2781.
[22] M. Irfan, M. Nadeem, Q. Syed, *Braz. J. Microbiol.*, **2013**, 45, 457-465.
[23] S. Mukhopadhyay, B. Nandi, *J. Sci. Ind. Res*., **1998**, 58, 107-111.