Annals of Biology **38** (2) : 177-180, 2022

Impact Score : 0.32 (Scopus)

Rice Straw Valorization for Ethanol Production through Dilute Acid Pre-treatment

POONAM MAAN* AND R. S. SENGAR

Department of Agricultural Biotechnology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250 110 (Uttar Pradesh), India
**(e-mail : prm.iitr@gmail.com; Mobile : 94124 72292)*

(Received : March 10, 2022; Accepted : May 16, 2022)

ABSTRACT

Burning of agricultural residues after harvesting in the rice field has created many health and environmental issues. Therefore, the main focus of research was to utilize the crop residues for production of ethanol. The pre-treatment of rice straw using different concentrations of dilute sulfuric acid was undertaken. 2% H_2SO_4 concentration worked as effective for rice straw pre-treatment at 121°C temperature, 15 psi pressure, 60 min time and 5% solid loading. Cellulose fraction of pre-treated rice straw was increased 25% as compared to control. The acid pre-treated rice straw showed the saccharification efficiency of 28.5±1.5% after enzymatic saccharification with crude cellulase enzyme in 24 h at dose of 15 IU/g and substrate concentration of 4%.

Key words : Rice straw, valorization, ethanol production, dilute acid pre-treatment

INTRODUCTION

Due to the present scenario of exponential population growth and consumption of energy, the sources of fossil fuels are declining rapidly. Fossil fuels are considered to be the main cause of ecological pollution and climate change (Chu *et al.*, 2020; Sonne *et al.*, 2021). The interest must be in production of biofuels from feed stocks like agro-residues biomass such as bagasse and straw, or cellulosic food wastes, forestry waste materials or algae, etc. (Lorenci *et al.*, 2020; Xia *et al.*, 2021). Rice straw (RS) is a lignocellulosic material and promising feedstock for ethanol production and it does not has competition with food because it is a byproduct of food crop (Soam *et al.*, 2018). Rice straw is abundantly available in India and has been identified as the potential feedstock for ethanol production (Kapoor *et al.*, 2018). Rice straw comprises 36-40% cellulose, 15-20% hemicellulose and 20-23% lignin (Raj *et al.*, 2015). Cellulose and hemicelluloses are interlinked by hydrogen bonding in biomass. Lignin is a heteropolymer, amorphous in nature and composed of mainly three phenyl propanoid; p-coumarin, coniferyl and sinapyl alcohol. Cellulose is major source of hexose sugar, while hemicellulose is the major source of pentose sugars. Lignin acts as glue which keeps cellulose and hemicellulose intact and provides the structural strength to the plant tissue and fibers. Pre-treatment is an essential step for conversion of agro-rersidues into ethanol. Pre-treatment is done for loosening the structural components of agro-residues and to make cellulose and hemicellulose amenable to enzymatic reactions (Singh *et al.*, 2016). Dilute acid treatment extract usually pentose sugar and a little lignin from the lignocellulosic biomass, leaving the cellulose and lignin in biomass (Gabriela *et al.*, 2015). The major drawback of acidic pre-treatment is the formation of inhibitory by products such as the furans, furfurals and phenolics, etc. These inhibitory byproducts affect the fermentation and saccharification process negatively and need to be removed (Jampatesh *et al.*, 2019).

Therefore, the chemical pre-treatment of rice straw with dilute H_2SO_4 was optimized. The enzymatic saccharification of pre-treated rice straw was done. Moreover, this study was undertaken to utilize and convert the rice straw to bioethanol and to reduce the environmental problems caused by the burning of this waste biomass.

MATERIALS AND METHODS

Rice straw was subjected to different concentrations of dilute H_2SO_4 i. e. 1, 2, 3, 4 and 5% separately, at a fixed solid loading of 10% initially. The experiments were performed

at 121°C and 15 psi pressure for 30, 60 and 90 min. Then, solid loading was tested at four levels i. e. 5, 10, 15 and 20%. The neutral detergent fiber fraction (NDF), acid-detergent fiber fraction (ADF), cellulose, hemicellulose and acid-detergent lignin (ADL) content of rice straw were determined using Van Soest method before and after pre-treatment.
Saccharification experiment of untreated and pre-treated (under optimized conditions) rice straw was performed in 100 ml Erlenmayer flasks at crude cellulose enzyme dose of 15 IU/g and substrate concentration of 4%. The pH and reaction temperature were fixed at 5 and 50°C, respectively. The reaction mixture was sealed and incubated at 50 °C under continuous agitation (150 rpm) for 24 h. Hydrolysates were transferred in screw-capped tubes, heated in a boiling water bath for 15 min and centrifuged to remove solid particles. The supernatant was used for analysis of released sugars. Released sugars were estimated by DNS method. The control experiments were carried out separately either by avoiding cellulose, enzyme or the pre-treated substrate. Saccharification yield was calculated from released sugar.

RESULTS AND DISCUSSION

The biomass sample i.e. rice straw was treated with different doses of dilute acid i. e. 1 to 5% H_2SO_4 at 121±2°C in an autoclave for 30, 60 and 90 min at 10% solid loading. The plot of H_2SO_4 doses versus NDF content for rice straw indicated that NDF content increased with increasing the dose of H_2SO_4 from 1 to 5% when treatment time was 30 min. At treatment time of 60 and 90 min, the NDF content initially increased with increasing the dose of H_2SO_4 from 1 to 2%, beyond that it started to decrease (Fig. 1A).
The plot of H_2SO_4 doses versus ADF content for rice straw indicated that the ADF content increased with increasing the dose of H_2SO_4 from 1-5% at 30 and 60 min time period of treatment. While at treatment time of 90 min, the ADF content increased with increasing the dose of H_2SO_4 from 1-4% and beyond that it started to decrease (Fig. 1B).
The cellulose content of rice straw increased with increasing the dose of H_2SO_4 from 1 to 5% when treatment time was 30 min (Fig. 2A). At treatment time of 60 and 90 min, the

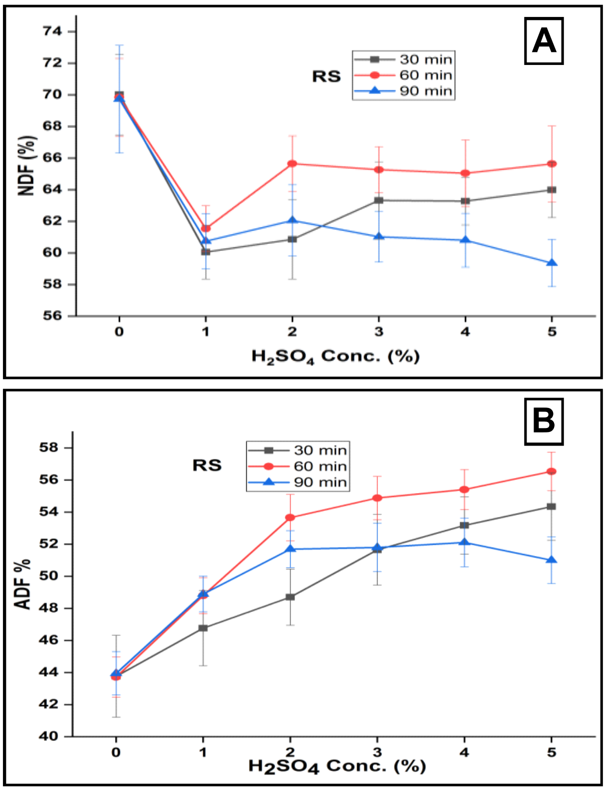

Fig. 1. Effect of H_2SO_4 dose on NDF (A) and ADF (B) content of rice straw.

cellulose content increased with increasing the dose of H_2SO_4 up to 2% and it started to decrease slightly while further increasing the dose. The plots of H_2SO_4 doses versus hemicellulose content indicated that the hemicellulose content of rice straw adopted a decreasing trend with increasing the dose of H_2SO_4 from 1 to 5% at every time period of treatment (Fig. 2B).
The lignin content of rice straw increased with increasing the dose of H_2SO_4 from 1 to 5% at every time period of treatment i. e. 30, 60 and 90 min (Fig. 3). The increase in lignin content during acid treatment indicated residual lignin deposition.
Delignification of rice straw biomass at 2% dose of H_2SO_4 for 60 min showed the apparent increase in cellulose fraction from 38.60 to 46.42% (about 20% rise), while decrease in hemicellulose fraction from 26.25 to 11.99% (about 54% fall) and the lignin content increased from 5.18 to 7.24% (about 40% rise), respectively.
Pre-treatment was required for loosening the complex structure of lignocellulosic biomass. Moreover, a suitable pre-treatment method was important for solubilization and alteration

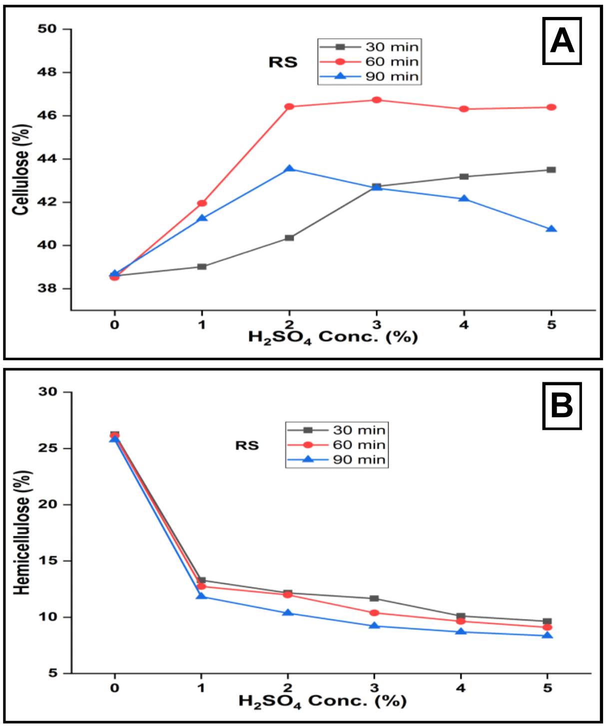

Fig. 2. Effect of H_2SO_4 dose on cellulose (A) and hemicellulose (B) content of rice straw.

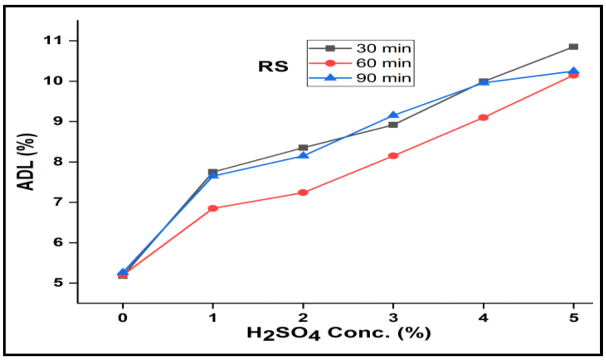

Fig. 3. Effect of H_2SO_4 dose on ADL content of rice straw.

of cellulose and hemicellulose components of agro-residues into fermentable sugars (Sun *et al.,* 2019). Among the acids, H_2SO_4 was one of the most widely adopted chemicals to treat the lignocellulosic biomass. Acid pre-treatment removed mainly hemicellulose, exposing more cellulose to enzyme actions with little effect on lignin degradation (Kshirsagar *et al.*, 2015). Sahoo *et al.* (2018) demonstrated that rice grass produced more saccharification yield when it was pre-treated with dilute acid as compared to alkali pre-treated. Dilute sulfuric acid removed the acid-soluble lignin and hemicellulose by breaking of the β-O-4 linkages in lignocellulosic biomass, as a result, the residual solid fraction became rich in cellulose and acid-insoluble lignin (Kapoor *et al.*, 2017).

Rice straw in different solid loading i. e. 5, 10, 15 and 20% was treated with optimized conditions i. e. 2% H_2SO_4 at 121±2°C in an autoclave for 60 min (Table 1). The highest cellulose and lowest lignin content i. e. 48.25 and 7.15%, respectively, were found at 5% solid loading.

The efficiency of dilute sulfuric acid pre-treatment to alter cellulose and to reduce lignin content in rice straw for using it as substrate for enzymatic saccharification was investigated. The saccharification rate of untreated rice straw substrate was found 14.5%. The maximum saccharification rate i. e. 28.5% was found with rice straw pre-treated with 2% H_2SO_4 at 121±2°C for 60 min at 5% solid loading. The result showed that untreated rice straw was attacked poorly by the crude cellulase enzymes compared with the pre-treated ones. The low saccharification value of untreated rice straw was because the untreated lignocellulosic biomass had a high amount of lignin, which did not support the penetration of the large protein molecules of enzymes to tight network of biomass cell wall. Therefore, efficient enzymatic hydrolysis of lignocellulosic biomass needed a suitable pre-treatment method.

CONCLUSION

From the above studies, it was found that the optimum conditions for acid pre-treatment of

Table 1. Effect of solid loading on NDF, ADF, cellulose, hemicellulose and ADL content of rice straw

Solid loading (%)	NDF (%)	ADF (%)	Cellulose (%)	Hemicellulose (%)	ADL (%)
5	68.05±2.15	55.40±1.45	48.25	12.65	7.15
10	65.65±2.5	53.66±1.6	46.42	11.99	7.24
15	62.95±1.5	52.70±1.56	44.75	10.25	7.95
20	58.96±1.4	50.61±1.45	42.14	8.35	8.47

Treatment conditions : H_2SO_4 Conc. 2%, Temp. 121°C at 15 psi, Time 60 min.

rice straw were at 2% H_2SO_4 concentration, 60 min time of treatment, 5% solid loading and 121°C at 15 psi temperature. Cellulose content in acid pre-treated rice straw increased by 25%; due to dissolution of amorphous material. The saccharification yield of acid pre-treated rice straw increased from 14.5 to 28.5% as compared to untreated sample. From the above studies, it was concluded that efficient reaction conversions were strongly affected by acid concentration and time of pre-treatment. Solid loading slightly affected them. This study determined that dilute acid pre-treatment of rice straw offered a good option for amplified enzymatic hydrolysis efficiency. Rice straw acted as a good and cheap substrate for bioethanol production.

ACKNOWLEDGEMENT

The authors like to acknowledge University Grants Commission, New Delhi for providing financial support during this research work.

REFERENCES

Chu, Q., Wang, R., Tong, W., Jin, Y., Hu, J. and Song, K. (2020). Improving enzymatic saccharification and ethanol production from hardwood by deacetylation and steam pre-treatment : insight into mitigating lignin inhibition. *ACS Sustain. Chem. Eng.* **8** : 17967-17978.

Gabriela Piccolo Maitan-Alfenas, Evan Michael Visser, Rafael Ferreira Alfenas, BráulioRis, G. Nogueira, GuilhermeGalvão de Campos, Adriane Ferreira Milagres, Ronald P. de Vries and ValériaMontezeGuimarães (2015). The influence of pre-treatment methods on saccharification of sugarcane bagasse by an enzyme extract from *Chrysoporthe cubensis* and commercial cocktails : A comparative study. *Bioresource Tech.* **192** : 670-676.

Jampatesh, S., Sawisit, A., Wong, N., Jantama, S. S. and Jantama, K. (2019). Evaluation of inhibitory effect and feasible utilization of dilute acid-pretreated rice straws on succinate production by metabolically engineered *Escherichia coli* AS1600a. *Bioresource Tech.* **273** : 093-102.

Kapoor, M., Semwal, S., Gaur, R., Kumar, R., Gupta R. P. and Puri, S. K. (2018). The pre-treatment technologies for deconstruction of lignocellulosic biomass. In : *Waste to Wealth.* pp. 395-421. Springer, Singapore.

Kapoor, M., Soam, S., Agrawal, R., Gupta, R. P., Tuli, D. K. and Kumar, R. (2017). Pilot scale dilute acid pre-treatment of rice straw and fermentable sugar recovery at high solid loadings. *Bioresource Tech.* **224** : 688-693.

Kshirsagar, S. D., Waghmare, P. R., Loni, P. C., Patil, S. A. and Govindwar, S. P. (2015). Dilute acid pre-treatment of rice straw, structural characterization and optimization of enzymatic hydrolysis conditions by response surface methodology. *RSC Adv.* **5** : 46525-46533.

Lorenci, W. A., Dalmas, N. C. J., de Souza, P., Vandenberghe, L., de Carvalho, N. D. P., Novak, S. A. C., Letti, L. A. J., Karp, S. G., Zevallos, T. L. A. and Soccol, C. R. (2020). Lignocellulosic biomass : Acid and alkaline pre-treatments and their effects on biomass recalcitrance-conventional processing and recent advances. *Bioresour. Technol.* **304** : 122848.

Raj, T., Kapoor, M., Gaur, R., Christopher, J., Lamba, B., Tuli, D. K. and Kumar, R. (2015). Physical and chemical characterization of various Indian agricultural residues for biofuels production. *Energy and Fuels* **29** : 3111-3118.

Sahoo, D., Ummalyma, S. B., Okram, A. K., Pandey, A., Sankar, M. and Sukumaran, R. K. (2018). Effect of dilute acid pre-treatment of wild rice grass (*Zizania latifolia*) from Loktak Lake for enzymatic hydrolysis. *Bioresource Technol.* **253** : 252-255.

Singh, R., Srivastava, M. and Shukla, A. (2016). Environmental sustainability of bioethanol production from rice straw in India : A review. *Renewable Sustain. Energy Rev.* **54** : 202-216.

Soam, S., Kapoor, M., Kumar, R., Gupta, R. P., Puri, S. K. and Ramakumar, S. S. V. (2018). Life cycle assessment and life cycle costing of conventional and modified dilute acid pre-treatment for fuel ethanol production from rice straw in India. *J. Cleaner Prod.* **197** : 732-741.

Sonne, C., Xia, C. and Lam, S. S. (2021). Ancient oaks of Europe are archives-protect them. *Nature* **594** (7864) : 495.

Sun, S. N., Chen, X., Tao, Y. H., Cao, X. F., Li, M. F., Wen, J. L., Nie, S. X. and Sun, R. C. (2019). Pre-treatment of *Eucalyptus urophylla* in γ-valerolactone/dilute acid system for removal of non-cellulosic components and acceleration of enzymatic hydrolysis. *Indus. Crops Products* **132** : 21-28.

Xia, C., Lam, S. S. and Sonne, C. (2021). Seize China's momentum to protect pangolins. *Sci.* **371**(6535) : 1214.