International Conference on Advanced Technologies for Industrial Pollution Control (ATIPC-2018)
December 17-19, 2018

Biodegradation of Cellulose using Aspergillus sp. for Extraction of Fermentable Sugars

Swagata Das, Shubhalakshmi Sengupta, Papita Das*, Siddhartha Datta

Masters of Engineering, Jadavpur University, Kolkata, India, dasswagata600@gmail.com
Post doctoral Fellow, Jadavpur University, Kolkata, India, sengupta.shubha@gmail.com
Professor, Jadavpur University, Kolkata, India, papitasaha@gmail.com
Professor, Jadavpur University, Kolkata, India, s.datta@gmail.com

Abstract- Industrial jute wastes had been collected as jute serves as a potential source for lignocellulosic biomass. Lignocellulosics contain a high amount of cellulose. Cellulose is a hard crystalline structure and it also contains a huge amount of fermentable sugars such as glucose, xylose and arabinose. These are the major sugars for bioconversion of the lignocellulosics to biofuels. Therefore, effort was put in to focus on increasing the biodegradation of cellulose for the release of the sugars. Enzymatic saccharification using *Aspergillus sp.* was applied in the process. Prior to enzymatic hydrolysis, pretreatments such as dilute acid treatment and alkali treatment were done. These were then subjected to enzymatic saccharification and the release of the simple sugar (hexose) was estimated. The results varied in each case- acid treatment of the jute caddies yielded a good concentration of glucose than the other pretreatment i.e. 2.81 g/L.

Keywords— lignocellulosics, biodegradation, enzymatic saccharification, hexose

I. Introduction

Production of bioethanol is a long way preventive measure that has been taken up by all developing nations to maintain a sustainable environment. Long before, first generation biofuels were produced from food crops which were rich in starch or sugars. But the food vs fuel controversy lead to the termination of the process as important food crops were being utilized for the production of biofuels rather than consumption of the food crops [1].

This concern led to the use of materials from where a large amount of sugars can be extracted. Sugars are the main component of the production of biofuels. A large amount of fermentable sugars ensures good yield of biofuels. Therefore, keeping in mind about the environmental concerns and sustainable waste management, new innovative technologies were developed [2]. The second generation biofuels came into limelight. These 2G biofuels were generally produced from leftover crop residues or industrial waste. Certain industries in India, produce a huge amount of waste which becomes a major threat to the environment. Managing them is a matter of great concern. So, technologies developed on the utilization of these wastes [3].

'Lignocellulosic' wastes as the term suggests are rich in cellulose. Cellulose is a hard crystalline structure. The degradation of cellulose releases the fermentable sugars such as glucose, xylose, arabinose etc. So, efforts are implied to utilize wastes which are rich in cellulose. Different chemical pretreatments are done to loosen the structure of the lignocellulosic material. Recent technologies focus on the utilization of microorganisms for biodegradation of the cellulose structure. Use of microorganisms in the process lowers the cost of production of biofuels. Microoragnisms such as *Trichoderma reseii, Aspergillus sp.,* has found application in the process [4].

In the experiment conducted, efforts have been put in to utilize the jute wastes which are rich in cellulose, for extraction of the major fermentable sugar – glucose. Prior to biodegradation by microorganism, chemical pretreatments have been implied as the

Corresponding author **:** Supriyo Goswami

need for the loosening of the hard crystalline structure of cellulose [5]. The process was followed by using *Aspergillus sp.,* for biodegradtion. After five consecutive days, the results were estimated to analyse the concentration of glucose produced in each case.

II. MATERIALS & METHODS

Materials used:

The huge amount of wastes that are generated regularly from jute industries (jute caddies) were used for the experiment. The jute caddies were manually trimmed to small sizes for better handling during the experiment.

Reagents used:

Sulphuric acid, Sodium hydroxide, Anthrone reagent, Sodium nitrite, Potassium chloride, Magnesium sulphate, Ferrous Sulphate, Potassium di-phosphate, Peptone, Agar agar, Czapek Dox medium, Di-nitro salicyclic acid, Phenol, Sodium sulphite, Rochelle salt were purchased from Merck,India.

Chemical Pretreatments:

Alkali treatment of jute caddies

5grams of jute caddies were alkali treated. 5% alkali treatment was conducted and the sample was left for 15 minutes standing time followed by filtering with water to remove the excess alkali and oven drying [6].

Acid treatment of jute caddies

5grams of the jute caddies were taken up for the acid pretreatment. Dilute acid pretreatments i.e. 0.5% acid, was performed. The sample was left for 15minutes followed by proper washing away of the acid and drying [7].

Cellulose estimation:

After the chemical pretreatments were conducted and the samples were oven dried, Anthrone method was followed to estimate the cellulose concentration in the samples (treated and non-treated) [8].

Enzymatic saccharification:

The non treated along with the chemically treated jute caddies were subjected for enzymatic saccharification. A basal media was prepared which was free of nitrogen source and *Aspergillus sp.* was used for biodegradation. The samples were left for consecutive five days to observe full growth of the microbe [9].

Glucose analysis:

After the full growth of the microbe, the solution was estimated for the concentration of glucose produced by using DNS method [10].

III RESULTS & DISCUSSION

Cellulose estimation:

Non-treated jute caddies

The non treated jute caddies were estimated for cellulose using the Anthrone method. The concentration of cellulose for the non-treated samples was the minimum. The crystalline nature of cellulose is not easily breakable as no chemical pretreatments were conducted on the sample. Therefore, the concentration of cellulose yield was only 2.81 g/L.

Alkali treatment of jute caddies

The alkali pretreatment was conducted to loosen the cystallinity of cellulose such that the yield of the fermentable sugars is easy and the sugars can be utilised for the production of biofuels. The alkali treatment breaks the structure of cellulose and releases the probable sugars [11]. The cellulose estimated in case of this pretreatment was more than the non-treated samples. The concentration of cellulose produced was 3.82 g/L.

Acid treatment of jute caddies

Dilute acid is preferred because strong acid solutions may destroy the crystallinity of cellulose. 0.5% acid solution was prepared for utilization in the experiment. The acid gives better results than the alkali. The acid ruptures the structure of

Corresponding author **:** Supriyo Goswami

cellulose easily and results in higher concentration of cellulose [12]. The concentration of cellulose yield was 4.12 g/L.

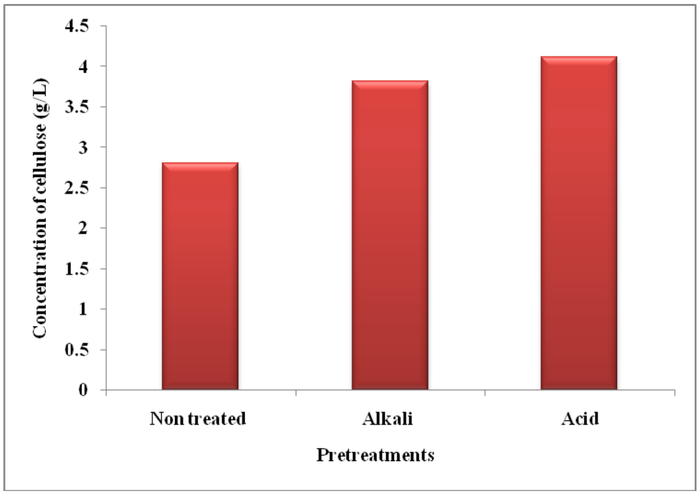

Fig 1: Cellulose estimation

Reducing Sugar Analysis:

The concentration of glucose released after the incubation period of five consecutive days was analysed using the DNS method.

Non-treated jute caddies

As no pretreatment was given to the sample, the concentration of glucose released after biodegradation by *Aspergillus sp.* was found to be minimum than the other two pretreatments. The concentration was found to be 1.67 g/L.

Alkali treatment of jute caddies

The alkali pretreatment was done to decrease the tensile strength of the fiber. The alkali pretreated sample was then subjected to biodegradation. *Aspergillus sp.* which was left for an incubation period of five days yielded better results in the concentration of glucose than the non treated jute caddies. The alkali treatment focuses on the release of glucose from the cellulose as well as the hemicelluloses structure [7]. Moreover, the degradation of the cellulose structure by the microorganism increased the glucose concentration. Therefore, the yield was more than the non-treated. The concentration was found to be 2.06 g/L.

Acid treatment of jute caddies

More previously stated, the acid treatment has proved to produce better cellulose than the other two treatments, hence it was probable that an increase in the concentration of glucose will been observed. *Aspergillus sp.* which has been used during this experiment, showed a similar observation. The concentration of glucose was more than the other two treatments [13]. The concentration was found to be 2.81 g/L.

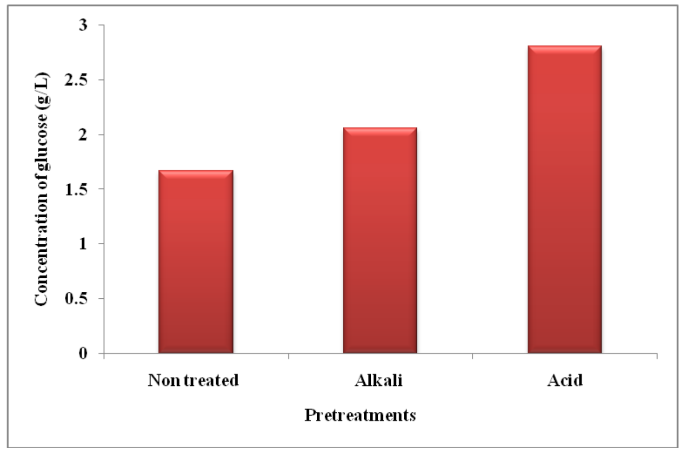

Fig 2: Glucose concentration

IV .CONCLUSION

The release of the hexose sugar can be utilised for the production of bioethanol following a microbial pathway. These sugars on fermentation can then be used as biofuels which will not add on to the greenhouse gases and environmental pollution thereby also managing waste feedstocks from industries for conversion to fuels. In this experiment, the best result was found in case of the acid treated jute caddies i.e. 2.81 g/L.

ACKNOWLEDGMENT

The present research work is supported by Department of Chemical Engineering, Jadavpur University, by providing all necessary instruments and chemicals for the research.

REFERENCES

[1] Miah, R., Siddiqa, A., Tuli, J.F., Barman, N.K., Dey, S.K., Adnan, N., Yamada, M. and Talukder, A.A., 2017. Inexpensive

Corresponding author : Supriyo Goswami

Procedure for Measurement of Ethanol: Application to Bioethanol Production Process. Advances in Microbiology, *7*(11), p.743.

[2] Wyman, C.E., Cai, C.M. and Kumar, R., 2017. Bioethanol from Lignocellulosic Biomass. Encyclopedia of Sustainability Science and Technology, pp.1-27.

[3] Bergeron, P., 2018. Environmental impacts of bioethanol. In Handbook on Bioethanol (pp. 89-103). Routledge.

[4] Juneius, C.E.R. and Kavitha, J., 2017. Bioconversion of Cellulosic Waste into Bioethanol—A Synergistic Interaction of Trichoderma Viride and Saccharomyces Cerevisiae. In Bioremediation and Sustainable Technologies for Cleaner Environment (pp. 201-211). Springer, Cham.

[5] Kumar P, Barrett DM, Delwiche MJ, Stroeve P (2009) "Methods for pre treatment of ligno cellulosic biomass for efficient hydrolysis and bio fuel production". Ind Eng Chem Res 48(8): 3713-3729.

[6] Darmanto, S., Rochardjo, H.S., Jamasri and Widyorini, R., 2017, January. "Effects of alkali and steaming on mechanical properties of snake fruit (Salacca) fiber." In *AIP Conference Proceedings* (Vol. 1788, No. 1, p. 030060). AIP Publishing.

[7] Anwar, Z., Gulfraz, M., Imran, M., Asad, M.J., Shafi, A.I., Anwar, P. and Qureshi, R., 2012. "Optimization of dilute acid pretreatment using response surface methodology for bioethanol production from cellulosic biomass of rice polish." Pak. J. Bot, 44(1), pp.169-176.

[8] Ribeiro, I.A., Bronze, M.R., Castro, M.F. and Ribeiro, M.H., 2016. Selective recovery of acidic and lactonic sophorolipids from culture broths towards the improvement of their therapeutic potential. Bioprocess and biosystems engineering, *39*(12), pp.1825-1837.

[9] Pierce, B.C., Agger, J.W., Wichmann, J. and Meyer, A.S., 2017. "Oxidative cleavage and hydrolytic boosting of cellulose in soybean spent flakes by *Trichoderma reesei* Cel61A lytic polysaccharide monooxygenase." Enzyme and microbial technology, 98, pp.58-66.

[10] Fu, C.C., Hung, T.C., Chen, J.Y., Su, C.H. and Wu, W.T., 2010. Hydrolysis of microalgae cell walls for production of reducing sugar and lipid extraction. Bioresource Technology, 101(22), pp.8750-8754.

[11] Manna, S., Saha, P., Chowdhury, S., Thomas, S. and Sharma, V., 2017. Alkali Treatment to Improve Physical, Mechanical and Chemical Properties of Lignocellulosic Natural Fibers for Use in Various Applications. Lignocellulosic Biomass Production and Industrial Applications, pp.47-63.

[12] Lavarack, B.P., Griffin, G.J. and Rodman, D., 2002. The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose, glucose and other products. Biomass and bioenergy, *23*(5), pp.367-380.

[13] Yoon, S.Y., Han, S.H. and Shin, S.J., 2014. The effect of hemicelluloses and lignin on acid hydrolysis of cellulose. Energy, 77, pp.19-24.