# Journal of Environmental Science and Technology

ISSN 1994-7887

science alert

ANSInet
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OPEN ACCESS Journal of Environmental Science and Technology

ISSN 1994-7887
DOI: 10.3923/jest.2019.157.163

# Research Article
# Bioethanol Production from Banana Pseudostem by Using Separate and Cocultures of Cellulase Enzyme with *Saccharomyces cerevisiae*

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## Abstract

**Background and Objective**: The lignocellulosic composition and availability of banana pseudostem is giving the selective choice for the renewable use of it from waste to valued product. The aim of the study was to improve bioethanol production by using separate and cocultures of cellulase enzyme with *Saccharomyces cerevisiae*. **Materials and Methods:** The raw material contains of 42.35% cellulose, 22.63% hemicellulose, 15.36% lignin, 8.79% extractives and 18.70% ash. Hot compressed water (HCW) and dilute $H_2SO_4$ pretreatment was performed to break down the lignin structure. Cellulase enzyme *Aspergillus niger* broken the β-glycosidic bonds and delivered the fermentable sugar, which was fermented to ethanol. The fermentation was carried out on the hydrolysate by cultured yeast (*S. cerevisiae* from grapes). **Results:** The coculture fermentation in Simultaneous saccharification and fermentation (SSF) was found to be promising with maximum yield of ethanol (8.28 and 5.96 g $L^{-1}$ of BP pretreated at 200°C and 0.5% of $H_2SO_4$, respectively) at 48 h than as compared to the separate hydrolysis and fermentation (SHF). **Conclusion:** The apparent result of the study observed that highest yield of ethanol was attained in a shorter duration in the coculture system containing cellulase enzyme and *S. cerevisiae* of grapes.

Key words: Banana pseudostem, bioethanol, hot compressed water treatment, $H_2SO_4$ pretreatment, yeast culture, simultaneous saccharification and fermentation

Citation: Md. Zahidul Islam, Mohammad Asadullah Asad, Md. Tanvir Hossain, Shujit Chandra Paul and S.M. Asaduzzaman Sujan, 2019. Bioethanol production from banana pseudostem by using separate and cocultures of cellulase enzyme with *Saccharomyces cerevisiae*. J. Environ. Sci. Technol., 12: 157-163.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Sustainable energy is now capturing a good share of the worldwide headlines because of depletion of fossil fuel resources, fluctuation in crude oil prices and environmental issues. Biofuels (bioethanol, biodiesel, biohydrogen etc.) can be a good alternative of fossil fuels. Among all these, bioethanol is the majority (~90%) of the biofuel used worldwide. It is considered as eco-friendly and non-toxic automobile fuel of renewable resource with a possible potential to minimize particulate emissions in compression-ignition engines[1,2]. Over the past 15 years, studies have shown that diesel or gasoline replacement by biofuels causes a net average reduction of greenhouse gases of 71% for bioethanol and 54% for biodiesel[3]. The plant biomass can be considered as an excellent source of biofuel. Using biomass to extract energy or chemical products provide additional advantages such as the release of no additional $CO_2$ into the atmosphere, which also meet the objectives of Kyoto protocol. As starch and sugar-based feedstocks can not be used for bioethanol production due to its impact on food price and food scarcity. So, cellulosic materials are preferred feedstocks than others due to their long term bio-production, large scale and continuous supply and low price since they have been primarily considered as waste or byproduct. Almost 50% of the world biomass is considered as lignocellulosic biomass and its total annual production was estimated to be 10-50 billion tons approximately[4].

Banana is an important fruit crop in tropical and the subtropical regions of the world, mainly grown in the Asian, South American and African continents. It is a very popular fruit in Bangladesh and cultivated almost everywhere round the year. About 1.00 Mt of bananas are produced annually. Pseudostem is the cylindrical part of banana plant, made of overlapping leaf sheaths and 20-50 cm in diameter. In the harvesting time, normally the banana tree is cut and the bottom part of the stem and rhizome becomes untouched. From every ton of bananas harvested, approximately 4 tonnes of agricultural residues are generated of which 75% is pseudostem[5]. Since BP contains a low amount of lignin[6], it could serve as a good substrate for bioethanol production.

According to several studies of pretreatment methods, till now acid pretreatment is considered as one of the most important techniques and aims for high yields of sugars from lignocellulosic biomass. The biomass pre-treatment with $H_2SO_4$ 2% m/m resulted in an ethanol production of $22.1 \pm 0.8$ g $L^{-1}$ with respective values[7] of $Y_{P/RS} = 0.47 \pm 0.03$ g $g^{-1}$, where hydrolysis with 5% $H_2SO_4$ showed 8-9% ethanol production[8]. Again, the hydrolysate with 3% m/m NaOH gave maximum ethanol (17.1 g $L^{-1}$) with yield (84%) and productivity (0.024 g%/h) after 72 h[9]. In another experiment, the maximum yield by using dry pseudostem was 0.288 g $g^{-1}$ of waste, while by using BP and WS produced 0.19 and 0.2 g $g^{-1}$ ethanol, respectively[10]. For this purpose, the material was hydrolyzed by both acid and enzyme to obtain fermentable sugars and the bioethanol was obtained from fermentation. The innovation of this study is that the use of coculture enzyme (*A. niger*) and yeast (*S. cerevisiae*-cultured from grapes) for the production of ethanol from banana pseudostem. The objective of this work was to evaluate the effect of enzyme and laboratory cultured yeast for the production of bioethanol along with the study of efficiency of simultaneous process over separate hydrolysis and fermentation process.

## MATERIALS AND METHODS

**Location:** Institute of Fuel Research and Development, Bangladesh Council of Scientific and Industrial Research, 1205 Dhaka, Bangladesh; Duration: March, 2017-January, 2018.

**Biomass enzyme and culture organisms:** In the study, raw banana pseudostem was collected from Mirpur, Dhaka, Bangladesh and the undesirable materials, plant parts especially the thread like parts were removed. The solid portion was then separated from water and dried in a solar dryer so that the moisture content is constant before the pretreatment. The dried banana pseudostem was grounded to fine powder using electrically operated laboratory crusher and then ball milled to reduce the size of the particles and thus increasing the surface area of the powder. The fine particles were sieved through vibratory screen separator to obtain <80 mesh size.

The cellulase enzyme of *Aspergillus niger* (Product No. 22178) was procured from Sigma-Aldrich, Germany whereas the *Saccharomyces cerevisiae* of grapes was cultured on potato dextrose agar (PDA) media in the laboratory. The media containing 40 g $L^{-1}$ agar in distilled water, which was sterilized in an autoclave at 121°C for 15 min, by acidifying (at pH 3.5) the medium with 10% tartaric acid. The solid media got by pouring the liquid solution into petri dishes and allowing to get solidify.

**Yeast culture from the grapes was performed in 2 ways:**

- **In anaerobic condition:** The *Saccharomyces cerevisiae* colony was picked and introduced into the petri dishes by using a sterilized inoculating loop and stored in empty air-tight zip bag

- **In salt (0.85% NaCl) solution:** The grapes containing the colony of *Saccharomyces cerevisiae* was smashed in water and about 0.2 mL of the solution was placed into the petri dish by spreading over the surface of media with a glass rod. The petri dishes were then sealed and incubated for 48 h at a temperature of 25°C

**Experimental design:** Two modes of bioconversion methodologies for ethanol production were trialed in the present study. Mode I comprised of a separate hydrolysis and fermentation (SHF) process using enzyme and cellulase yeast. Mode II was designed to conduct a simultaneous saccharification and fermentation (SSF) process using the selected cellulase enzyme with yeast. Pretreatment of Biomass was performed before the 2 modes of experiments.

**Pretreatment:** The lignin structure of the fine powdered sample was broken down in two ways namely, hot compressed water (HCW) pretreatment and acid pretreatment. In hot compressed water pretreatment, banana pseudostem powder was suspended in distilled water at a solid-to-liquid ratio of 1:15 at varying temperature (120, 140, 160, 180 and 200°C) for 15 min. Upon completion of the set time, the reactor was cooled down to 30°C within 5 min by rapid cooling. After separating the residue by filtration, the water soluble fraction is stored in refrigerator with 5 mL additional distilled water[11]. Acid pretreatment was performed both by 0.25 and 0.5% (v/v) sulfuric acid for 20 min at a solid-to-liquid ratio of 1:10. The mixture was filtered and then the filtrate was further hydrolyzed by autoclaving at 121°C for 30 and 60 min, respectively. After the pretreatment, by soaking the cellulosic residue in distilled water and incubating in water bath at 50°C for 30 min, the mixture was filtered.

**Separate Hydrolysis and Fermentation (SHF)**

**Enzymatic hydrolysis of substrates:** About 10 g of treated substrate was mixed with 200 mL of 0.1 M citrate buffer (pH 4.8) and added 3, 4 or 5 U $mL^{-1}$ enzyme solution. Then, pH 5.0 was adjusted and the flask was incubated on a rotary shaker at 37°C, 100 rpm for 24 h. The mixture was boiled for 2 min to denature enzyme, centrifuged at 5000 rpm for 15 min to collect the supernatant solution for fermentation[12].

**Ethanol fermentation:** The collected supernatant solution was taken in a conical flask. The flask containing the saccharified sample was aseptically inoculated with 4 colony of 48 h old yeast culture of *S. cerevisiae* and maintained pH 4. Then, the flask was incubated at 33°C under anaerobic conditions for 5 days. Samples were withdrawn from the fermenting media at regular intervals of time (24 h) for analysis.

**Simultaneous Saccharification and Fermentation (SSF):** The SSF represents a single step process in which sugars get released by enzymatic hydrolysis and are simultaneously fermented by yeasts in the same medium. At first, 10 g of treated substrate was mixed with 200 mL of 0.1 M citrate buffer (pH 4.8) solution. About 20 mL enzyme (5 U $mL^{-1}$ de-ionized water) solution was added and inoculated with 4 colony of 48 h old yeast culture of *S. cerevisiae*. The pH of mixture was adjusted at 5.0 and the flask was incubated on a rotary shaker at 37°C, 100 rpm for 5 days. Samples were collected from the second day on 24 h interval for analysis.

**Analysis methods:** Banana pseudostem was subjected to proximate analysis such as moisture[13], ash[14], volatile matter[15] and fixed carbon content[16]. Ultimate analysis (C, H, N, S) was done by the organic elemental analyzer (Flash 2000, Thermo Scientific, USA) according to ASTM method[17]. The composition of Banana pseudostem was analyzed for extractive content[18], holocellulose[19], cellulose[20], hemicellulose[21] and lignin[22-24].

The sugars and produced bioethanol were detected and estimated by reverse phase HPLC (Ultimate 3000, Thermo Scientific) with RI (Shodex RI-101) detector. The sugar monomers and ethanol were detected by using the column HyperREZ XP which is specialized for fermentation broth analysis. The kinetic parameters of ethanol fermentation were determined as follows[25]:

$$\text{Ethanol concentration (Ec)} = \frac{\text{Ethanol produced (g)}}{\text{Volume of reaction mixture (L)}}$$

$$\text{Ethanol productivity (Ep)} = \frac{\text{Ethanol produced (g)}}{\text{Volume of reaction mixture (L)} \times \text{Time (h)}}$$

$$\text{Ethanol yield (Ey)} = \frac{\text{Ethanol produced (g)}}{\text{Weight of substrate (g)}}$$

**Statistical analysis:** Three tests were done for each experiment and the obtained results were analyzed by using one way analysis of variance (ANOVA) at a significant level of $p<0.05$ and the group means were compared with Duncan's Multiple Range Test (DMRT).

## RESULTS

**Analysis of banana pseudostem:** The compositional analysis of banana pseudostem is showed in Table 1. From Table 1, it is clear that the total cellulose contains of the pseudostem is about 14% while the lignin content is about 6% along with 5% of moisture.

### Sugar yield

**Sugar yield on HCW, acid and autoclave pretreatment:** The effects of pretreatments were investigated by analyzing the HPLC data. Table 2 summarizes the concentration of dextrose and xylose in the water soluble fraction of HCW treated samples. The production of sugars was higher in the BP pretreated at 200°C (dextrose 1.04 and xylose 11.81 g $L^{-1}$ pretreated liquid) than at 180°C. The highest amount of xylose (2.97 g $L^{-1}$ pretreated liquid) and dextrose (1.45 g $L^{-1}$ pretreated liquid) was produced by treating with 0.5% sulfuric acid (Table 2) on the acid pretreatment. Table 2 also shows that after being autoclaved at 121°C for 60 min, highest yields of sugars were observed (dextrose 0.97 and xylose 2.60 g $L^{-1}$ pretreated liquid), compared to the yields of autoclaving for 30 min. In overall, at temperature of 200°C, sulfuric acid concentration of 0.50% and autoclave time at 121°C of 60 min gave the maximum yield of sugar.

**Effect of enzyme:** After enzymatic hydrolysis for 24 h, HCW treated substrate at 180°C, produced the dextrose and xylose with the concentration of 0.61 and 1.74 g $L^{-1}$ at 5 U $mL^{-1}$, respectively. At 200°C, the produced sugars were dextrose 6.47 g $L^{-1}$ and xylose 2.44 g $L^{-1}$ at 5 U $mL^{-1}$, respectively (Fig. 1a).

However, in the acid and the autoclaved pretreatment (60 min), the amount of resulting sugars (dextrose and xylose were 4.86 and 7.07 g $L^{-1}$ at 0.25% $H_2SO_4$ and 4.68 and 4.78 g $L^{-1}$ at 0.5% $H_2SO_4$, respectively) at 5 U $mL^{-1}$ (Fig. 1b) were higher compared to HCW process.

**Bioethanol production:** The optimization studies showed that simultaneous saccharification and fermentation (SSF) of banana pseudostem to ethanol was feasible with the cellulase enzyme *A. niger* and yeast strain *S. cerevisiae* of grapes. Maximum yield of ethanol was 8.28 and 5.96 g $L^{-1}$ of BP at 48 h of fermentation for pretreated BP at 200°C and 0.5%

Table 1: Chemical composition of banana pseudostem

| | Moisture | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameters | WB | DB | Holo-C | Cellulose | Hemi-C | Lignin | Ash | VM | FC | Extractives | C | H | N | S |
| Value (%) | 94.45 | 4.73 | 60.14 | 42.35 | 22.63 | 15.36 | 18.7 | 74 | 7.3 | 8.79 | 33.36 | 4.88 | 1.85 | 0 |

WB: Wet basis, DB: Dry basis, Holo-C: Holocellulose, Hemi-C: Hemicellulose, VM: Volatile matter, FC: Fixed carbon, C: Carbon, H: Hydrogen, N: Nitrogen, S: Sulphur

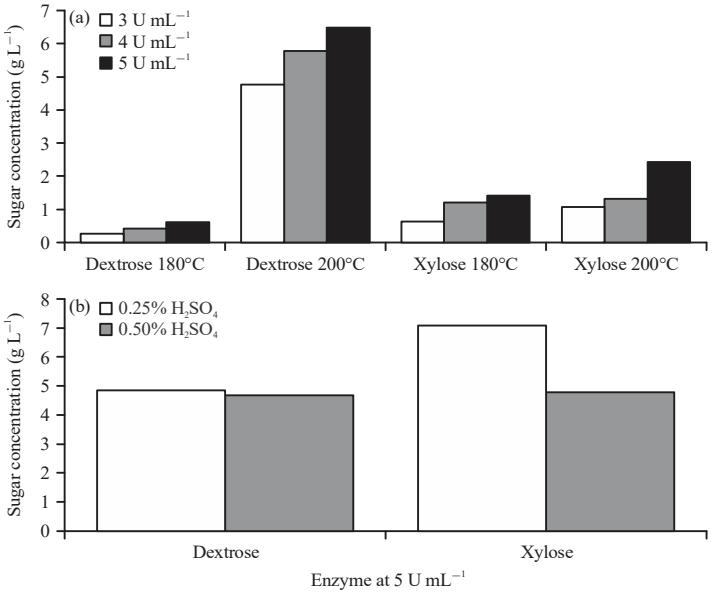


Fig. 1(a-b): Sugar concentration changes in pretreatment (a) HCW with respect to various concentrations of enzyme (24 h) and (b) Acid and autoclave at 5 U $mL^{-1}$ of enzyme (1 h)

Values represent the average of 3 measurements ±SEM

Table 2: Effect of pretreatment on sugar concentration

| Factors | Dextrose (g $L^{-1}$) | Xylose (g $L^{-1}$) |
|---|---|---|
| **Temperature (°C)** | | |
| 180 | 0.23±0.02[b] | 2.24±0.04[a] |
| 200 | 1.04±0.03[b] | 11.81±0.66[a] |
| **Sulfuric acid concentration (%)** | | |
| 0.25 | 1.18±0.07[a] | 0.94±.06[a] |
| 0.50 | 1.45±0.05[b] | 2.97±0.05[a] |
| **Autoclave time at 121°C** | | |
| 30 min | 0.16±0.01[a] | 0.33±0.05[a] |
| 60 min | 0.97±0.06[b] | 2.6±0.05[a] |

Values are the mean of three replicates±SEM, means followed by the same letter within treatment do not differ significantly ($p<0.05$)

Table 3: Ethanol production in SHF/SSF processes at 48 h hydrolyzed with 5 U $mL^{-1}$ of enzyme

| Process | Ec | Ep | Ey |
|---|---|---|---|
| **SHF** | | | |
| 200°C | 2.34±0.12[a] | 0.05±0.002[b] | 0.05±0.01[b] |
| 0.5% $H_2SO_4$ | 4.93±0.25[a] | 0.10±0.005[b] | 0.10±0.02[b] |
| **SSF** | | | |
| 200°C | 8.28±0.42[a] | 0.17±0.16[b] | 0.18±0.80[b] |
| 0.5% $H_2SO_4$ | 5.96±0.20[a] | 0.13±0.12[b] | 0.13±0.58[c] |

Ec: Ethanol concentration (g $L^{-1}$), Ep: Ethanol productivity (g $L^{-1}$ $h^{-1}$), Ey: Ethanol yield (g $g^{-1}$), SHF: Separate hydrolysis and fermentation, SSF: Simultaneous saccharification and fermentation, values are the mean of three replicates±SEM, mean followed by the same letter within treatment do not differ significantly ($p<0.05$)

$H_2SO_4$, respectively. In the SHF process, a maximum of 2.34 and 4.93 g $L^{-1}$ ethanol was produced at 48 h of fermentation for BP pretreated at 200°C and 0.5% $H_2SO_4$, respectively. The ethanol productivity and yield at 48 h is shown in Table 3.

## DISCUSSIONS

The compositional analysis of banana pseudostem suggests that in addition to volatile matter and fixed carbon, it is rich in cellulose, hemicellulose and ash. There are some physicochemical, structural and compositional factors which hinder the digestibility of lignocelluloses. The pretreatments enhanced the susceptibility of the hemicellulose and lignin[26]. Sugars mainly resulted from some easily solubilized polysaccharides and hardly from crystalline cellulose, which can be degraded usually at higher temperatures. In case of the acid pretreatment, significant variation was found between the substrates treated with 0.25 and 0.5% $H_2SO_4$ for 20 min. Since acid pretreatment hydrolyzes the cellulosic material better but it produces some by-products, e.g., furfural and 5-hydroxy furfural, those are known to inhibit the fermentation step[27]. Table 2 also shows that after being autoclaved at 121°C for 60 min, highest yields of sugars were observed (dextrose 0.97 and xylose 2.60 g $L^{-1}$ pretreated liquid), compared to the yields of autoclaving for 30 min. Thus, it substantiates that the amount of sugar released increases with time and the pretreatment temperature basically dominated the pattern of sugar production for an HCW process. As the temperature of 200°C and sulfuric acid concentration of 0.50% showed maximum sugar yield, in the study, these 2 conditions were considered for optimization of ethanol production. Fermentation was completed after 48 h, where ethanol reached a maximum concentration of 8.28 g $L^{-1}$ with complete depletion of glucose. This indicates that the consumption of glucose by yeast cells was virtually in sync with the ethanol production. The current results clearly demonstrated the saccharification potential of cellulase enzyme of *A. niger*, where the performance of the strain in co-fermentation with *S. cerevisiae* was significantly higher than their respective individual fermentation, which is also similar to the previous studies[28-30]. The results suggest that *S. cerevisiae* can grow well in the hydrolysate medium used in this study and is able to convert glucose in the hydrolysate to ethanol completely. The finding of the current study is higher ethanol concentration in shorter period which has a direct impact on process economic. While comparing the produced ethanol concentration with other lignocellulosic biomass, such as wheat straw, rice straw and bagasse[31-34], banana pseudostem offers a significant advantage as an ideal substrate for bioethanol production, especially in banana producing countries. In the future, further improvements can be carried out by choosing a hyper cellulase producing strain capable of fermenting both pentose and hexose sugars to increase ethanol production to competitive position with other ethanol industry.

## CONCLUSION

The results demonstrated that banana pseudostem could be efficiently utilized for bioethanol production. The preliminary data of the BP showed the probability for the saccharification and later by the study, it was achieved the expected amount fermentable sugar. Though the lignin and the ash content were the primary main concern, by using proper pretreatment method, the hurdles were overcome. To produce highest yield of bioethanol from BP, SSF technique used in this study had shown to be not only a most efficient way but also time-saving process till now for the future scale-up production of bioethanol. This study will also solve the problem of safe disposal of other agricultural waste.

## ACKNOWLEDGMENTS

The authors are thankful to all members of Institute of Fuel Research and Development, Bangladesh Council of Scientific and Industrial Research, Dhaka, for the support of this research.

## SIGNIFICANCE STATEMENT

This study discovered the utilization of enzyme and laboratory synthesized yeast that can be beneficial for production of bioethanol. This study will help the researchers to uncover the critical areas of bioethanol production by using laboratory synthesized yeast that many researchers were not able to explore.

## REFERENCES

1. Balat, M. and H. Balat, 2009. Recent trends in global production and utilization of bio-ethanol fuel. Applied Energ., 86: 2273-2282.
2. Hernandez, L. and V. 2009. Use of bioethanol for sustainable electrical energy production. Int. J. Hydrogen Energy, 34: 7041-7050.
3. Koh, L.P. and J. Ghazoul, 2008. Biofuels, biodiversity and people: Understanding the conflicts and finding opportunities. Biol. Conserv., 141: 2450-2460.
4. Srivastava, N., R. Rawat, H.S. Oberoi and P.W. Ramteke, 2015. A review on fuel ethanol production from Lignocellulosic biomass. Int. J. Green Energy, 12: 949-960.
5. Filho, L.C.G., G.A.A. Fischer, N. Sellin, C. Marangoni and O. Souza, 2013. Hydrolysis of banana tree pseudostem and second-generation ethanol production by *Saccharomyces cerevisae*. J. Environ. Sci. Eng. A, 2: 65-69.
6. Hammond, J.B., R. Egg, D. Diggins and C.G. Coble, 1996. Alcohol from bananas. Bioresour. Technol., 56: 125-130.
7. Souza, E.L., G.F. Liebl, C. Marangoni, N. Sellin, M.S. Montagnoli and O. Souza, 2014. Bioethanol from fresh and dried banana plant pseudostem. Chem. Eng. Trans., 38: 271-276.
8. Mishra, M.S. ands S. Sheet, 2014. Production of bioethanol from banana pseudostemwaste via dilute acid hydrolysis and fermentation by *Aspergillus niger*. http://www.academia.edu/4037924
9. Ingale, S., S.J. Joshi and A. Gupte, 2014. Production of bioethanol using agricultural waste: Banana pseudo stem. Braz. J. Microbiol., 45: 885-892.
10. Meena, R.K., R. Sahu, P. Shukla and S. Thakur, 2015. Bio-ethanol production from lignocellulosic banana waste using co-culture techniques. Curr. Trends Biotechnol. Pharm., 9: 259-265.
11. Kumagai, A., S.H. Lee and T. Endo, 2016. Evaluation of the effect of hot compressed water treatment on enzymatic hydrolysis of lignocellulosic nanofibrils with different lignin content using a quartz crystal microbalance. Biotechnol. Bioeng., 113: 1441-1447.
12. Maurya, N., R. Sirohi and V. Prakash, 2014. Bioethanol production by *A. niger* produced cellulase by solid state fermentation. Int. J. Sci. Res., 3: 807-810.
13. TAPPI., 2013. Sampling and testing wood pulp shipments for moisture. TAPPI Test Methods TAPPI T 210 cm, Tappi Press, Atlanta, GA., USA.
14. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton, 2008. Determination of ash in biomass: Laboratory Analytical Procedure (LAP). Technical Report No. NREL/TP-510-42622, National Renewable Energy Laboratory, Golden, CO., USA., January 2008.
15. ASTM., 2011. Standard test method for volatile matter in the analysis sample of coal and coke. ASTM D3175, ASTM International, West Conshohocken, PA.
16. Naik, S., V.V. Goud, P.K. Rout, K. Jacobson and A.K. Dalai, 2010. Characterization of Canadian biomass for alternative renewable biofuel. Renewable Energy, 35: 1624-1631.
17. ASTM., 2007. Standard test methods for instrumental determination of C, H, N in petroleum products and lubricants. ASTM D5291-02, ASTM International, West Conshohocken, PA.
18. Sluiter, A., R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton, 2008. Determination of extractives in biomass. Laboratory Analytical Procedure (LAP) Issue Date: 7/17/2005 Technical Report NREL/TP-510-42619, http://www.nrel.gov/biomass/pdfs/42619.pdf
19. ASTM., 1978. Standards test method for holocellulose in wood. ASTM D1104-56, ASTM International, West Conshohocken, PA.
20. TAPPI., 2009. Alpha-, beta- and gamma-cellulose in pulp. TAPPI Test Methods TAPPI T 203 cm99, Tappi Press, Atlanta, GA., USA.
21. Ayeni, A.O., O.A. Adeeyo, O.M. Oresegun and T.E. Oladimeji, 2015. Compositional analysis of lignocellulosic materials: Evaluation of an economically viable method suitable for woody and non-woody biomass. Am. J. Eng. Res., 4: 14-19.
22. TAPPI., 2015. Acid-insoluble lignin in wood and pulp. TAPPI Test Methods, TAPPI T 222 om02, Tappi Press, Atlanta, GA., USA.
23. TAPPI., 1991. Acid-soluble lignin in wood and pulp. TAPPI., Useful Methods UM 250, Technical Association of the Pulp and Paper Industry, Atlanta, GA., USA.
24. Tina, E., 1996. Determination of acid-soluble lignin in biomass. LAP-004, Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory (NREL), USA.

25. Abate, C., D. Callieri, E. Rodriguez and O. Garro, 1996. Ethanol production by a mixed culture of flocculent strains of *Zymomonas mobilis* and *Saccharomyces* sp. Applied Microbiol. Biotechnol., 45: 580-583.
26. Adrados, B.P., P. Choteborska, M. Gable and G. Zacchi, 2005. Ethanol production from non-starch carbohydrates of wheat bran. Bioresour. Technol., 96: 843-850.
27. Pothiraj, C., R. Arumugam and M. Gobinath, 2014. Sustaining ethanol production from lime pretreated water hyacinth biomass using mono and co-cultures of isolated fungal strains with *Pichia stipitis*. Bioresour. Bioprocess., Vol. 1. 10.1186/s40643-014-0027-3.
28. Unrean, P. and S. Khajeeram, 2015. Model-based optimization of *Scheffersomyces stipitis* and *Saccharomyces cerevisiae* co-culture for efficient lignocellulosic ethanol production. Bioresour. Bioprocess., Vol. 2. 10.1186/s40643-015-0069-1.
29. Hendriks, A.T.W.M. and G. Zeeman, 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol., 100: 10-18.
30. Abouzied, M.M. and C.A. Reddy, 1986. Direct fermentation of potato starch to ethanol by cocultures of *Aspergillus niger* and *Saccharomyces cerevisiae*. Applied Environ. Microbiol., 52: 1055-1059.
31. Abbi, M., R.C. Kuhad and A. Singh, 1996. Bioconversion of pentose sugars to ethanol by free and immobilized cells of *Candida shehatae* (NCL-3501): Fermentation behaviour. Process Biochem., 31: 555-560.
32. Saha, B.C., L.B. Iten, M.A. Cotta and Y.V. Wu, 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Process Biochem., 40: 3693-3700.
33. Oberoi, H.S., P.V. Vadlani, K. Brijwani, V.K. Bhargav and R.T. Patil, 2010. Enhanced ethanol production via fermentation of rice straw with hydrolysate-adapted *Candida tropicalis* ATCC 13803. Process Biochem., 45: 1299-1306.
34. Dawson, L. and R. Boopathy, 2007. Use of post-harvest sugarcane residue for ethanol production. Bioresour. Technol., 98: 1695-1699.