

Structural Changes of Rice Straw Pre-Treated with *Paenibacillus* and *Aspergillus fumigatus*

Stella Matthews*

Strategic Resource Research Centre, Malaysian Agricultural Research and Development Centre, Serdang, 43400, Malaysia

Abstract. Lignocellulose biomass is one of the most abundant yet underutilized resources in the world due to the strong chemical structures that are recalcitrant for degradation. Microbial pre-treatment is suggested as one of the potential, eco-friendly and efficient methods of pre-treatment for biodegradation of rice straw. In this study, the structure of rice straw was analysed using frontier transformed infrared spectroscopy (FT-IR) after the microbial pre-treatment for two weeks. The functional groups of lignin, cellulose and hemicellulose showed degradation after the microbial pre-treatment consisting of *Paenibacillus* and *Aspergillus fumigatus*. The disintegration of rice straw structure was observed via scanning electron microscope (SEM). Fibre analysis revealed the changes in the fibre content after microbial pre-treatment.

Keywords. *Lignocellulose, microorganisms, pre-treatment*

*Correspondence:
stella@mardi.gov.my

1. Introduction

Lignocellulose is the plant biomass that mainly consists of carbohydrate polymers (cellulose and hemicellulose) and aromatic polymer (lignin). Cellulose is the main component of lignocellulose, which is coated with hemicellulose and protected by lignin on the outer layer. Cellulose is a linear polymer comprising β (1 $\rightarrow$ 4) linked D-glucose units. Hemicelluloses are branched polymers, whereas lignins are cross-linked macromolecules composed of phenylpropanoid units that are resistant to biodegradation.

Pre-treatment of lignocellulose is crucial, as it allows the breakdown of cellulose, hemicellulose, and lignin into small units. The aim of pre-treatment is to facilitate the efficacy of enzymatic hydrolysis by improving accessibility towards cellulose fractions. Methods of pre-treatment include physical, chemical, physico-chemical, and biological to alter the composition and structure of lignocellulosic biomass. Pre-treatment increases surface area, decrystallizes cellulose, solubilizes hemicellulose and lignin, alters the structure of lignin, and forms furfural. Physical pre-treatment techniques include milling, grinding, ultrasonic radiation, centrifugal grinding, and extrusion (Ravindran and Jaiswal, 2016).

Chemical pre-treatments include pre-treatments using dilute acid, acid-acetone, ionic liquids, alkaline potassium permanganate, organosolv, metal chlorides, and plasma pre-treatment (Ravindran and Jaiswal, 2016). Physico-chemical pre-treatments include steam explosion, hot water application, wet oxidation, ammonia fibre expansion, super critical CO₂ explosion, integrated hydroxyl radicals, and hot water pre-treatment (Ravindran and Jaiswal, 2016). Other pre-treatments are thermal expansion pre-treatment and microwave pre-treatment (Ravindran and Jaiswal, 2016). Chemicals are reported as the highest cost contributor to the pre-treatment process apart from equipment cost in bioethanol production (Arora et al., 2015). Biological pre-treatment refers to fungal, microbial consortium, and enzymatic pre-treatment. It weakens the fractions of straw biomass with lignin degrading microorganisms and removes a substantial quantity of lignin from biomass (Ghaffar et al., 2015).

Rice straw is one of the toughest plant residues to be decomposed due to the recalcitrant lignocellulosic structure. Straw is reportedly rich in cellulose (40%), hemicellulose, (26%) and lignin

(9%). It is a postharvest waste that has been produced abundantly and underutilized in Malaysia. About two million metric tonnes of rice straw is produced per season from 700,000 hectares of plantation area. The strong chemical structure of the lignocellulose component of rice straw is the main obstruction for its degradation that results in open field burning to prepare the land for the next cropping cycle. Straw biomass has a great prospective for bioconversion. Physical, chemical, and biological pre-treatments were suggested to allow for rapid and efficient degradation of rice straw for various biotechnology applications, such as biofuel production (Nurul atika et al., 2014), biorefinery (Sweeney and Xu 2012), bio-products (Ghaffar et al., 2015), feedstock for the production of lipids and chemicals and lignocellulose enzyme production (Ravindran and Jaiswal, 2016). It has been reported that Asia could be the major potential producer from straw biomass that was estimated to be up to 291 GL/year of bioethanol (Ghaffar et al., 2015).

Biodegradation of rice straw is hardly determined by visual observation because very minimal changes can be noticed in the physical appearance of rice straw. Thus, more sophisticated instrumental analysis using scanning electron microscope analysis and FT-IR is used to analyse biomass structure and monitor the changes that occur from pre-treatments. Qualitative and quantitative analytical techniques, such as FT-IR and SEM, provide valuable information in terms of chemical and morphological properties of biomass after biological pre-treatment (Ghaffar et al., 2015). This paper elaborates the changes in structures and chemical composition of rice straw pre-treated with a microbial consortium, which consists of *Paenibacillus* and *Aspergillus fumigatus*.

2. Material and Methods

2.1 Microbial Inoculum Preparation

Bacterial and fungal isolates from soil that were screened earlier as cellulose degraders were identified as *Paenibacillus* and *Aspergillus fumigatus* using 16S rDNA identification technique. Pure culture of bacteria was grown on nutrient broth amended with 10% yeast extract,

10% glucose, and 10% peptone for 48 hours, and the cells were harvested by centrifugation at 9000 rpm for five minutes. Fungal culture was grown in potato dextrose agar until sporulation. Later the spores were collected in 1% of tween 80.

2.2 Rice Straw Inoculation

Rice straw saturated in water overnight was used for the experiment. Excessive water was discarded, and about 50% of the moisture content of the rice straw was maintained throughout the experiment. A mixed culture of *Paenibacillus* and *Aspergillus fumigatus* was served as the biological pre-treatment, whereas untreated rice straw was used as a control. Each treatment was prepared in three replications. Rice straw was dried in an oven at 50°C and sent for analysis after two weeks of treatment with the microbial consortium.

2.3 Structural and Chemical Analysis of Rice Straw

Structural and chemical changes of treated and untreated rice straw were characterized using FT-IR (Perkin Elmer Spectrum 100 FT-IR Spectrometer equipped with ATR), whereas acid detergent fibre, neutral detergent fibre, and lignin content were analysed using a hot extractor by Foss (fibertec system 2010). For FT-IR analysis, the sample was transferred to an ATR top plate, and the wet samples were dried by hand dryer. FT-IR spectra were acquired at the region of 4000-650 cm⁻¹, with a total of four scans per measurement and a resolution of 4 cm⁻¹. OPUS FT-IR software was used as a tool for the analysis of IR spectra. The morphological changes of rice straw structure before and after the pre-treatment were also observed at 500X magnification (particle size 100µm) using a scanning electron microscope (Hitachi E-10-10).

3. Results

3.1 Structural and Chemical Analysis of Rice Straw

The FTIR analysis was done mainly to identify functional groups present in treated and untreated rice straw.

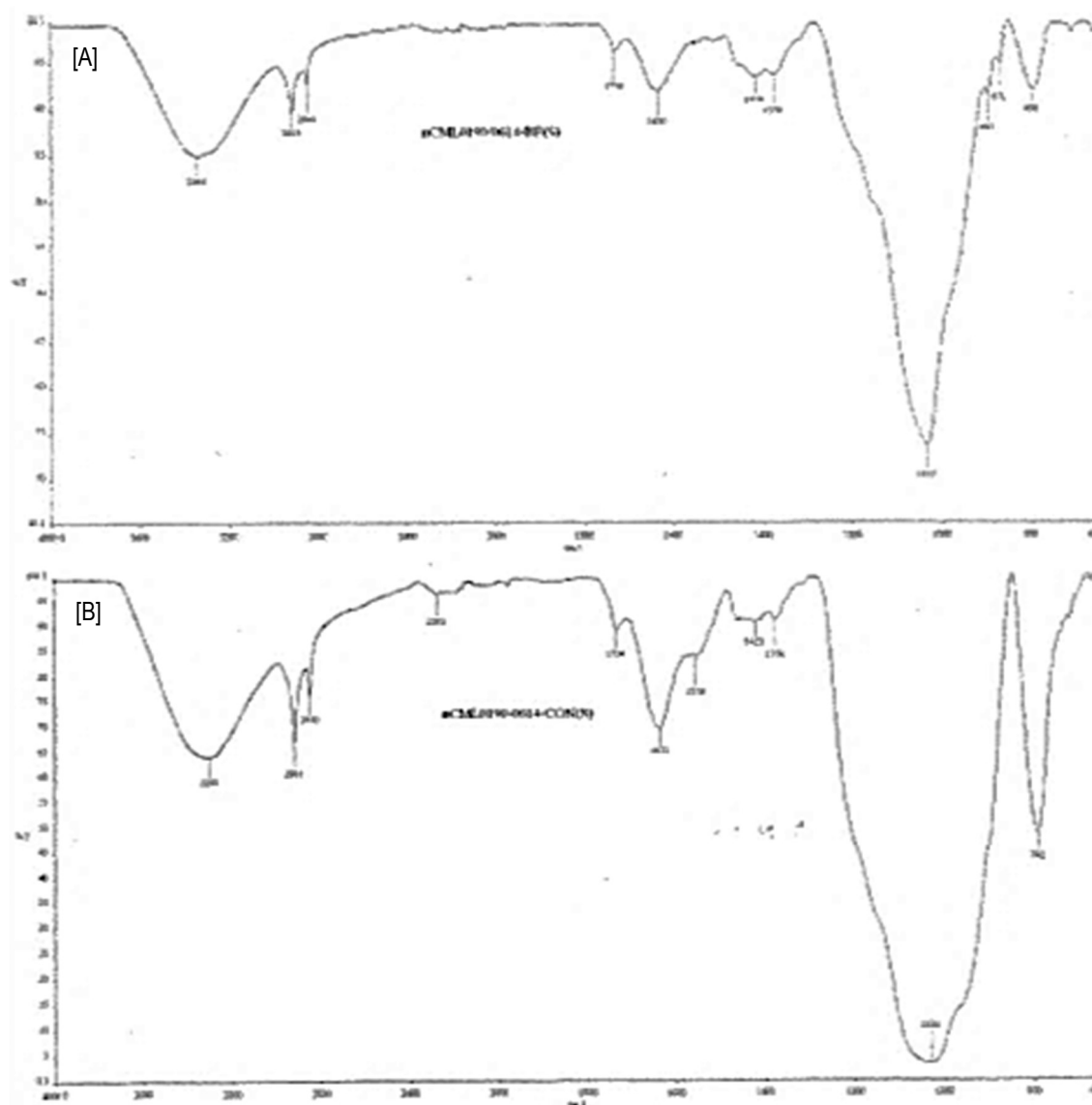


Figure 1: FT-IR image of rice straw treated with microorganisms (top) and untreated rice straw (bottom)

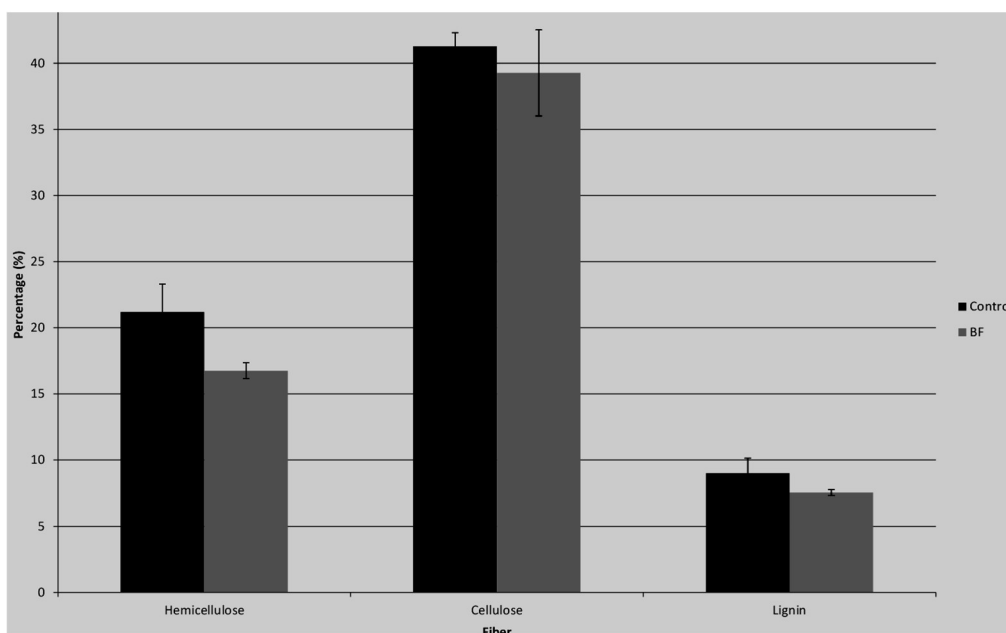
Figure 1 shows the FT-IR spectra of rice straw treated with microorganism (spectrum a) and untreated rice straw (spectrum b). The fingerprint region between 3400 cm^{-1} and 650 cm^{-1} comprises bands assigned to the main components of rice straw, such as cellulose, hemicellulose and lignin. In general, some differences were detected in the spectrum in terms of intensity of the band and disappearance of bands after treatment. The absence of three bands at 2283 cm^{-1} , 1554 cm^{-1} , and 792 cm^{-1} indicates that these components were eradicated completely after the treatment with microorganisms.

An increase in the intensity of the transmission percentage was observed at 2918 cm^{-1} , 2850 cm^{-1} , 1734 cm^{-1} , 1637 cm^{-1} , 1423 cm^{-1} , 1378 cm^{-1} , and 1031 cm^{-1} . Some bands were also observed to be shifted to a lower wavenumber value (cm^{-1}). A decline in the intensity of absorption bands at 3299 cm^{-1} , 2918 cm^{-1} , 2849 cm^{-1} , 1734 cm^{-1} , 1636 cm^{-1} , 1031 cm^{-1} , and 792 cm^{-1} indicate that the specific functional groups in rice straw were reduced after the treatment with microorganisms. The chemical changes in band position 1734 cm^{-1} are assigned to the carbonyl stretching attributed to aromatic skeletal vibrations in lignin structures.

Table 1

Functional Groups of Rice Straw Polymers

Wavenumber (cm ⁻¹)	Untreated rice straw (T %)	Rice straw treated with microbes (T %)	Functional group	Polymer	Reference
3299-3344	65	85	OH stretching of hydroxyl group	Cellulose	(Hsu et al., 2010)
				Lignin	(Supitcha, 2014)
2918-2919	68	89	CH stretching of alkyl group	Cellulose & Hemicellulose	(Bahrin et al., 2012)
2849-2850	78	93	CH stretching of alkyl group	Cellulose & Hemicellulose	(Supitcha, 2014)
2283	95	Missing peak	Possible functional group could not be identified	-	(Bahrin et al., 2012)
1734	88	96	C=O stretching of carbonyl group	Lignin	-
				Hemicellulose	(Prihardi, 2013)
1636-1637	70	91	OH bending	Lignin	(Guo et al., 2008)
1554	82	Missing peak	Anti-symmetric-COO-stretching of salts of carboxylic acid	-	(Bahrin et al., 2012)
1419-1423	90	92	C-O stretching / OH deformation	Cellulose	(Nurul atika et al. 2014)
1378	90	93	CH ₃ deformation	-	-
1031-1035	8	33	Anti-symmetric P-O stretching	Cellulose & Hemicellulose	(Hsu et al., 2010)
792-897	48	95	CH out of plane deformation	Lignin	(Bahrin et al., 2012)

**Figure 2:** Fibre content in rice straw treated with microbial consortium

The region between 3281 cm^{-1} and 3332 cm^{-1} was assigned to hydrogen bonds-OH stretching on the cellulose surface (Prihardi, 2013). Table 1 shows the functional groups and related polymers in rice straw based on the band position (cm^{-1}).

3.2 Fibre Analysis

Figure 2 illustrates hemicellulose, cellulose, and lignin content in rice straw treated with *Paenibacillus* and *Aspergillus fumigatus*. The results did not show significant change in two weeks, but there was a slight reduction in the fibre content of rice straw after biological treatment. Hemicellulose was reduced to about 21%, lignin

to about 16%, and cellulose to about 5% in two weeks.

3.3 Scanning Electron Microscope

Figure 3 shows the morphological changes of rice straw treated and untreated with microorganisms via scanning electron microscope observation. The structure of untreated rice straw looks intact without any disruption on its surface, whereas rice straw treated with microorganisms looks damaged. This could be due to the enzymatic activity of microorganisms that could have removed the external fibres in rice straw. The disrupted fibres of rice straw are obvious in Figure 3[A], [C], and [D].

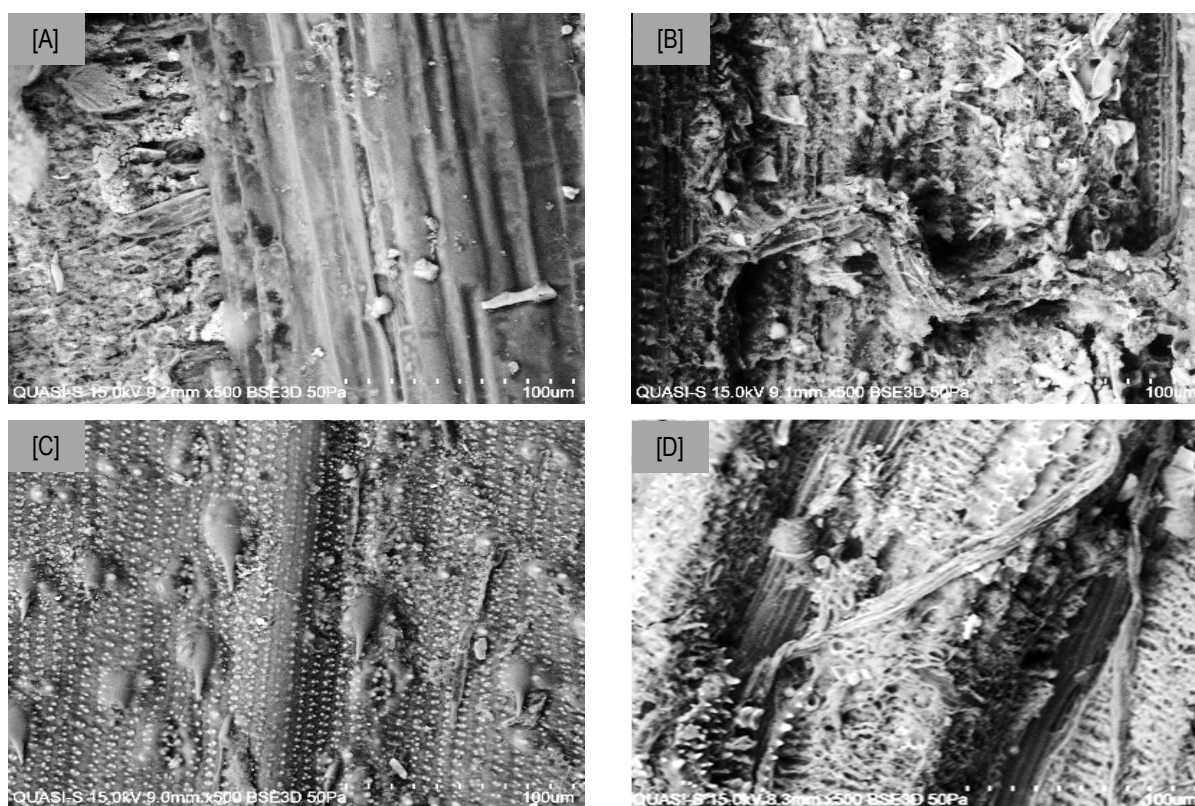


Figure 3: Images of scanning electron microscope at x100 magnification. [A]: Rice straw treated with *Paenibacillus* and *Aspergillus fumigatus*. [B]: Rice straw treated with water (control). [C]: Rice straw treated with *Paenibacillus* only [D]: Rice straw treated with *Aspergillus fumigatus* only

4. Discussion

Lignin is a three dimensional, highly cross-linked macromolecule composed of three phenylpropane monomers, namely *p*-coumaryl alcohol, coniferyl alcohol, and synapyl alcohol by enzymatic polymerization. A vast number of functional groups and linkages of lignin make it a highly irregular complex polymer. FTIR can be used to study the functional groups of lignocellulose biomass and the transformation caused by different treatments. FTIR spectra confirm the ability of the microorganisms to degrade the lignin, hemicellulose and cellulose content of rice straw. The absorption bands are associated with the presence of lignocellulose component. Cellulose is found by glycosidic linkages and hydroxyl groups with a small amount of carboxyl, while hemicellulose and lignin are pre-dominated by ether bonds and carboxyl bonds. The increase in transmission percentage reflects the low absorption of infrared light by the functional groups in rice straw due to degradation and disintegration. The missing bands confirm complete degradation of that compound. The unidentified functional group detected at the wavelength of 2283 cm^{-1} has not been reported previously. Anti-symmetric-COO-stretching of salts of carboxylic acid from rice straw at the wavelength of 1554 cm^{-1} was also reported for the first time in this study. This functional group might be related to lignin, as it was previously reported that, the band at 1511 cm^{-1} could be assigned to aromatic skeleton vibrations in the lignin fractions of rice straw (She et al., 2012). However, the unidentified functional groups and compounds could be determined by using other analytical tools, such as Raman Spectroscopy, Fluorescence Spectroscopy, and Nuclear Magnetic Resonance Spectroscopy (Jason et al., 2014).

The ideal pre-treatment process should be designed to remove lignin and to disintegrate cellulose structures without loss of cellulose and hemicellulose parts. Microbial treatment is able to delignify rice straw but retains the cellulose content. Pre-treatment of straw is essential to expose the cellulose structure and increase the enzymatic hydrolysis efficiency. In general, microbial pre-treatment in this study resulted in the disappearance of most of the lignocellulosic bands. Chemical pre-treatments demand high

chemical charges and energy input to attain complete lignin removal. It is also an energy intensive method that generates toxic by-products. In contrast, microbial pre-treatment does not involve chemicals and is an exclusively green and environmentally sound method. Since the degradation process could be lengthy with microbial pre-treatment, an integration of physical and microbial pre-treatment is suggested for a more efficient degradation. Biological pre-treatment is not very successful on an industrial scale due to the slow pre-treatment rate. Biological pre-treatment combined with mild physical, chemical, or mechanical pre-treatment allows efficient bio-conversion of straw biomass to bio-products (Ghaffar et al., 2015). In this study, rice straw was not ground or cut into small fragments prior to microbial treatment. It was also not exposed to steam or high temperature to facilitate the degradation. However, microorganisms alone were able to disintegrate the lignocellulose component effectively in two weeks. High microbial population integrated with minimal physical pre-treatment and increased time of treatment could pave the way for a better degradation process.

Scanning electron microscope also reveals the destruction of the outer layer (lignin) by microbial treatment. *Paenibacillus* sp. has been reported recently for the degradation of pulp wastes (Raj et al., 2014) and for the production of xylanase (Sanchez et al., 2005). Similarly, *Aspergillus fumigatus* is also reported as cellulolytic fungi (Sarkar and Aikat, 2014). Though studies have been conducted on mixed bacterial and fungal cultures, no studies were found on the effect of *Paenibacillus* and *Aspergillus fumigatus* consortium on the chemical and physical structure of rice straw. Hence, this report would be the first of this kind to study the effect of *Paenibacillus* sp. and *Aspergillus fumigatus* consortium on rice straw structure. The results show the potential of the microbes to delignify rice straw without much disruption of its cellulose content. This criterion is very important for enzymatic saccharification. Pre-treatment is generally done to destroy the lignin and expose cellulose and hemicellulose to convert it into fermentable sugar for the production of biofuel and enzymes. The removal of lignin by microbial treatment

could assist and accelerate the penetration of enzymes to carbohydrates. The reduction of lignin component to about 16 % and cellulose component to about 5 % shows the ability of the consortium for efficient delignification of rice straw.

5. Conclusion

Microbial pre-treatment is one of the effective and pro-environment methods of rice straw delignification and degradation. Rice straw, a recalcitrant lignocellulose biomass, can be disintegrated to cellulose, hemicellulose, and lignin according to the demand by manipulation of the time of treatment and incorporation of physical pre-treatment. In this study, a mixed culture of

Paenibacillus and *Aspergillus fumigatus* was confirmed to have the potential to degrade the complex lignocellulose structure of rice straw with minimal cost and low energy consumption. However, an integration of physical and biological pre-treatment of rice straw is required to speed up the degradation process and improve the efficiency of enzymatic hydrolysis in order to make the bioconversion a real success.

Acknowledgement

The authors would like to thank the Malaysian Agriculture Research and Development Institute for providing funds to conduct the research.

References

- Arora, R., Behera, S. and Kumar, S. (2015), "Bio-prospecting thermophilic/thermotolerant microbes for production of lignocellulose ethanol: A future perspective", *Renewable and Sustainable Energy Reviews*, 51, pp. 699-717
- Bahrin, E.K., Baharuddin, A.S., Ibrahim, M.F., Abdul Razak, M.N., Sulaiman, A., Abd-Aziz, S., Hassan, M.A., Shirai, Y. and Nishida, H. (2012), "Physicochemical property changes and enzymatic hydrolysis enhancement of oil palm empty fruit bunches treated with superheated steam", *Bioresources*, 7(2), pp. 1784-1801
- Ghaffar, S.H., Fan, M. and McVicar, B. (2015), "Bioengineering for utilisation and bioconversion of straw biomass into bio-products", *Industrial Crops and Products*, 77, pp. 262-274
- Guo, G.L., Chen, W.H., Chen, W.H., Men, L.C. and Hwang, W.S. (2008), "Characterization of dilute acid pretreatment of silvergrass for ethanol production", *Bioresource Technology*, 99, pp. 6046-6053
- Hsu, T.C., Guo, G.L., Chen, W.H. and Hwang, W.S. (2010), "Effect of dilute acid pretreatment of rice straw in structural properties and enzymatic hydrolysis", *Bioresource Technology*, 101, pp. 4907-4913
- Jason, S.L., Seema, S., Blake, A.S., Robert, J.H. (2014), "Assessment of Lignocellulosic Biomass Using Analytical Spectroscopy: an Evolution to High-Throughput Techniques", *Bioenerg. Res.*, 7, pp. 1-23
- Nurul Atika, M.R., Umi Kalsom, M.S., Rosfarizan, M. and Suraini A.A. (2014), "Effects of chemical and thermal pretreatments on the enzymatic saccharification of rice straw for sugars production", *Bioresources*, Vol. 9 No.1, pp. 510-522
- Prihardi, K. (2013), "Synergistic effects of pre-treatment process on enzymatic digestion of rice straw for efficient ethanol fermentation", in Petre, M. (Ed.) *Environmental Biotechnology - New Approach and Perspective Applications*, DOI: 10.5772/54949, Available at: <http://www.intechopen.com/books/environmental-biotechnology-new-approaches-and-prospective-applications/synergistic-effects-of-pretreatment-process-on-enzymatic-digestion-of-rice-straw-for-efficient-ethanol>
- Raj, A., Kumar, S., Haq, J. and Kumar Singh, S. (2014), "Bioremediation and toxicity reduction in pulp and paper mill effluent by newly isolated ligninolytic *Paenibacillus* sp.", *Ecological Engineering*, 71, pp. 355-362
- Ravindran, R. and Jaiswal, A.K. (2016), "A comprehensive review on pre-treatment strategy

- for lignocellulose food industry waste: Challenges and opportunity", *Bioresource Technology*, Vol. 199, pp. 92-102
- Sanchez, M.M., Fritze, D., Blanco, A., Spoer, C., Tindall, B.J., Schumann, P., Kroppenstedt, R.M., Diaz, P. and Pastor, F.J.J. (2005), "*Paenibacillus barcinensis* sp. nov., a xylanase producing bacterium isolated from a rice field in the Ebro River delta", *International Systematic and Evolutionary Microbiology*, 55, pp. 935-939
- Sarkar, N. and Aikat, K. (2014), "*Aspergillus fumigatus* NITDGPKA3 provides for increased cellulase production", *International journal of Chemical Engineering*, Available at: <http://dx.doi.org/10.1155/2014/959845>
- She, D., Nie, X.N., Xu, F., Geng, Z.C., Jia, H.T., Jones, G.L. and Baird, M.S. (2012), "Physio-chemical characterization of different alcohol soluble lignins from rice straw", *Cellulose Chemistry and Technology*, Vol. 46 No. (3-4), pp. 201-219
- Supitcha, R. (2014), "Rapid preparation of biosorbents with high ion exchange capacity from rice straw and bagasse for removal of heavy metals", *The Scientific World Journal*, Available at: <http://dx.doi.org/10.1155/2014/634837>
- Sweeney, M.D. and Xu, F. (2012), "Biomass converting enzymes as industrial biocatalysis for fuels and chemicals: Recent developments", *Catalysts*, Vol. 2 No. 2, pp. 244-263