Combination of Biological and Hydrothermal Pretreatment of Mixed Rice Biomass for Fermentable Sugars Production

Ang Kian Thing¹ and Saleha Shamsudin²

¹,²School of Bioprocess Engineering, UniMAP, Malaysia
²COE for Biomass Utilization, School of Bioprocess Engineering, UniMAP, Malaysia.

Email: saleha@unimap.edu.my

Abstract. Rice biomass is one of the most staple agricultural by-products in Malaysia. In this study, mixed rice biomass (MRB) which combines rice husk and straw in certain ratio was used to produce the fermentable sugars. White-rot fungal strain, P. chrysosporium was used in biological pretreatment for 7 days (30°C). The pretreatment was proceeded by hydrothermal autohydrolysis for further removal of hemicellulose. The reducing sugar of soluble solid mixed rice biomass after autohydrolysis process was determined (0.225g sugar/g MRB). The characteristic of raw and insoluble solid pretreated MRB was observed by Scanning Electron Microscopy (SEM) and the functional groups changes were determined by Fourier Transformed Infrared (FTIR). The parameters of enzymatic saccharification were optimized using Central Composite Design for Response Surface Methodology by setting the concentration of commercial cellulase enzyme used, Celluclast 1.5L from 5 FPU/g to 15 FPU/g and incubation times from 24 to 72 hours. It was discovered that fermentable sugars production was increased from 0.098g sugar/g MRB (0.125 g/L) to 0.248g sugar/g MRB (0.319g/L) at optimized condition 82 hours incubation time and 10 FPU/g enzyme concentrations. Hence, the total reducing sugar produced was calculated as 0.473g sugar/g MRB and saccharification was determined as 66.31%.

1. Introduction
Lignocellulosic material (LCM) is one of the major sources of agricultural residues. It is most plentiful and low price biomass available to the world [1]. In every year, almost 600 million tonnes of rice is produce in the worldwide [2]. Generally for every 1kg of paddy grain harvested about 1-1.5kg of the rice straw and 200g of the rice husk are obtained [3,4]. Therefore, LCM as green chemicals are encourage, could provide renewable and environmental friendly process system in overall for generation of value added products [5]. In this study, mixed rice biomass (the combination of rice straw and rice husk in certain ratio) which is abundant will be the probable raw material source for future fermentable sugar production.

The lignocellulosic is formed by three main structural polymers which is cellulose, hemicelluloses and lignin and small quantities of other compounds bound in the matrix [6]. These three polymers form a structure called microfibrils. Microfibrils can undergo any sustainable and suitable process to be converted into useful biochemicals and biomaterials such as sugars and could replace traditional feedstock petroleum-based product processing [7]. Pretreatment is the important step where the biomass can be broken down and give maximal sugar productivity and minimal loss of sugar during the enzymatic hydrolysis [8]. Autohydrolysis is one of the physicochemical pretreatment processes. It is an environmentally friendly technology using water or steam to pretreat the LCM; no chemical will
be used in this process. In this process, LCM is treated with high temperature (170°C-200 °C) and high pressure for few minutes [9]. Autohydrolysis is efficient in modifying lignin, hydrolyzing the hemicellulose, decrease the crystallinity of cellulose and its degree of polymerization [10]. Biological pretreatment is another pretreatment method to degrade lignins and hemicelluloses with low energy requirements [11]. It is a safe and environmental-friendly method to remove lignin. However biological pretreatment need longer time than others pretreatment method especially fungi, which slowly colonized and decomposed biomass feedstock [12]. According to Zheng et al., [13] white rot fungi are more promising basidiomycetes for biological pretreatment by degrade the lignin component actively.

2. Materials and methods

2.1 Raw materials preparation

Rice husk and straws were supplied by local factory, Padi Bernas National Berhad (BERNAS) located at Simpang Empat, Perlis. The sample was weighted and dried for 24 hours at 60°C until constant weight. The rice husk and straw was ground (RT-34, Taiwan) and sieved (Retsch AS200, Germany) to particular sized (0.36 mm - 1.00 mm) [14].

2.2 Pretreatment

2.2.1 Biological Pretreatment

One litre of culture medium contains: 24g of Potato Dextrose Broth, 19.6g of (NH₄)₂SO₄, 7g of yeast extract, 4.2g of CoCl₂, 4.2g of Urea, 4.2g of MgSO₄.7H₂O, 0.028g of CaCl₂, 0.07g of FeSO₄.7H₂O and 0.019g of ZnSO₄.7H₂O. All the medium, flask and material use were autoclaved (Hirayama HVE, Malaysia) at 121°C for 20 min. Potato Dextrose Agar (PDA) plates was used to maintain *P. chrysosporium*, a white-rot fungus at 30 °C in incubator (Binder BD115, Germany). After 7 days of growth, sporulated agar plates was obtained and used immediately or stored at 4 ºC [15]. 30 mL of sterile distilled water was added to each sporulated agar plates. The spores were then scraped to form a spore suspension. 10 mL of spore suspension will be added to 100 mL of culture medium. The flasks were kept in the incubator shaker (DaihanWIS-20, Korea) for 7 days at 30°C and 150 rpm speed to get the homogeneous spores suspension [16]. The mixed rice biomass was controlled at 60% humidity (20% broth medium: 80% distilled water) for solid state fermentation. 5 g of mixed rice in ratio of 3 :1 (rice straw to rice husk) was placed in a conical flask for solid state fermentation at 30 ºC for 7 days [17].

2.2.2 Hydrothermal Pretreatment

Hydrothermal was carried out by using the fermented samples under condition 190°C for 10 min [12]. For 10% solid loading, 30g of the mixed rice biomass was added to 300 mL of distilled water [18]. Ice cold water will be used to cooled down the reactor and stop the reaction immediately. The remaining liquid after the process was used to determine the amount of reducing sugar by DNS.

2.3 Optimization of Enzymatic Saccharification

Celluclast 1.5L and 20 ml Citrate buffer (50mmol L⁻¹, pH 4.8) were added to 1g dry pretreated sample from autohydrolysis. The flasks were kept in incubator shaker at 50°C and 150 rpm. The variables were incubation times and enzyme loading. Central Composite Design (CCD) was used to design the experiment. The time range were set between 24-72 hours and enzyme loadings were between 5 FPU/g to 15 FPU/g [19].

2.4 Analysis

Dinitrosalicylic acid (DNS) method was used to determine the amount of total reducing sugars [20]. SEM (Jeol JSM-6460LA, Japan) was operated at 10kV accelerating voltage to shows the changes in
the structural properties. FTIR (Perkin Elmer Spectrum65, USA) was used to characterize the pretreated and untreated of insoluble solid mixed rice biomass. The changes of chemical structure and functional group in raw and pretreated mixed rice biomass were analyzed under region of 600 – 4000 cm\(^{-1}\) which is a range that performs the best structural characteristics for cellulose. The chemical composition of five samples which were rice straw, rice husk, and mixed rice biomass with ratio of 1:3, 1:1 and 3:1 (rice husk: rice straw) were determined by standard AOAC protocol method. This selective hydrolysis method of biomass was analyzed via neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) methods of analyses.

3. Results and Discussions

3.1 The Characterization of Raw and Pretreated Rice Biomass for Synthesizing Rich-Cellulose Mixed Rice Biomass.

3.1.1 Analysis of lignocellulose content

Table 1 shows that the raw rice husk had higher cellulose and lignin content but lower content of hemicellulose than the raw rice straw. However in mixed rice biomass with ratio 3:1 (rice straw: rice husk), it had the highest cellulose and lignin content among other ratio of combination thus it is more suitable ratio to be used for producing higher of reducing sugars. Based on saccharification result, this composition had produced the highest concentration of reducing sugars. Thus mixed rice biomass with the ratio 3:1 (rice straw: rice husk) is the best selection for enhancing reducing sugar production. Since FTIR and SEM result was based on this selection, therefore only mixed rice biomass with ratio 3:1 (rice straw: rice husk) was observed in FTIR and SEM.

| Sample           | Chemical Composition (%) |
|------------------|--------------------------|
| RRS              | Cellulose = 37.83        |
|                  | Hemicellulose = 32.06    |
|                  | Lignin = 8.37            |
| RRH              | Cellulose = 47.07        |
|                  | Hemicellulose = 16.61    |
|                  | Lignin = 22.57           |
| RRS:RRH (3:1)    | Cellulose = 42.49        |
|                  | Hemicellulose = 26.51    |
|                  | Lignin = 10.03           |
| RRS:RRH (1:1)    | Cellulose = 42.47        |
|                  | Hemicellulose = 23.89    |
|                  | Lignin = 14.08           |
| RRS:RRH (1:3)    | Cellulose = 45.17        |
|                  | Hemicellulose = 18.95    |
|                  | Lignin = 17.28           |

**RRS is Raw Rice Straw and RRH is Raw Rice Husk**

3.1.2 Scanning Electron Microscope (SEM)

Table 2 shows the micrographs of raw and pretreated mixed rice surfaces examined under SEM. The micrographs of pretreated sample was clearly demonstrated that pretreatment could alter the biomass structure. This observation is in agreement with Taniguchi et al., [21]. According to Chen et al., [22] pretreatment was an important step to improve sugar production and enhance saccharification. The untreated raw mixed rice had a rigid and highly ordered fibril structure with a layer of matrix material like lignin and silica (c). The image from c to f clearly shown that \( \text{P.chrysosporium} \) was grown on the
surface, the cell loosening of the fibers with a simultaneous increase in porosity. Taniguchi et al., [21] stated that subsequent treatment with white rot fungi caused a loosening of the networks of lignin and a partial collapse of the structure. Based on the observation from image g to i, hydrothermal pretreatment process caused the partial cracking and destruction to biomass surface. This observation is in agreement with Taniguchi et al., [21], stated that the yield of glucose from rice straw significantly increase after steam explosion pretreatment due to partial destruction of the lignin and hemicellulose was hydrolyzed. The SEM observations suggested that the combine biological and hydrothermal pretreatment resulted in an increase in the susceptibility of mixed rice biomass to enzymatic hydrolysis by destruction the structural networks that will preventing dispersion of cellulase in the mixed rice biomass.

Table 2. Scanning electron micrograph of untreated, biological and autohydrolysis pretreatment in magnification 100X, 500X, 1000X

| RS:RH(3:1)         | 100x | 500x | 1000x |
|--------------------|------|------|-------|
| Untreated mixed rice | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| Biological pretreatment | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |
| Autohydrolysis     | ![Image](image7.png) | ![Image](image8.png) | ![Image](image9.png) |

3.1.3 Fourier Transform Infrared Spectroscopy (FTIR)
The adsorption band intensity of 3447 cm\(^{-1}\) in biological pretreated sample indicated O-H stretch, was decreased from adsorption band intensity of 3471 cm\(^{-1}\) in untreated sample to adsorption band intensity of 3414 cm\(^{-1}\) in autohydrolysis pretreated sample (figure 1, table 3). However, autohydrolysis pretreated sample had broader peak compare to biological pretreated sample. This result indicated biological pretreatment had disrupted some of the hydrogen bonds in cellulose and autohydrolysis pretreatment had altered the structure of cellulose in biomass [23]. The adsorption band intensity of 2954 cm\(^{-1}\) in untreated sample was decreased to the adsorption band intensity of 2922 cm\(^{-1}\) in biological pretreated sample. According to Fatriasari et al. [24], this result was caused by some disturbances made by biological pretreatment to methylene and methyl group in cellulose. Autohydrolysis pretreated sample had similar result with biological pretreated sample, absorption band intensity of 2922 cm\(^{-1}\) which indicated that autohydrolysis had no effect in this case. The functional group of hemicellulose was represented by C=O stretch. The absorption band intensity of 1749 cm\(^{-1}\) for
untreated sample was slightly increased to absorption band intensity of 1753 cm\(^{-1}\) due to the grow of the fungi on the sample and then it was decreased to absorption band intensity of 1636.6 cm\(^{-1}\). This result shows that autohydrolysis pretreatment had successfully removed the hemicellulose and it was in agreement with [25]. The peak in range of absorption band intensity of 1620-1650 cm\(^{-1}\) which was referred to O-H bending vibration, shows that there was presence of absorbed water molecule on the surface of all samples. In addition, all samples had CH\(_2\) and CH\(_3\) deformation bending and C-O bending which were indicated by absorption band intensity of 1350-1470 cm\(^{-1}\) and absorption band intensity of 1320-1380 cm\(^{-1}\) respectively. The decrease of frequency from untreated sample to biological and autohydrolysis pretreated sample supported that lignin had been removed after pretreatment due to the deformation of C-H in lignin methoxyl group [26]. Nazarpour et al. [27] stated that C-O stretch and C-H deformation in cellulose and hemicellulose suggested that both pretreatment step had effectively change the structure of the mixed rice biomass. The functional group of silica, silixane was present on both untreated and biological pretreated sample but then they were being diminished in autohydrolysis pretreatment [28].

**Figure. 1** FTIR Spectroscopy for Raw and Pretreated Mixed Rice

**Table 3.** Peak assignments analysis of untreated and pretreated mixed rice biomass

| Untreated (cm\(^{-1}\)) | Biological (cm\(^{-1}\)) | Autohydrolysis (cm\(^{-1}\)) | Peak assignment | Frequency Range (cm\(^{-1}\)) |
|-------------------------|-------------------------|-----------------------------|-----------------|-----------------------------|
| 3471                    | 3447                    | 3414                        | O-H stretch, H bonded | 3200-3550                |
| 2954                    | 2922                    | 2922                        | H-C-H stretch    | 2850-3000                |
| 1749                    | 1753                    | 1746                        | C=O stretch      | 1665-1760                |
| 1653                    | 1642.11                 | 1636.6                      | O-H stretch      | 1620-1650                |
| 1381                    | 1376.1                  | 1367.6                      | C-H bending vibrations, C-O vibration | 1350-1470 |
| 1047.84                 | 1047.22                 | 1057.87                     | C-O stretch(ester) | 1000-1300                |
| 890                     | 840.58                  | 840.58                      | C-H deformation  | 675-900                   |
| 781.02                  | 781.02                  | 778.04                      | Si-O-Si          | 780-800                   |

### 3.2 Optimization of Enzymatic Saccharification

According results showed in table 4, the reducing sugars production can be represented by a reduced quadratic model in term of coded as shown in equation:

Final equation in terms of coded factors:

Reducing sugars production = \(0.019 * A^2 + 2.869 * 10^{-3} * B^2\)

\[+0.14 + 0.048 * A + 0.028 * B - 0.017 * A * B\]
Where,

\[ A = \text{Incubation time (hours)} \]
\[ B = \text{Enzyme concentration (FPU/g)} \]

In the empirical model equation above, \( A \) and \( B \) represent the linear coefficients of incubation time and enzyme concentration respectively, \( AB \) are the interactive coefficient of parameter and \( A^2, B^2 \) represent the quadratic coefficients. The model has a synergistic effect since it has a positive sign. Quadratic model equation is solved to obtain the optimum condition for reducing sugars production in the enzymatic saccharification by using time and enzyme concentration these two independent variables.

**Table 4. Central composite design with reducing sugars in experimental and predicted value**

| Std Run | Factor 1 A: Time | Factor 2 B: Enzyme conc (FPU/g) | Response 1 Reducing sugar (g/g) |
|---------|-----------------|-------------------------------|-------------------------------|
|         |                 |                               | Experimental | Predicted |
| 13      | 1               | 48                            | 10             | 0.171 | 0.140 |
| 11      | 2               | 48                            | 10             | 0.139 | 0.140 |
| 1       | 3               | 24                            | 5              | 0.084 | 0.069 |
| 4       | 4               | 72                            | 15             | 0.226 | 0.221 |
| 10      | 5               | 48                            | 10             | 0.140 | 0.140 |
| 12      | 6               | 48                            | 10             | 0.126 | 0.140 |
| 3       | 7               | 24                            | 15             | 0.173 | 0.159 |
| 9       | 8               | 48                            | 10             | 0.138 | 0.140 |
| 6       | 9               | 82                            | 10             | 0.248 | 0.246 |
| 2       | 10              | 72                            | 5              | 0.205 | 0.199 |
| 8       | 11              | 48                            | 17             | 0.180 | 0.185 |
| 5       | 12              | 14                            | 10             | 0.098 | 0.110 |
| 7       | 13              | 48                            | 3              | 0.102 | 0.106 |

Analysis of variance (ANOVA) was used to observe the fitness of the quadratic model and significance response towards the interaction of the each independent variable in the experiment. The significant of the model can be referred by F-value or Prob>F-values. From table 5, F-value of model was 22.53 that imply the model is significant. There is only 0.04% chance that a “Model F-Value” this large could occur due to noise. The Prob>F-values less than 0.05, which indicate that the model term were significance. F-value shows the effect of the variables to the response [29]. The higher the F-values will indicate that the effect of the variables affect the response was stronger. The F-value of 0.76 implies the Lack of Fit is insignificant, it is ensure that the model was good and the error was lowered.

For the reducing glucose production by enzymatic saccharification, \( A, B, A^2 \) are significant model terms whereas \( AB \) and \( B^2 \) are insignificant since the Prob>F-values is more than 0.05. Insufficient factors are negligible and could be discarded from the empirical model to yield a more simplified and refine model. The coefficient of determination of the model (R²) was 0.9415. It means that 94.15% of the reducing sugar production by optimization enzymatic saccharification was attributed from two factors which were incubation time and enzyme concentration used. Furthermore, the ‘Pred. R-squared’ and ‘Adj. R-squared’ were 0.7907 and 0.8997 respectively, which is in reasonable agreement. It were recognized generally a high level of correlation between experiment data and predicted data because the value is almost closer to 1 [30]. "Adeq Precision" measures the signal to noise ratio. Since
ratio greater than 4 is desirable, so the model show ratio of 16.433 indicates an adequate signal. This model can be used to navigate the design space.

Table 5. Analysis of Variance for reducing sugars production

| Source          | Sum of squares | Degree of freedom | Mean Square | F- Value | p-value prob>F |
|-----------------|----------------|-------------------|-------------|----------|----------------|
| Model           | 0.028          | 5                 | 5.677 x 10^-3 | 22.53    | 0.0004         |
| A - Time        | 0.019          | 1                 | 0.019       | 74.23    | ~0.001         |
| B – Enzyme Conc | 6.072 x 10^-3  | 1                 | 6.072 x 10^-3 | 24.10    | 0.0017         |
| AB              | 1.159 x 10^-3  | 1                 | 1.159 x 10^-3 | 4.60     | 0.0691         |
| A²              | 2.451 x 10^-3  | 1                 | 2.451 x 10^{-3} | 9.73     | 0.0169         |
| B²              | 5.725 x 10^-5  | 1                 | 5.725 x 10^{-5} | 0.23     | 0.6481         |
| Residual        | 1.764 x 10^-3  | 7                 | 2.519 x 10^4  | 0.76     | 0.5719         |
| Lack of Fit     | 6.407 x 10^-4  | 3                 | 2.136 x 10^4  | 0.86     |                |
| Pure Error      | 1.123 x 10^-3  | 4                 | 2.807 x 10^4  |          |                |

In optimization on the conditions of reducing sugar production, run 9 which indicate 82 hours incubation time and 10FPU/g enzyme concentration was enhanced the production of the reducing sugar. It can be seen that interactions between incubation time and enzyme concentration used, significantly influence the amount of reducing sugar production. The experiment was validated by performed the minimum incubation time and maximum enzyme concentration used which were 24 hours and 15FPU/g to achieve maximum reducing sugars production. The efficiency of mixed rice biomass after combination of hydrothermal and biological pretreatment was calculated as follows [31]:

\[
\text{Efficiency} \% = \frac{\text{Total yield of reducing sugar} \times 0.9 \times 100}{\text{Carbohydrate content of the biomass}}
\]  

(1)

The total reducing sugar production was obtained from the autohydrolysis process and enzymatic saccharification (highest reducing sugar yield). Table 6 showed that the yield of reducing sugar from the autohydrolysis liquid condensate was 0.225g/g and the highest reducing sugar production during enzymatic saccharification was 0.248 g/g.

Table 6. Total reducing sugar yield from pretreated mixed rice biomass.

| Condition                   | Reducing Sugar Yield (g/g) |
|-----------------------------|----------------------------|
| Autohydrolysis (190°C, 10min) | 0.287                     |
| Saccharification (Run 9)     | 0.248                     |
| Total                       | 0.535                     |

Carbohydrate content of the mixed rice biomass with ratio 3:1 (RS: RH) was obtained from the table 1. Table 7 showed the total carbohydrate from raw mixed rice biomass.

Table 7. Total carbohydrate from raw mixed rice biomass.

| Type of Carbohydrate | Carbohydrate Content (g/g) |
|----------------------|-----------------------------|
| Hemicellulose        | 0.425                       |
| Cellulose            | 0.265                       |
| Total                | 0.690                       |
4. Conclusion

Combination of biological pretreatment and autohydrolysis pretreatment had altered the chemical structure of mixed rice biomass by removing lignin, reducing crystallinity of cellulose, partial depolymerization of hemicellulose and hence increasing the fermentable sugars production in enzymatic saccharification. The results of SEM and FTIR show that chemical structure of untreated mixed rice had been modified from highly ordered structure to higher porosity during biological pretreatment and cracking surface layer present during autohydrolysis pretreatment. Therefore, combination of biological pretreatment and autohydrolysis pretreatment had increased the susceptibility of mixed rice biomass to enzymatic hydrolysis effectively. Reducing sugar content was determined as 0.287g sugar/g sample after the combination of biological pretreatment and autohydrolysis pretreatment. Fermentable sugars production was increased from 0.098g sugar/g sample to 0.248g sugar/g sample at 82 hour incubation time and 10 FPU/g enzyme concentrations in optimization on conditions of enzymatic saccharification. Hence, the total reducing sugar produced was calculated as 0.473g sugar/g sample and efficiency of total reducing sugar production was determined as 69.78%.

References

[1] Hsu T C, Guo G L, Chen W H and Hwang W S 2010 Bio. Tech. 101 4907–4913
[2] Food and Agricultural Organization (FAO) 2014 http://www.fao.org/publications/sofa/2014/en/
[3] Karimi K, Kheradmandinia S and Taherzadeh M J 2006 Biomass Bioenergy 30 247–253
[4] Yu J, Zhang J, He J, Liu Z and Yu Z 2009 Bioresource Techn. 100 903–908
[5] Reddy N and Yang Y 2005 Trends Biotechnol. 23 22–27
[6] Gupta P and Parkhey P 2014 Bioresour. Technol. 173 207–215
[7] Iqbal H M N, Kyazze G and Keshavarz T 2013 BioResources 8 3157–3176
[8] Roberto I C, Mussatto S I and Rodrigues R C L B 2003 Ind. Crops. Prod. 17 171–176
[9] Egués I, Sanchez C, Mondragon I and Labidi J 2012 Bioresource Tech. 103 239–248
[10] Pereira R L 2003 Quim. Nova 26 863–871
[11] Kumar P, Barrett D M, Delwiche M J and Stroeve P 2009 Ind. Eng. Chem. Research 48 3713–3729
[12] Gu T 2013 Spring. 158 107–125
[13] Zheng Y, Pan Z and Zhang R 2009 Int. J. Agr. Biol. Eng. 2 51–68
[14] Ong L G A, Chan C H and Chew A L 2012 J Med. Bio. 1 14–16
[15] Potumarthi R, Raju R, Nayak P and Jetty A 2013 Bioresource Tech. 128 113–117
[16] Yu H, Du W, Zhang J, Ma F, Zhang X and Zhong W 2010 Bioresource Tech. 101 6728–6734
[17] Zhang J, Ren X, Chen W and Bao J 2012 Front. Chem. Sci. Eng. 6 146–151
[18] Monavari S, Bennato A, Galbe M and Zacchi G 2010 Biotechnol. Prog. 26 1054–1060
[19] Taniguchi M, Suzuki H, Watanabe D, Sakai K, Hoshino K and Tanaka T 2005 J Biosci. Bioeng. 100 637–643
[20] Miller G L 1959 Anal. Chem. 31 3 426–428
[21] Taniguchi M, Takahashi D, Watanabe D, Sakai K, Hoshino K, Kouya T and Tanaka T 2010 J. Biosci. Bioeng. 110 449–452
[22] Chen W H, Pen B L, Yu C T and Hwang W S 2011 Bioresource Tech. 102 2916–2924
[23] Balasubramaniam M K and Rajarathinam R 2013 Saccharomyces Cerevisiae E 4047–4053
[24] Patrasari W, Syaifii W, Wistara N J, Syamsu K and Prasetya B 2014 Intern. J. Renew. Energy Dev. 3 133–143
[25] Rahnama N, Mamat S, Shah U K M, Ling F H, Rahman N A A and Ariff A B 2013 BioResources 8 2881–2896
[26] Ludueña L, Fasce D, Alvarez V A and Stefani P M 2011 BioResources 6 1440–1453
[27] Nazarpour F, Abdullah D K, Abdullah N, Motedayen N and Zamiri R 2013 Biomed. Res. Int. 2013 268349
[28] Lee J, Lee E K and Kim J 2003 Phy. Rev. A 1371 1–4
[29] Lokman I M, Rashid U and Taufiq Y Y H 2015 J. Chem. Eng. 23 1857–1864
[30] Ramli N A S and Amin N A S 2015 Energy Convers. Manage. 95 10–19
[31] Satheeba S V, Bhagat A K, Saranya S, Govindarajan G and Jebakumar S R D 2014 Int. Biodeterior. Biodegradation 96 144–151
[32] Zailani W W A, Mohd M A B, Razak R, Rozainy Z, Mohd T M F, Ahmad T M F and Hamzah H N 2016 Matec. Conf. 78. 01065