



Effect of microwave-assisted acid or alkali pretreatment on sugar release from Dragon fruit foliage

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Abstract

Agriculture residues are a promising feedstock for value-added products from lignocellulosic waste. However, pretreatment of lignocellulosic materials is essential to facilitate enzymatic hydrolysis and improve sugar yield. The objective of this study is to evaluate the effect of acid or alkali during microwave-assisted pretreatment of dragon fruit foliage (DFF) that would make hydrolysis process more efficient. In the present study, distilled water and three chemicals were examined for their effects on releasing monomeric sugar during microwave treatment. Microwave-assisted pretreatment namely microwave-distilled water (M-H₂O) (control); microwave-sulfuric acid (M-H₂SO₄); microwave-sodium hydroxide (M-NaOH); and microwave-sodium bicarbonate (M-NaHCO₃) pretreatment were performed using 5% (w/v) of DFF as substrate at 800 watt microwave power for 5 minutes exposure time. Highest yield of monomeric sugar was found at 15.56 mg/g using M-NaOH pretreatment at 0.1N NaOH. For M-H₂SO₄ pretreatment, 0.1N H₂SO₄ produced 8.2 mg/g of monomeric sugar. Application of M-NaHCO₃ pretreatment using 0.05N NaHCO₃ solution released 6.45 mg/g of monomeric sugar. While, soaking DFF in distilled water and subjecting to microwave irradiation released 6.6 mg/g of monomeric sugar. Treatments with the lowest concentration (0.01 N) of the three chemicals released only small quantities of total monomeric sugars and less than that with distilled water. The changes in the physical structure of DFF prior to and after the microwave-assisted pretreatment are also reported.

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Keywords

Microwave pretreatment

Monomeric sugar

Dragon fruit foliage

Morphological changes

Introduction

Bioethanol is an important liquid fuel of late because it has been used to power internal combustion engine vehicles (Zhou and Thomson, 2009). Presently, corn stover and sugarcane bagasse are the main feedstocks to produce most of the bioethanol (Balat and Balat, 2009). The substrates account for around 40–70% of the production cost of produced bioethanol by the aforementioned feedstocks (Quintero *et al.*, 2008). The mass production of bioethanol requires using cheaper and more abundant raw materials. Lignocellulosic biomass is a feasible option that can make production of bioethanol more economical and environmental friendly due to its low cost and availability in large quantities as a by-product of agricultural industry (Saga *et al.*, 2010). Dragon Fruit foliage (DFF) is one of the agricultural by-products obtained by pruning dragon fruit trees and is mostly treated as waste. It has been cultivated commercially in large scale farms in Malaysia.

Availability of these materials in large quantities and do not cause food shortage make it an attractive source to reduce sugar alcohol production cost (Saga *et al.*, 2010; Cardona *et al.*, 2010). Bioconversion of lignocellulose to biofuels or other chemical based fermentable sugar involves pretreatment process to facilitate the hydrolysis of carbohydrate polymers and magnify monomeric sugars yield for fermentation process. Currently, several pretreatment methods such as wet oxidation (Schmidt and Thomsen, 1998), hydrothermal treatment (Lu *et al.*, 2009), and steam explosion (Viola *et al.*, 2008) have been applied; however, these methods require severe operation conditions, very high pressures and heating. Microwave-assisted pretreatment is one of the promising methods due to high efficiency of microwave heating and is easy to implement (Ethaib *et al.*, 2015). A number of studies have been conducted and shown that microwave irradiation can change the ultrastructure of lignocellulose (Ooshima *et al.*, 1984; Ethaib, *et al.*, 2016) cleavage the bond

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among lignin, cellulose and hemicellulose, the main components in the lignocellulosic materials, and enhance sugar recovery from enzymatic hydrolysis by increasing the enzymatic susceptibility. It has been reported that using water during microwave pretreatment could enhance the enzymatic hydrolysis of lignocellulosic dry matter (Azuma *et al.*, 1984). Microwave irradiation can be easily combined with chemicals and accelerate the chemical reaction rate (Caddick, 1995). Combination microwave heating with acid or alkali might be a good alternative for pretreatment of biomass. Microwaves has been applied in pretreatment of various feedstocks and the ethanol yield was comparable to those from conventional pretreatment methods (Zhu *et al.*, 2006; Hu and Wen, 2008; Ma *et al.*, 2009).

This study aims to evaluate the acid/alkali effect on monomeric sugar yield during microwave pretreatment of DFF. Various combination of microwave heating and distilled water (M-H₂O), microwave-sulphuric acid (M-H₂SO₄), microwave-sodium hydroxide (M-NaOH) and microwave-sodium bicarbonate (M-NaHCO₃) were performed to evaluate the monomeric sugar yield.

Material and Method

Feedstock and selected characterization



Figure 1. Preparation steps of dragon fruit foliage

The DFF was obtained from Agriculture Park of University Putra Malaysia (UPM), Serdang, Malaysia. The harvested DFF was washed with distilled water vigorously to remove all undesirable matters such as sand particles and cut to get average particle size of 1-2 cm (Figure 1). The cut DFF was then dried in an oven with the temperature of 105°C for 24 h. Subsequently, DFF samples were stored in a freezer at -20°C until subsequent experiments.

The chemical components of raw feedstock DFF, consisting mainly of cellulose, hemicelluloses, lignin, and ash, were analyzed by determining the neutral detergent fiber (NDF), the acid detergent fiber (ADF), the acid detergent lignin (ADL) and ash (Van Soest *et al.*, 1991). The chemical identification of elements and their concentration in DFF was carried out by using Energy Dispersive X-Ray Spectroscopy (EDX) to verify silica content. This test was accomplished by NORAN System 7 X-ray Microanalysis (Thermo scientific, USA).

Microwave-assisted pretreatment

Microwave-assisted pretreatment experiments were performed in modified domestic microwave oven (Panasonic, NN-ST340M). The microwave oven has 2.45 GHz magnetron and a maximal operation power of 800 watt. A 1000 ml round-bottom flask was placed inside the microwave cavity and connected to a condenser cooled by tap water at ambient temperature. The pretreatment experiments were performed at 800 W microwave power for 5 minutes and 5% (w/v) substrate concentration and all experiments were duplicated. Oven-dried samples were immersed in 100 ml of distilled water or chemical solution in the round-bottom flask. The chemical solutions prepared by diluting sulphuric acid, sodium hydroxide, and sodium bicarbonate (R and M Chemicals, Malaysia) with deionized water to get concentrations of 0.01N, 0.05N and 0.01N. Upon treatment, mixtures were filtered through filter paper (Double Ring 102, China) to separate solid residue and liquid (filtrate). The filtered solid fraction were washed with distilled water to remove the acid/alkali solution and dried at 60°C until constant weight. The filtrate was collected for pH value measurement and then neutralized before monomeric sugar analysis.

Sugar analysis

For sugar determination, samples were filtered using nylon membrane 0.45 µm syringe filter (Phenex, England) prior to HPLC analysis. The HPLC system (Alltech ELSD 2000, ALT, USA) was equipped with a refractive index detector RI and sugar column Rezex RPM-Monosaccharide Pb+2 column (Phenomenex, USA). The mobile phase was deionized water with the flow rate of 0.6 ml/in and 20 µL of samples were injected. Standard solutions of D-(+)-glucose, D-(+)-xylose, and L-(+)-arabinose (Sigma Aldrich Chemical Inc., Germany) at known concentrations were injected to establish retention times and standard curves.

Scanning electron microscopy (SEM) analysis

Physical changes in the raw and microwave-chemical pretreated materials were observed by Scanning Electron Microscope (SEM) (S-3400N, Hitachi, Japan). Images of the surfaces of the raw and microwave pretreated materials were taken at various magnifications. The specimens to be coated were mounted on a conductive tape and coated with gold palladium using Emetic K550X automatic sputter coater and observed using a voltage of 5-10 kV.

Results and Discussion

Chemical composition and elemental analysis of feedstock

DFF was characterized by determining ADF and NDF to evaluate the cellulose and hemicellulose, and lignin content. The potential carbohydrates of the substrate accounted for 32.4% distributed between cellulose, 17.5% and hemicellulose, 14.9%. Other components such as lignin and ash were 11.5% and 15.2% w/w, respectively. These results refer to the low amount of lignin fraction that could reflect positively on the released sugar from cellulose and hemicellulose for easy delignification process of lignin. The elemental analysis of DFF shows that there is no trace of silica compound and the main elements are only carbon and oxygen.

Effect of pretreatment on the hydrolysate pH

DFF The pH of the pretreatment hydrolysate can indicate the presence of sugar degradation products and inhibitors. Table 1 shows the distribution of pH value of the liquors before and after the pretreatment for the effect of acid or alkali of microwave pretreatment. In the case of M-H₂O pretreatment, the pH value of the filtrate obviously decreased from 6.86 prior the pretreatment to 4.7 at the end of pretreatment. Addition of chemicals showed different effects on pH of the microwave-assisted filtrate. Lower concentration of sulphuric acid resulted in more increment of pH values of hydrolysate after pretreatment process. Same observation was found for the samples treated with 0.01N of NaOH where the pH dropped from 12.25 to 9.5. In contrast, M-NaHCO₃ pretreatment where the baking soda was the treatment solution resulted in more alkaline pH as compared to the initial pH. Using 0.1N and 0.05N of sodium bicarbonate to treat DFF sample increased pH from 8.38 and 8.46 before pretreatment to 9.7 and 9.65 after pretreatment, respectively. While, performing the pretreatment with 0.01N of NaHCO₃ slightly dropped the final hydrolysate pH.

The fluctuation of final pH values during microwave-assisted pretreatment could be related to several reasons. This decrease in pH is usually attributed to formation of organic acids, such as, acetic acid, lactic acid, etc. (Hoekman *et al.*, 2011), due to the hydrolysis of acetyl groups of hemicelluloses and degradation of mono sugar released during the pretreatment. It is of interest that the pH values of the solution become higher after M-H₂SO₄ and somehow of M-NaHCO₃ pretreatment. This arises from the fact that biomass generally possesses buffering capacity which can prevent acid production and make the pretreatment less severe (Öhgren *et al.*, 2007). During the alkaline pretreatment, hydroxide ions attacked lignin by splitting ether bonds in the delignification process (Gierer, 1985). Moreover, acetic acid could be produced at severe conditions because of sugar degradation, which caused the lowering of pH (Pedersen and Meyer, 2010). As the temperature increase due to high microwave power, the acid proton performed as a catalyst to protonate the oxygen atom linkage between hemicellulose and cellulose. High temperature contributes to the alkaline degradation of biomass, causing the ester bonds among lignin, cellulose and hemicellulose to undergo cleavage. The intense alkaline environment can cause degradation and produce more acid substances because of more uronic acid substitutions and the acetyl groups on hemicellulose can be hydrolyzed to form uronic acid and acetic acid throughout the intensive alkaline environment (Chang and Holtzapfle, 2000; Karimi, *et al.*, 2006). In summary, increasing acid or alkaline concentration is conducive to the destruction of the biomass even though the buffering capacity is exhibited by the biomass (Chen *et al.*, 2012).

Table 1. The pH values of pretreatment liqueur before and after pretreatment of DFF

Pretreatment	Chemical concentration (N)	Initial pH	Final pH
M-H ₂ O	-	6.9	4.7 ± 0.1
	0.1	1.3	1.5 ± 0.2
M-H ₂ SO ₄	0.05	1.7	2.3 ± 0.0
	0.01	2.1	3.8 ± 0.0
M-NaOH	0.1	12.8	12.4 ± 0.0
	0.05	12.6	12.0 ± 0.2
	0.01	12.3	9.5 ± 0.3
M-NaHCO ₃	0.1	8.4	9.7 ± 0.0
	0.05	8.5	9.7 ± 0.2
	0.01	8.5	8.4 ± 0.2

Effect of chemicals on solid loss

The solid loss of DFF is an important factor to investigate the effectiveness of the pretreatment. Figure 2 shows the percentage loss of solid after microwave pretreatment. It can be clearly observed that increasing the concentration of chemicals (H_2SO_4 and NaOH) decreased the percentage of solid loss. Highest solid loss of 12.4% was observed with 0.1N NaOH. It was reported, solid loss came from the solubility of the compounds such as lignin and also partial solubilisation of hemicelluloses during pretreatment with alkali. The alkali treatment caused swelling, leading to separation of structural linkages between lignin and carbohydrates (Komolwanich *et al.*, 2014). Solid loss of 11.6% was found when 0.1N H_2SO_4 was used as solvent and this could be attributed to the degradation of DFF by microwave-acid pretreatment where the acidic environment is able to partially fractionate hemicellulose (Chen *et al.*, 2012).

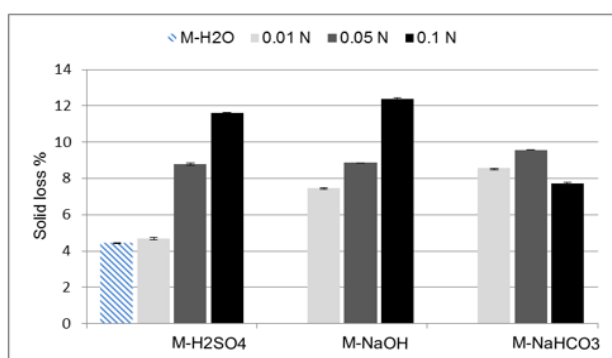


Figure 2. Solid loss after microwave-assisted pretreatment

Both of M- H_2SO_4 and M-NaOH pretreatment showed a proportional relationship between solid loss percentage and chemical concentration. The solid loss values for the pretreatment with 0.01, 0.05, and 0.1N H_2SO_4 are 4.7, 8.8, and 11.6%, respectively; while, similar concentrations of NaOH resulted in 7.5, 8.9, and 12.4% loss, respectively. The microwave assisted pretreatment with 0.1N $NaHCO_3$ resulted in solid loss of 7.7%, with no significant difference with treatment at 0.01N (8.6%). This trend is perhaps because sodium bicarbonate is an amphoteric compound where the aqueous solutions of sodium bicarbonate are mildly alkaline because of formation carbonic acid and hydroxide ion. The increase in the concentration of this sodium bicarbonate will increase the formation of carbonic acid thus decreasing the alkalinity effect and neutralizing the solution (Housecroft and Sharpe, 2004). Pretreatment using distilled water showed the lowest percentage of solid loss at 4.42% compared with other microwave-chemical pretreatment of DFF. Therefore, the use of chemicals could enhance the following hydrolysis step as a result to delignification.

Effect of chemical on releasing sugar from microwave-assisted pretreatment

In the present study, the recovery of fermentable sugar of DFF was compared by using different chemicals. Table 2 shows the monomeric sugar yield after microwave-assisted pretreatment of DFF. The total monomeric sugar yield was found at 6.6 mg/g when distilled water was used. The maximum reducing sugar yield of 15.56 mg/g was obtained with 0.1N from sodium hydroxide solution. Decreasing concentration of NaOH negatively affected the monomeric sugar yield. These results can be attributed to polar characteristics of NaOH, it has a higher dipole moment 6.89 Debye, compared to H_2SO_4 and deionized water, which are 3.09 and 2.12, respectively (Keshwani, 2009; Saifuddinomanbhai and Refalhussain, 2014). The higher polarity can solubilize lignin fraction and facilitate biomass hydrolysis to produce high amount of monomeric sugar higher (Fan *et al.*, 1987). Pretreatment with lower concentration of NaOH yielded less sugar compared to with the pretreatment using higher concentration. It could be due to the fact that NaOH cannot disrupt the recalcitrant structure of lignocellulose material thus would not improve monomeric sugar recovery during pretreatment step.

Table 2. Monomeric sugar yield after microwave-assisted pretreatment of DFF

Pretreatment	Chemical Concentration (N)	Glucose (mg/g)	Xylose (mg/g)	Arabinose (mg/g)	Total (mg/g)
M-H ₂ O	-	6.6	ND*	ND	6.6
	0.1	2.6	5.6	ND	8.2
M-H ₂ SO ₄	0.05	4.6	ND	ND	4.6
	0.01	1.23	ND	ND	1.23
	0.1	12.96	0.23	2.37	15.56
M-NaOH	0.05	7.28	ND	ND	7.28
	0.01	3.68	ND	ND	3.68
	0.1	ND	ND	ND	ND
M-NaHCO ₃	0.05	6.45	ND	ND	6.45
	0.01	3.0	ND	ND	3.0

* ND: Not detected

The maximum yield of xylose (5.6 mg/g) was obtained for 0.1N H_2SO_4 -microwave pretreatment. It has been known that xylose comes from the hydrolysis of hemicellulose (Balat *et al.*, 2008). Total monomeric sugar yield from M- H_2SO_4 pretreatment indicated that the generated glucose partially stems from the hydrolysis of amorphous cellulose (Rosa *et al.*, 2010) and partially from hemicellulose (Gómez *et al.*, 2006). Pretreatment using M- $NaHCO_3$ did not favor the release of monomeric sugar at 0.1N concentration, although lower concentrations of $NaHCO_3$ released monomeric sugars in a similar

way with identical concentrations of NaOH and H₂SO₄. It was obvious that M-NaOH pretreatment was the most effective to release maximum quantity of monomeric sugars from DFF. Pretreatment with H₂SO₄ partially hydrolyzed the hemicellulose fraction and this was confirmed by the presence of xylose in the sugar analysis. Total monomeric sugar yield when biomass was subjected to microwave-assisted chemical pretreatment was less than that obtained with M-H₂O. The study showed that the synergistic effect of microwave and chemicals could enhance the monomeric sugar yield from Dragon fruit foliage, when appropriate quantities of either NaOH or H₂SO₄ were used.

Morphological characteristics of biomass during microwave-assisted chemical pretreatment

The changes in the physical structure of DFF, prior to and after pretreatment, were characterized by using SEM as shown in Figure 3. The texture of raw DFF (Figure 3A) is compact and covered with a thin film, which might be the waxy layer. After DFF was treated in M-NaOH pretreatment, huge voids appeared on the surface and many granules appeared in these voids (Figure 3B), indicating breakdown of the lignin structure and cellulose destruction (Zhu *et al.*, 2006). This observation was confirmed by the glucose yield data in Table 2. An obvious fragmentation of DFF surface could be seen in Figure 3C and D, when biomass was pretreated with M-H₂SO₄ or M-NaHCO₃. Microwave irradiation in acidic or alkali environment increased lignocellulose surface destruction and accessible area favoring the next enzymatic hydrolysis step. The rough surface generated from the pretreatment led to increase in the surface area thereby enhancing the accessibility to cellulolytic enzymes at the saccharification stage. Similar structural changes were earlier reported for sugarcane bagasse pretreated with combination of alkaline, acid and microwave irradiation (Binod *et al.*, 2012) and for corncob subjected to microwave-assisted NaOH pretreatment (Boonsombuti *et al.*, 2013).

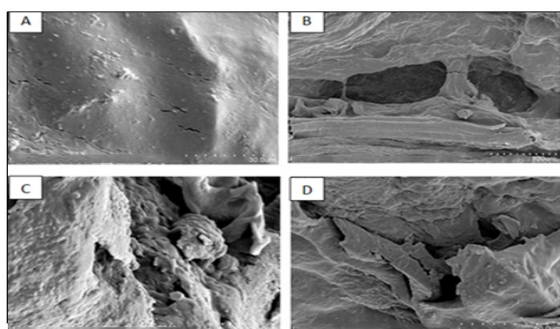


Figure 3. SEM images of DFF (A) raw (X 1500) and pretreated in (B) 0.1N

Conclusion

The impact of acid or alkali on the release of monomeric sugars during microwave-assisted pretreatment of DFF was investigated. Pretreatment with 0.1N NaOH under microwave irradiation (800W; 5 min.) gave maximum total monomeric sugar of 15.56 mg/g. Treatment using similar concentration of H₂SO₄ yielded 8.2 mg/g comprising of glucose and xylose. Pretreatment with M-NaHCO₃ (0.05N) yielded 6.45 mg/g of total monomeric sugar which did not significantly differ from distilled water. Generally, the type of chemicals and its concentration substantially affected the release of fermentable sugars from biomass during microwave-assisted pretreatment. However, the study of investigation the relationship between these chemicals and microwave-assisted pretreatment operating conditions such as microwave power, exposure time, and solid loading may need to be done.

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References

- Azuma, J., Tanaka, F. and Koshijima, T. 1984. Enhancement of enzymatic susceptibility of lignocellulosic wastes by microwave irradiation. *Journal of Fermentation Technology* 62(4): 377-384.
- Balat M, Balat H. and Oz C. 2008. Progress in bioethanol processing. *Progress in Energy and Combustion Science* 34(5): 551-573.
- Balat, M. and Balat, H. 2009. Recent trends in global production and utilization of bio-ethanol fuel. *Applied Energy* 86(11): 2273-2282.
- Binod, P., Satyanagalakshmi, K., Sindhu, R., Janu, K. U., Sukumaran, R. K. and Pandey, A. 2012. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. *Renewable Energy* 37(1): 109-116.
- Boonsombuti, A., Luengnaruemitchai, A. and Wongkasemjit, S. 2013. Enhancement of enzymatic hydrolysis of corncob by microwave-assisted alkali pretreatment and its effect in morphology. *Cellulose* 20(4): 1957-1966.
- Caddick, S. 1995. Microwave assisted organic reactions. *Tetrahedron* 51(38): 10403-10432.
- Chang, V. S. and Holtzapple, M. T. 2000. Fundamental factors affecting biomass enzymatic reactivity. *Twenty-First Symposium on Biotechnology for Fuels*

- and Chemicals, p. 5-37. Fort Collins, Colorado: Springer Science and Business Media.
- Chen, W., Ye, S. and Sheen, H. 2012. Hydrolysis characteristics of sugarcane bagasse pretreated by dilute acid solution in a microwave irradiation environment. *Applied Energy* 93: 237-244.
- Ethaib, S., Omar, R., Kamal, S. M. M., and Biak, D. R. A. 2015. Microwave-assisted pretreatment of lignocellulosic biomass—a review. *Journal of Engineering Science and Technology* 1: 97-109
- Ethaib, S., Omar, R., Mazlina, M. K. S., Radiah, A. B. D., and Syafie, S. 2016. Microwave-assisted Dilute Acid Pretreatment and Enzymatic Hydrolysis of Sago Palm Bark. *BioResources* 11(3):5687-5702
- Fan, L.T., Gharpuray, M.M. and Lee, Y.H.1987. Cellulose hydrolysis. *Biotechnology monographs*. Vol. 3. United States: Springer-Verlag, New York.
- Gámez, S., González-Cabriales, J. J., Ramírez, J. A., Garrote, G. and Vázquez, M. 2006. Study of the hydrolysis of sugar cane bagasse using phosphoric acid. *Journal of Food Engineering* 74(1): 78-88
- Gierer, J. 1985. Chemistry of delignification. *Wood Science and Technology* 19(4): 289-312.
- Hoekman, S. K., Broch, A. and Robbins, C. 2011. Hydrothermal carbonization (HTC) of lignocellulosic biomass. *Energy and Fuels* 25(4): 1802-1810.
- Housecroft, C. E. and Sharpe, A. G. 2004. *Inorganic Chemistry* 2nd ed, p. 173–204. New Jersey: Prentice Hall.
- Hu, Z. and Wen, Z. 2008. Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment. *Biochemical Engineering Journal* 38(3): 369-378
- Karimi, K., Kheradmandinia, S. and Taherzadeh, M. J. 2006. Conversion of rice straw to sugars by dilute-acid hydrolysis. *Biomass and Bioenergy* 30(3): 247-253.
- Keshwani, D. R. 2009. Microwave pretreatment of switchgrass for bioethanol production. USA: North Carolina State University, PhD dissertation.
- Komolwanich, T., Tatijareen, P., Prasertwasu, S., Khumsupan, D., Chaisuwan, T., Luengnaruemitchai, A. and Wongkasemjit, S. 2014. Comparative potentiality of kans grass (*Saccharum spontaneum*) and giant reed (*arundo donax*) as lignocellulosic feedstocks for the release of monomeric sugars by microwave/chemical pretreatment. *Cellulose* 21(3): 1327-1340.
- Lu, X., Zhang, Y. and Angelidaki, I. 2009. Optimization of H₂SO₄-catalyzed hydrothermal pretreatment of rapeseed straw for bioconversion to ethanol: Focusing on pretreatment at high solids content. *Bioresource Technology* 100(12): 3048-3053.
- Ma, H., Liu, W. W., Chen, X., Wu, Y. J. and Yu, Z. L. 2009. Enhanced enzymatic saccharification of rice straw by microwave pretreatment. *Bioresource Technology* 100(3): 1279-1284
- Öhgren, K., Bura, R., Saddler, J. and Zacchi, G. 2007. Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. *Bioresource Technology* 98 (13): 2503-2510.
- Ooshima, H., Aso, K., Harano, Y. and Yamamoto, T. 1984. Microwave treatment of cellulosic materials for their enzymatic hydrolysis. *Biotechnology Letters* 6(5): 289-294.
- Pedersen, M. and Meyer, A. S. 2010. Lignocellulose pretreatment severity—relating pH to biomatrix opening. *New Biotechnology* 27(6): 739-750.
- Quintero, J. A., Montoya, M. I., Sánchez, O. J., Giraldo, O. H. and Cardona, C. A. 2008. Fuel ethanol production from sugarcane and corn: comparative analysis for a Colombian case. *Energy* 33(3): 385-399
- Rosa, M. F., Medeiros, E. S., Malmonge, J. A., Gregorski, K. S., Wood, D. F., Mattoso, L. H. C. and Imam, S. H. 2010. Cellulose nanowhiskers from coconut husk fibers: Effect of preparation conditions on their thermal and morphological behavior. *Carbohydrate Polymers* 81(1): 83-92
- Saga, K., Imou, K., Yokoyama, S. and Minowa, T. 2010. Net energy analysis of bioethanol production system from high-yield rice plant in Japan. *Applied Energy* 87(7): 2164-2168
- Zhou, A. and Thomson, E. 2009. The development of biofuels in Asia. *Applied Energy* 86: S11-S20.
- Saifuddinomanbhay and Refalhussain 2014. Microwave-assisted alkaline pretreatment and microwave assisted enzymatic saccharification of oil palm empty fruit bunch fiber for enhanced fermentable sugar yield. *Chemical Science Transactions* 3(1): 350-358.
- Schmidt, A. S. and Thomsen, A. B. 1998. Optimization of wet oxidation pretreatment of wheat straw. *Bioresource Technology* 64(2): 139-151.
- Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74(10): 3583-3597.
- Viola, E., Cardinale, M., Santarcangelo, R., Villone, A. and Zimbardi, F. 2008. Ethanol from eel grass via steam explosion and enzymatic hydrolysis. *Biomass and Bioenergy* 32(7): 613-618.
- Zhu, S., Wu, Y., Yu, Z., Wang, C., Yu, F., Jin, S. and Zhang, Y. 2006. Comparison of three microwave/chemical pretreatment processes for enzymatic hydrolysis of rice straw. *Biosystems Engineering* 93(3): 279-283.