Ionic Solution Pretreatment of Oil Palm Empty Fruit Bunch to Produce Sugars

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Ferric (III) chloride, when prepared as an ionic solution, was used to pretreat oil palm empty fruit bunch (OPEFB) before undergoing enzymatic hydrolysis to convert into value-added products. The pretreatment was assisted with microwave irradiation to improve the degradation of recalcitrant structure of lignocellulosic materials with minimum time. The effects of salt concentration, temperature, and duration of pretreatment on the chemical composition of OPEFB and total reducing sugar (TRS) yield were investigated. The results revealed that the best pretreatment occurred at pretreatment time of 10 min, 100 °C, and ferric chloride concentration of 10 w/v%. The TRS yield achieved using the pretreated OPEFB was approximately 0.485 g/g, which was three times higher than the non-pretreated OPEFB, which was only 0.154 g/g. Thus, the ionic solution pretreatment method is a promising alternative for replacing other pretreatment methods.

Keywords: Format; Lignocellulosic biomass; Ionic solution pretreatment; Ferric chloride solution; Microwave irradiation

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INTRODUCTION

Oil palm is one of the important crops in Southeast Asian countries, as it generates increased income every year to these countries. Malaysian Palm Oil Board reported that oil palm trees planted area in Malaysia has reached 5.9 million hectares (ha) in 2019. As the oil palm plantation area increased, various biomass residues generated from oil palm industry also increased throughout the year. Oil palm empty fruit bunches (OPEFB), are the remains of the fresh fruit bunches after the fruits have been removed for oil pressing. It is the most abundant biomass waste generated at all palm oil mills. The amount of OPEFB throughout 2017 were around 15.8 to 17 million tons per year (Derman *et al.* 2018). Thus, huge amounts of OPEFB should be converted into value-added products, such as bioethanol, bio-oil, and sugars to reduce environment problems.

The utilization of OPEFB as a renewable carbon resource has attracted much attention to produce valuable products such as sugars. Lignocellulosic biomass is made up of complex biopolymers that consist of three major components of lignin, cellulose, and hemicellulose. However, conversion of lignocellulosic biomass, such as EFB, into sugars is still a challenge because the pretreatment methods currently available are still far from efficient and economical. Currently, there are two types of routes for EFB conversion. The first type is to de-polymerize lignocellulose directly without differentiating its components and only regard biomass as a mixture of C, H, and O elements; the degraded products are still complex and require an upgrading process; the methods used usually include gasification and thermal pyrolysis (Li and Chen 2018; Ferreira *et al.* 2020). The second type of conversion involves fractionation of lignocellulose biomass to lignin, cellulose, and hemicellulose (Hassan *et al.* 2020). Pretreatment is the most important process in the bioconversion of lignocellulose for sugars production. The purpose of pretreatment is to remove the recalcitrant lignin to ease the biomass hydrolysis process, improve the enzymatic digestibility, and increase the yield of reducing sugars produced. There are three kinds of lignocellulose pretreatment methods, which are physical (microwave, ultrasound, pyrolysis, *etc.*), chemical (dilute acid, mild alkali, ionic liquids, *etc.*) and physicochemical (steam explosion, oxidative, wet oxidation, *etc.*). However, different biomasses will present different standards of hemicellulose or lignin removal and thus different enzymatic digestibility, even under the same pretreatment process at the same conditions (Kang *et al.* 2012).

Dilute acid pretreatment is the most frequently used method to convert biomass to fermentable sugars by hydrolyzing hemicellulose using dilute acid followed by enzymatic hydrolysis. However, this method may generate toxic substances, which will inhibit fermentation. In addition, dilute acid pretreatment causes corrosion. Thus, expensive materials for construction are required, which lead to the increase in capital cost. Besides the aforementioned methods, imidazole-type ionic liquids have also been used in pretreatment processes (Cao *et al.* 1994; Binder and Raines 2010; Muranaka *et al.* 2015; Kuroda *et al.* 2016; Qian *et al.* 2016). Unlike an ionic liquid, which is expensive (Wan *et al.* 2018) and has melting point below the boiling point of water, an ionic solution is simply a solution containing ions where ionic compound is dissolved in water; dissociating into cations and anions.

Recently, the trend to use ionic solutions, such as $ZnCl_2$, and $FeCl_3$ aqueous solutions, in the pretreatment of biomass for producing value-added product such as bioethanol has become attractive. An ionic solution has the ability to increase cellulose and hemicellulose conversion rates as well as improve hydrolysis yields (Liu *et al.* 2009; Hassan *et al.* 2020). Additionally, inorganic salt solutions are less corrosive and can achieve higher rate of enzymatic digestibility than inorganic acids and it can be recycled (Liu *et al.* 2009; Kamireddy *et al.* 2013; Hassan *et al.* 2020). A Lewis acid, such as $FeCl_3$, has been proposed to be the most effective inorganic salt that can replace acid pretreatment (Liu *et al.* 2009; Zhu *et al.* 2020). $FeCl_3$ can undergo hydrolysis and generate hydronium ions, leading to depolymerisation of hemicellulose. $FeCl_3$ is able to degrade hemicellulose into monomeric and oligomeric sugars in liquid hydrolysate with a large amount of xylose and produce cellulose-rich solid substrate that can be converted to glucose in enzyme hydrolysis process. Moreover, pretreatment using these ionic solutions results in a significant improvement of the enzyme activity due to the metal cations forming complexes with the lignin, reducing the unproductive enzyme–lignin links (Kamireddy *et al.* 2013).

A perusal of published literature showed that a majority of the current pretreatment methods are energy intensive in nature (Kang *et al.* 2013; Liu *et al.* 2009). Thermal energy is crucial in accelerating the hydrolytic process, which results in substantial breakdown of the lignocellulosic structure, hydrolysis of the hemicellulose fraction and depolymerization of the lignin components. Thus, in this study a ferric salt solution and microwave heating were combined during the pretreatment, as this approach mediates in breaking of bonds among the molecules. The influence of salt concentration, temperature, and pretreatment time towards enzymatic hydrolysis of pretreated OPEFB were also investigated. The effectiveness of pretreatment process is measured in terms of total reducing sugar (TRS) yield.

EXPERIMENTAL

$FeCl_3$ Pretreatment

A 250-mL three-necked flask was used for pretreatment of OPEFB. The solid-to-liquid ratio was set at 1:10, and 5 g of dry biomass was added in 50 mL prepared $FeCl_3$ solution. The $FeCl_3$ solution concentrations were varied (5, 10, and 15% w/v). The flask was placed in a microwave oven to heat at various desired temperatures (75, 90, and 100 °C) at different pretreatment times (5, 10, and 15 min). Upon the end of reaction, the flask was then cooled to below 40 °C by placing it inside the water bath. The treated OPEFB fibres were then washed with water to remove $FeCl_3$ until pH reached 7. The pretreated solid samples were dried at 60 °C and then stored in a plastic container for later use. The mean values of results were calculated from duplicate analysis.

Composition Analysis of OPEFB

Holocellulose content was determined using the procedure used by Teramoto *et al.* (2009), and α-cellulose amount was determined using TAPPI T203 cm-09 (2009). The National Renewable Energy Laboratory standard method (Sluiter *et al*. 2008) was used to determine lignin amount inside the OPEFB sample. The mean values of results were calculated from duplicate analysis.

Enzymatic Hydrolysis

Enzymatic hydrolysis of OPEFB samples was performed to obtain the TRS yield. The hydrolysis of OPEFB samples was performed in 250-mL conical flasks containing sodium citrate buffer (pH 4.8, 0.05 M), 100 µL of sodium azide (2% w/v), and Cellic CTec2 enzymes (20 FPU/g OPEFB). Cellic CTec2 is an enzymes blend consist of cellulases, ß-glucosidases, and hemicellulose. The enzymatic hydrolysis was performed at temperature of 50 °C. The sampling was performed every 1, 4, 24, 48, 72 h of enzymatic hydrolysis and the TRS yields were analyzed at the time mentioned using 3,5-dinitrosalicylic acid (DNS) method. Then, TRS was then calculated accordingly to Eq. 1:

$$\textbf{TRS yield}\ (\tfrac{g}{g}) = \frac{\boldsymbol{Sugar\ recovered}\left(\frac{g}{L}\right) \times \boldsymbol{Volume\ of\ solvent\ used}\ (L)}{\boldsymbol{Mass\ of\ OPEFB\ used}\ (g)} \tag{1}$$

All hydrolysis experiments were conducted in triplicate, and errors were within ± 5%. The data are presented in average values.

RESULTS AND DISCUSSION

Analysis of OPEFB Composition Before and After Pretreatment

Based on the data in Table 1, the pretreated OPEFB had a higher content of cellulose and lignin than non-pretreated OPEFB, where the treated OPEFB contained 65.6% of cellulose and 18.9% of lignin. The results in Table 2 also shows that there was a high loss of hemicellulose in pretreated OPEFB compared to the non-pretreated one, which was from 23.41 ± 0.59% to 3.78 ± 0.38%. The data strongly demonstrated that $FeCl_3$ solution has the ability to solubilize hemicellulose compound in OPEFB at a higher degree. This result agrees with that of Zhang *et al.* (2018), who determined that $FeCl_3$ pretreatment could easily and effectively solubilize hemicellulose in lignocellulosic sugarcane bagasse. The increase in lignin and cellulose content after $FeCl_3$ pretreatment can be ascribed to the solubilization of hemicellulose fraction. Similar lignin and cellulose content increment phenomenon were reported by Moodley and Kana (2017) during the pretreatment of sugarcane leaves waste using microwave assisted-$FeCl_3$ pretreatment.

Various reaction mechanisms have been suggested for metal salts. Metal chlorides such as $FeCl_3$ dissociate into complex ions in aqueous solvent such as water. Fe^{3+} can form coordinate covalent bonds with six water molecules as monodentate ligands. This metal cation behaves as a Lewis acid in an aqueous environment, which facilitate the cleavage of the glycosidic linkages (Kamireddy *et al.* 2013). Meanwhile, the resulting water molecule from the hydrated cation species plays a key role in forming xylose by participating as nucleophiles (Zhu *et al.* 2020; Moodley *et al.* 2020). Secondly, $FeCl_3$ can undergo hydrolysis and generate hydronium ions, leading to depolymerisation of hemicellulose by selective hydrolysis of glycosidic linkages to form acetic and uronic acids. The released acids could also catalyse hemicellulose hydrolysis (Loow *et al.* 2015). Thirdly, Fe^{3+} cations are good electron acceptors and able to coordinate with oxygen donor atoms of carbohydrates and their derivatives without losing the protons that make up the hydroxyl groups of the ligand to form saccharide-metal cation intermediate, leading to reduced activation energy requirements for biomass hydrolysis (Loow *et al.* 2015; Zhu *et al.* 2020).

Table 1. Chemical Composition of OPEFB Before and After Pretreatment

Samples	Chemical Composition of OPEFB (%)		
	Cellulose	Hemicellulose	Lignin
Non-pretreated	39.57 ± 0.78	23.41 ± 0.59	16.84 ± 0.02
Treated	65.56 ± 0.34	3.78 ± 0.38	18.94 ± 0.02

TRS Yield Comparison of Pretreated and Non-pretreated OPEFB

Figure 1 illustrates the TRS yield of pretreated and non-pretreated OPEFB. Both curves show the same trend but at different rates.

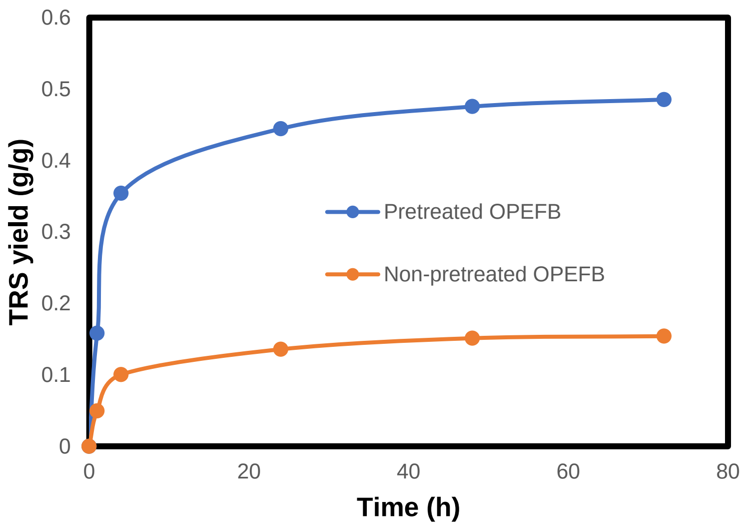

Fig. 1. TRS yield of pretreated and non-pretreated OPEFB

The rate of enzymatic hydrolysis increased rapidly initially but gradually decreased after 20 h. The finding shows that a high amount of reducing sugars can be harvested from the pretreated OPEFB compared to the non-pretreated OPEFB. The maximum reducing sugar generated from the pretreated OPEFB was 0.485 g/g, which was three times higher than the non-pretreated OPEFB where only 0.154 g/g of sugar was produced. The data obtained remarkably proved microwave assisted ionic solution pretreatment method was effective in removing the recalcitrant structure of OPEFB, which in turn facilitated and accelerated the enzymatic hydrolysis.

Effect of $FeCl_3$ Concentration on TRS Yield

Figure 2 illustrates the TRS yield against time at constant temperature (100 °C) and pretreatment time (10 min). The findings show that the trend of TRS yield for different $FeCl_3$ concentrations (0%, 5% w/v, 10% w/v, and 15% w/v) were almost similar. Initially, the total reducing sugar yield increased consistently until it reached 72 h of hydrolysis. The maximum sugar yield was achieved at 10% w/v, followed by the 15% w/v and 5% w/v $FeCl_3$ concentrations. The final amount of reducing sugars produced were 0.4924 g/g, 0.4642 g/g, and 0.2935 g/g, respectively. Meanwhile, the use of hot liquid water (0% of $FeCl_3$) during pretreatment yielded low TRS accumulation, as only 0.1571 g/g TRS was produced.

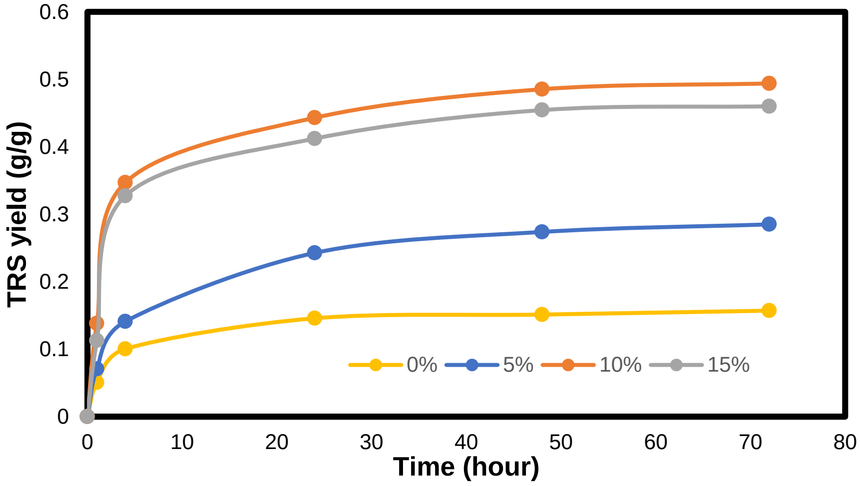

Fig. 2. Effect of $FeCl_3$ concentration on TRS yield; pretreatment was performed at temperature of 100 °C and for 10 min

The use of a higher amount of $FeCl_3$ in the pretreatment resulted in higher accumulation of TRS. The TRS yield increased from and 0.2935 ± 0.0120 g/g to 0.4924 ± 0.0021 g/g when the $FeCl_3$ concentration was increased from 5 to 10%. High concentration of Fe^{3+} in pretreatment solution accelerated the generation of reactive H_3O^+ (Ravindran *et al.* 2017). Thus, substantial removal of hemicellulose was achieved, which enhanced enzymatic hydrolysis by improving the enzymes access. However, low sugar yield was obtained when the $FeCl_3$ concentration was further increased to 15% w/v with TRS yield of 0.4642 ± 0.0059 g/g. A similar observation was reported by Kang *et al.* (2015), where $FeCl_3$ was used during the pretreatment to enhance the enzymatic hydrolysis of *Miscanthus* straw. They found that the glucose conversion yield was negatively affected when high loading of $FeCl_3$ was used. In their study, the increase of the $FeCl_3$ concentration beyond 2.0% caused much glucose loss, as almost 80% of glucan was hydrolyzed. It is concluded that pretreatment at 10% w/v $FeCl_3$ is suitable to obtain higher yield of reducing sugars compared to 5% w/v and 15% w/v $FeCl_3$.

Effect of Pretreatment Temperature and Pretreatment Time on Total Sugar Yield

Figures 3 and 4 illustrate the time course of enzymatic hydrolysis of OPEFB at different pretreatment temperatures. From these graphs, all the total reducing sugar yield trends for different pretreatment temperatures and time were almost similar. Initially, the TRS yield increased gradually until it reached 72 h of hydrolysis.

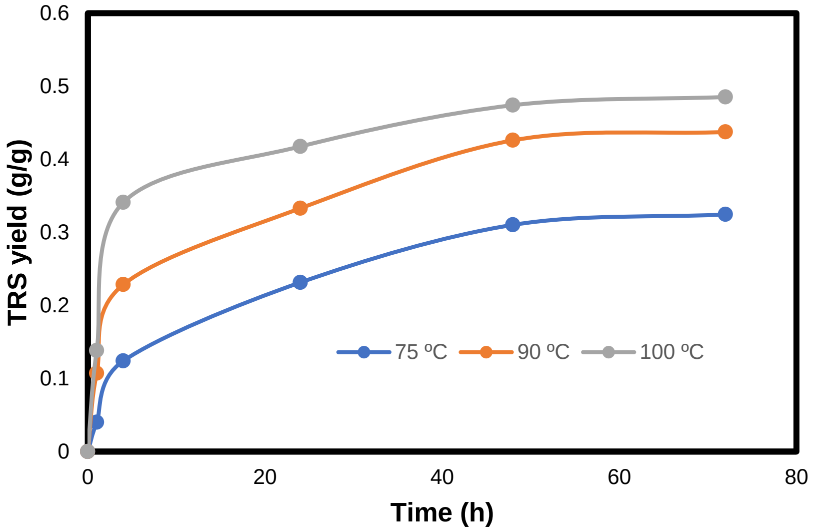

Fig. 3. Effect of temperature on TRS yields; pretreatment was performed using 10% w/v of $FeCl_3$ and pretreatment time of 10 min

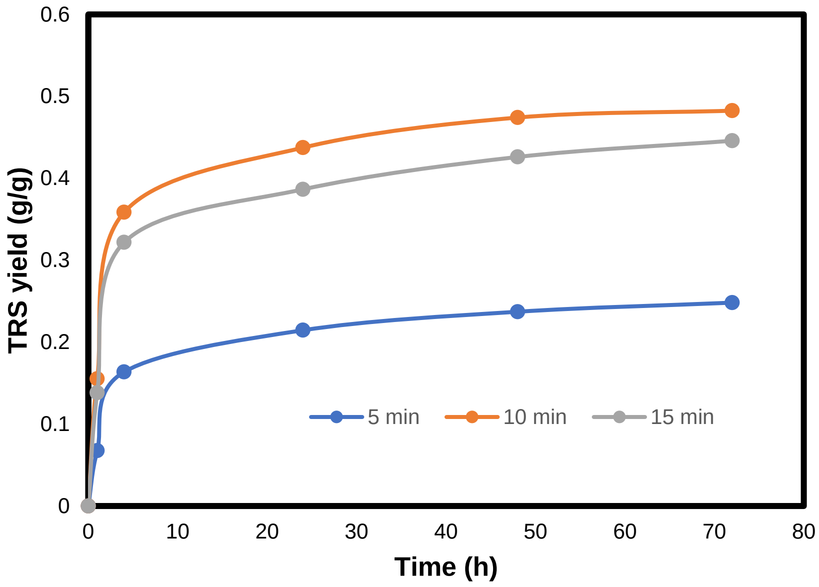

Fig. 4. Effect of pretreatment time on the enzymatic hydrolysis of OPEFB; pretreatment was performed at $FeCl_3$ concentration of 10% w/v and pretreatment time of 10 min

The optimum TRS yield was achieved at pretreatment temperature of 100 °C, followed by 90 °C and 75 °C, with the amount of reducing sugar produced of 0.4839 ± 0.0020 g/g, 0.4317 ± 0.0081 g/g, and 0.3189 ± 0.0079 g/g, respectively. The maximum TRS yield was achieved by pretreating at 10 min, followed by 15 min and 5 min, with the amount of reducing sugar produced at 0.4853 ± 0.0040 g/g, 0.4416 ± 0.0059 g/g, and 0.2413 ± 0.0098 g/g, respectively.

It can be observed that, at temperature of 100 °C, 0.4839 g/g of TRS was produced, which was much higher than 90 °C with 0.4317 g/g of TRS produced. However, pretreatment time for 10 min generated more TRS yield compared to pretreatment time of 15 min, where 0.4853 g/g of TRS was produced at 10 min, with only 0.4416 ± 0.0059 g/g of TRS at 15 min. This scenario could be ascribed to cellulose degradation, which was the main cause of slightly lower TRS yield. Overall, the combination of high pretreatment temperature and longer pretreatment time was not a good choice. It is suggested to slightly decrease the pretreatment time when pretreating at high temperature to obtained high yield of TRS. Finally, the best pretreatment temperature and pretreatment time were set at 100 °C for 10 min.

CONCLUSIONS

1. Factors such as pretreatment time, temperature, and ferric chloride concentration influenced the total sugar yield, and ionic solution pretreatment was best performed at 10 min, 100 °C, and 10% w/v, respectively.
2. Pretreatment at the recommended process variables achieved a TRS yield of 0.4853 g/g, which was three times higher than the non-pretreated OPEFB, for which only 0.1541 g/g was obtained.
3. The remarkable difference in reducing sugars yield between the pretreated and non-pretreated OPEFB showed that ionic solution pretreatment can improve the enzymatic hydrolysis process.
4. Overall, ionic solution pretreatment method is a potential and promising alternative pretreatment method.

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REFERENCES CITED

Binder, J. B. and R. T. Raines. (2010). “Fermentable sugars by chemical hydrolysis of biomass,” *Proc. Natl. Acad. Sci. U.S.A.*, 107, 4516-4521.

Cao, N.-J., Xu, Q., Chen, C.-S., Gong, C. S., and Chen, L. F. (1994) “Cellulose hydrolysis using zinc chloride as a solvent and catalyst,” *Appl. Biochem. Biotechnol.* 45, 521-530. DOI:10.1007/BF02941827

Derman, E., Abdulla, R., Marbawi, H., and Sabullah, M. K. (2018). "Oil palm empty fruit bunches as a promising feedstock for bioethanol production in Malaysia," *Renewable Energy* 129(Part A), 285-298. DOI: 10.1016/j.renene.2018.06.003

Ferreira, M. F. P., Oliveira, B. F. H., Pinheiro, W. B. S., Correa, N. F., França, L. F., and Ribeiro, N. F. P. (2020). "Generation of biofuels by slow pyrolysis of palm empty fruit bunches: Optimization of process variables and characterization of physical-chemical products," *Biomass and Bioenergy* 140, Article ID 105707. DOI: 10.1016/j.biombioe.2020.105707

Hassan, N., Anwar, N. A. K. K., and Idris, A. (2020). "Strategy to enhance the sugar production using recyclable inorganic salt for pre-treatment of oil palm empty fruit bunch (OPEFB)," *BioResources* 15(3), 4912-4931. DOI: 10.15376/biores.15.3.4912-4931

Kamireddy, S. R., Li, J., Tucker, M., Degenstein, J., and Ji, Y. (2013). "Effects and mechanism of metal chloride salts on pretreatment and enzymatic digestibility of corn stover," *Industrial & Engineering Chemistry Research* 52(5), 1775-1782. DOI: 10.1021/ie3019609

Kang, K. E., Jeong, G. T., Sunwoo, C., and Park, D. H. (2012). "Pretreatment of rapeseed straw by soaking in aqueous ammonia," *Bioprocess and Biosystems Engineering* 35, 77–84. DOI: 10.1007/s00449-011-0606-z

Kang, K. E., Park, D. H., and Jeong, G. T. (2013). "Effects of inorganic salts on pretreatment of Miscanthus straw," *Bioresource Technology* 132, 160-165. DOI: 10.1016/j.biortech.2013.01.012

Kuroda, K., K. Inoue, K. Miyura, K. Takada, K. Ninomiya and K. Takahashi. (2016). "Enhanced hydrolysis of lignocellulosic biomass assisted by a combination of acidic ionic liquids and microwave heating," *J. Chem. Eng. Japan* 49, 809-813. DOI: 10.1252/jcej.15we292

Li, Y. H., and Chen, H. H. (2018). "Analysis of syngas production rate in empty fruit bunch steam gasification with varying control factors," *International Journal of Hydrogen Energy* 43(2), 667-675. DOI: 10.1016/j.ijhydene.2017.11.117

Liu, L., Sun, J., Li, M., Wang, S., Pei, H., and Zhang, J. (2009). "Enhanced enzymatic hydrolysis and structural features of corn stover by $FeCl_3$ pretreatment," *Bioresource Technology* 100(23), 5853-5858. DOI: 10.1016/j.biortech.2009.06.040

Loow, Y. L., Wu, T. Y., Tan, K. A., Lim, Y. S., Siow, L. F., Md. Jahim, J., and Teoh, W. H. (2015). "Recent advances in the application of inorganic salt pretreatment for transforming lignocellulosic biomass into reducing sugars," *Journal of Agricultural and Food Chemistry* 63(38), 8349-8363. DOI: 10.1021/acs.jafc.5b01813

Moodley, P., Sewsynker-Sukai, Y., and Kana, E. G. (2020). "Progress in the development of alkali and metal salt catalysed lignocellulosic pretreatment regimes: Potential for bioethanol production," *Bioresource Technology*, article no. 123372. DOI: 10.1016/j.biortech.2020.123372

Moodley, P., and Kana, E. G. (2017). "Microwave-assisted inorganic salt pretreatment of sugarcane leaf waste: Effect on physiochemical structure and enzymatic saccharification," *Bioresource Technology* 235, 35-42. DOI: 10.1016/j.biortech.2017.03.031

Muranaka, Y., Suzuki, T., Hasegawa, I., and Mae, K. (2015). "Saccharification of lignocellulosic biomass under mild condition using ionic liquid," *J. Chem. Eng. Japan*, 48, 774-781 (2015).

Ravindran, R., Sarangapani, C., Jaiswal, S., Cullen, P. J., and Jaiswal, A. K. (2017). "Ferric chloride assisted plasma pretreatment of lignocellulose," *Bioresource Technology* 243, 327-334. DOI: 10.1016/j.biortech.2017.06.123

Qian, E. W., Tominaga, H., Chen, T. L., and Isoe, R. (2016). "Synthesis of functional ionic liquids and their application for the direct saccharification of cellulose," *J. Chem. Eng. Japan*, 49, 466-474.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. (2008). *Determination of Structural Carbohydrates and Lignin in Biomass* (NREL/TP-510-42618), National Renewable Energy Laboratory Analytical Procedure, Golden, CO, USA.

TAPPI T203 cm-09 (2009). "Alpha-, beta- and gamma-cellulose in pulp," TAPPI Press, Atlanta, GA, USA.

Teramoto, Y., Lee, S. H., and Endo, T. (2009). "Cost reduction and feedstock diversity for sulfuric acid-free ethanol cooking of lignocellulosic biomass as a pretreatment to enzymatic saccharification," *Bioresource Technology* 100(20), 4783-4789. DOI: 10.1016/j.biortech.2009.04.054

Wan, H.-P., Hung, W.-C., Lin, U.-T., Chen, J.-Y., Yu, P.-J., and Yang, T.-Y. (2018). "Hydrolysis of lignocellulosic biomass in ionic solution," *J. Chem. Eng. Japan* 51(9), 786-793. DOI: 10.1252/jcej.17we151

Zhang, H., Lyu, G., Zhang, A., Li, X., and Xie, J. (2018). "Effects of ferric chloride pretreatment and surfactants on the sugar production from sugarcane bagasse," *Bioresource Technology* 265, 93-101. DOI: 10.1016/j.biortech.2018.05.111

Zhu, Z., Liu, Y., Yang, X., McQueen-Mason, S. J., Gomez, L. D., and Macquarrie, D. J. (2020). "Comparative evaluation of microwave-assisted acid, alkaline, and inorganic salt pretreatments of sugarcane bagasse for sugar recovery," *Biomass Conversion and Biorefinery*, 1-13. DOI:10.1007/s13399-020-00680-7

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