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Effects of ultrasonic chemical pretreatment on morphology and sugar production of Nipah fruit husk

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ABSTRACT

This work reports a study on the effects of several chemical pretreatment parameters such as solvent types, solvent concentration, liquid to solid ratio (LSR), temperature, time and sonication amplitude on total reducing sugar (TRS) production of nipah fruit husks using ultrasonic method. The pretreatment was carried out using hydrochloric acid, sulphuric acid, and citric acid for acid pretreatment; meanwhile sodium hydroxide and calcium hydroxide were used for alkaline pretreatment. Total reducing sugar produced from each pretreatment was estimated using DNS method. Screening results shown that nipah palm fruit husks pretreated with 40% w/v of sodium hydroxide, 10:1 of LSR, 50% of sonication amplitude at 80°C for 20 minutes showed the highest total reducing sugar (TRS), 78.79±1.0887 g/L. In fact, characterization of untreated and pretreated nipah fruit husks was done using scanning electron microscope (SEM) to observe the effect of each pretreatment on the morphology of the samples. Through SEM, nipah fruit husk pretreated with sodium hydroxide showed the greatest cell disruption in the structure which indicates that more fermentable sugar was released and thus promotes highest TRS yield.

1. Introduction

Bioethanol can be divided into first generation bioethanol (FGB) and second generation bioethanol (SGB). FGB originated from edible sources such as sugarcane and corns. However, it had disadvantages as the feedstock originated from food stream (Zheng et al., 2009). Thus, SGB overcomes the conflict between food and fuel as it is produced from non-edible source, lignocellulose biomass which includes hard/soft wood, agricultural wastes, municipal solid wastes and cellulose wastes (Zheng et al., 2009; Verma et al., 2011). Recently, the conversion of lignocellulosic biomass into biofuels had gained the attention of the researchers all over the world. This is due to the necessity of having energy sources which are inexpensive, ecofriendly, renewable and able to replace the conventional fossil fuels.

Lignocellulose biomass composed of cellulose, hemicellulose, lignin, extractives and ashes (Taherzadeh and Karimi, 2007). The long chain polymers which are cellulose and hemicellulose can be hydrolyzed into fermentable sugars (Chaturvedi and Verma, 2013). Fermentable sugars such as pentoses (C5) and hexoses (C6) will be utilized in the production of bioethanol. Lignin acts as barriers that prevent the degradation of cellulose and hemicellulose and cellulose by fungi and bacteria. It also hinders the hydrolysis of cellulose and hemicellulose. Thus, pretreatment is required to reduce the crystallinity of the lignocellulose and to remove the lignin allowing hydrolysis of fermentable sugars to take place (Asli et al., 2013).

A number of pretreatment methods are available which includes physical pretreatments (mechanical comminution, steam explosion, hot water), chemical pretreatment (acid, alkali, ionic liquids), biological pretreatment (enzymatic, microorganism) etc. (Sun and Cheng, 2002). Each of these pretreatments has their advantages and disadvantages and their suitability are depending on the type of lignocellulose biomass used as feedstock (Alvira et al., 2010).

The present work applied acid and alkali pretreatment on the nipah husk for the bioethanol production. The pretreatment was facilitated with ultrasonic wave. The use of ultrasound were previously used to enhance various processes and provided another options to conventional

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pretreatment of lignocellulose biomass (Rehman et al., 2013). In this work, from all of the solvent tested, the solvent that yielded the maximum total reducing sugar (TRS) went through the next screening. The effect of solvent concentration, liquid-to-solid ratio (LSR), temperature, time and sonication amplitude on the pretreatment were studied.

2. Materials and method

Nipah fruits (nypa fructicans) were obtained from a nipah plantation in Yan, Kedah. The collected fruit was washed to remove dirt from them. Then, they were chopped into smaller pieces and the husk was separated. Next, the husk was oven-dried at 45°C until the weight of the husks was constant. The dried husks was ground to fine powders and stored in air-tight containers at room temperature until further use. All chemicals use in this work was purchased from Fischer, Bendosen and Hmbg.

Finely grounded nipah fruit husks were pretreated using two types of alkali namely sodium hydroxide, NaOH and calcium hydroxide, $Ca(OH)_2$. Meanwhile acid pretreatment were done using hydrochloric acid, HCl, sulphuric acid, H_2SO_4 and citric acid, $C_6H_8O_7$. Both acid and alkaline pretreatment were conducted in an ultrasonic water bath with heating power of 500W and frequency 40Hz. The solvent which produced highest TRS was chosen for the subsequent screening: (1) concentration of solvent; (2) liquid to solid ratio (LSR); (3) temperature; (4) time; and (5) sonication amplitude. Next, the pretreated nipah fruit husk was filtered to separate the liquid and solid phase by using a vacuum pump. After that, the solid residues were thoroughly washed with distilled water until pH 7 and dried in oven at 45°C until constant weight. The dried solid residues were then kept in a sealed plastic bag at ambient room temperature until used for further analysis. Meanwhile, the liquid fraction was collected to test for sugar release.

2.1. Estimation of total reducing sugar (TRS)

Total reducing sugar (TRS) released after acid and alkaline pretreatment were estimated using DNS method (Miller, 1959).

2.2. Morphological characterization of untreated and pretreated nipah husks

Dried solid residues (treated and untreated nipah fruit husks) were characterized using scanning electron microscope (SEM) to observe the structure of nipah fruit husks before and after pretreatment. As nipah husks were non-conductive materials, the samples were coated with platinum using a sputter coater prior being observed under SEM.

3. Results and discussion

3.1. Effect of solvent types on the total reducing sugar (TRS)

Fig. 1 shows the effect of different solvent types on the total reducing sugar (TRS) obtained after pretreatment. Based on the bar graph, the highest amount of 3.5± 0.2646 g/L of TRS was achieved from the pretreatment of nipah husk with NaOH. Meanwhile, pretreatment of nipah husk with the other solvents produced very low amount of TRS. Previous study states that alkaline pretreatment is commonly more effective on hardwood, herbaceous crops, and agricultural residues. All these substrates contain a lower lignin content compared to softwood, which contain high amounts of lignin (Chen et al., 2013).

Nipah husks, considered as agricultural residues have low lignin content due to presence of high amount of ash and some other minor components (Tamunaidu and Saka, 2011). This explains why the acid pretreatment pretreated using HCl, H_2SO_4 or $C_6H_8O_7$ did not release high amount of reducing sugar. Through alkaline pretreatment, delignification process occurred which assumed to be from the saponification of intermolecular ester bonds cross-linking xylan hemicelluloses, lignin and hemiceluloses which will increases the porosity of the lignocellulosic materials (Sun and Cheng, 2002) (Singh and Trivedi, 2013).

As for $Ca(OH)_2$, even though it is a type of alkali, it did not produce as much sugar as NaOH. This was caused by the calcium ions ($Ca2+$) in $Ca(OH)_2$ carries two positive charges that can crosslink with the negatively-charged surface of biomass which will reduce the total solid loss thus, decrease the accessibility of carbohydrates from further hydrolysis into reducing sugar (Wang et al., 2008).

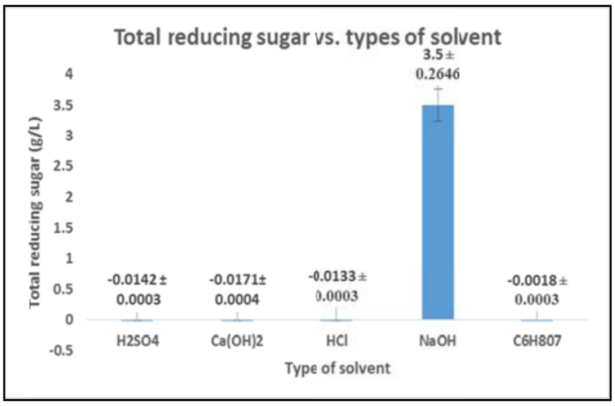

Fig. 1: Effect of solvent types on the total reducing sugar (TRS)

3.2. Effect of solvent concentration on the total reducing sugar (TRS)

By referring to Fig. 2, 40% w/v of NaOH showed the maximum amount of TRS, 26.025±0.5434 g/L as compared to other concentrations of NaOH. TRS increased with the increasing of NaOH concentration from 10% w/v up to 40 % w/v; higher concentration of NaOH resulted in greater lignin removal.

Therefore, more cellulose was available to be hydrolysed into reducing sugar (Akbarningrum et al., 2012). However, TRS decreased significantly when the concentration of NaOH reached 50 % w/v. Intensive biomass solubilization from higher NaOH concentrations may resulted in higher total solid loss which will lowered the total carbohydrates available for sugar production (Wang et al., 2008).

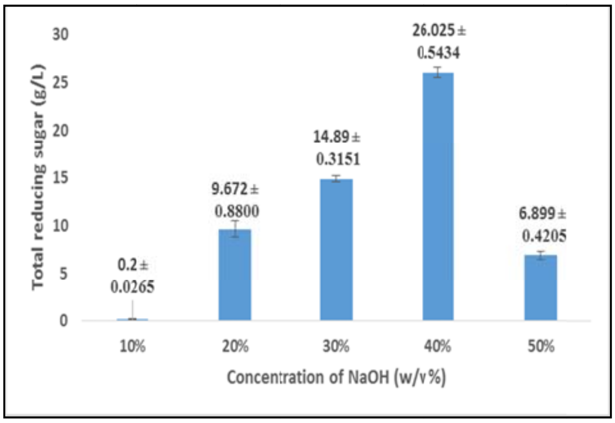

Fig. 2: Effect of solvent concentration on the total reducing sugar (TRS)

3.3. Effect of liquid to solid (LSR) on the total reducing sugar (TRS)

For the effect of LSR on TRS, highest TRS of 64.566± 0.8498 g/L was shown by nipah husk that was pretreated with LSR of 10:1 (1g of nipah husk pretreated with 10 ml of NaOH). Fig. 3 revealed that the TRS was decreased with the increasing of LSR. Higher LSR will reduce the concentration of NaOH in the liquid phase. This will further lead to decrease in the delignification rate of nipah husks during pretreatment (Zhao et al., 2009). The lower delignification rate, the lower the amount of TRS produced.

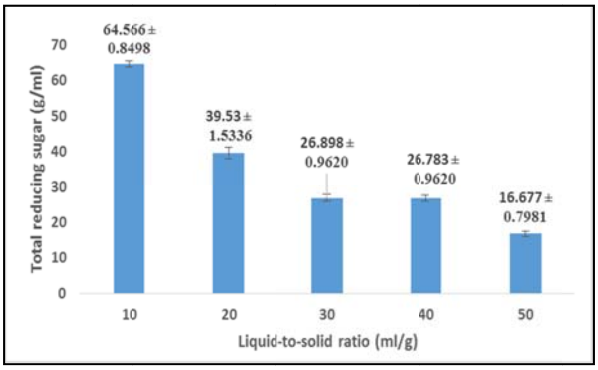

Fig. 3: Effect of liquid to solid ratio on the total reducing sugar (TRS)

3.4. Effect of temperature on the total reducing sugar (TRS)

The effect of temperature on TRS was shown in Fig. 4. The TRS was significantly increased with the increasing of pretreatment temperature. The lowest amount of TRS, 25.531±0.5986 g/L was achieved at temperature of 40°C. This due to insufficient heat supply will slow down the breakdown of cellulose, and thus less hemicellulose was hydrolyzed into glucose at lower temperature. The highest TRS amount of 64.566±1.0405 g/L was observed at 80°C which is the highest temperature attained by the ultrasonic water bath. The relationship between pretreatment temperature and NaOH concentration with TRS can be related with Arrhenius theory as shown in equation (1) and (2).

$$k = k0 \quad (1)$$

$$-rA = k.C \quad (2)$$

where:

$-rA$ = the rate of reaction
K = reaction rate constant
C = concentration
T = temperature

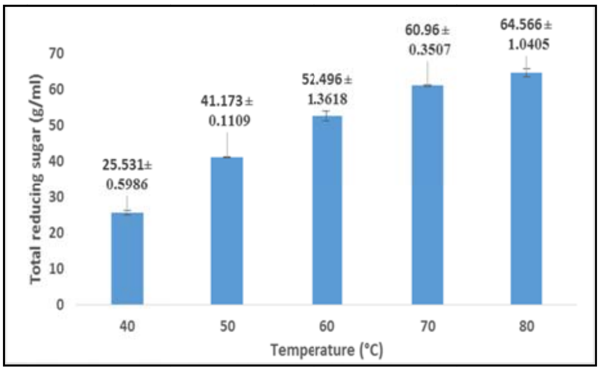

Fig. 4: Effect of temperature on the total reducing sugar (TRS)

When the pretreatment temperature was elevated, the reaction rate constant (k) became higher. Thus, at the same concentration of NaOH (C), the rate of reaction (-rA) will also increases. Increases in the rate of reaction will causes the delignification in nipah fruit husk occurs rapidly. Therefore, more lignin will be removed and more cellulose was hydrolyzed into reducing sugar (Fatmawati et al., 2012).

3.5. Effect of time on the total reducing sugar (TRS)

As shown in Fig. 5, the TRS increased significantly to maximum of 94.596±0.7107 g/L at pretreatment time of 20 minutes. Sun and Tomkinson, (2002), states that the amount of sugar release was increased with the increasing of sonication duration, and thus decreased the crystallinity of cellulose and hence increased the amount of lignin removal. However, lengthening the sonication exceeding certain amount of time will cause the sugar to thermal degradation. This explains why TRS decreased slightly when pretreatment time was lengthen to 25 minutes.

3.6. Effect of sonication amplitude on the total reducing sugar (TRS)

Fig. 6 showed the highest amount of TRS, 78.79±1.0887 g/L was obtained at sonication amplitude of 50%. However, further increased in amplitude of 60% resulted in significant reduction of TRS to 42.941±0.2510 g/L.

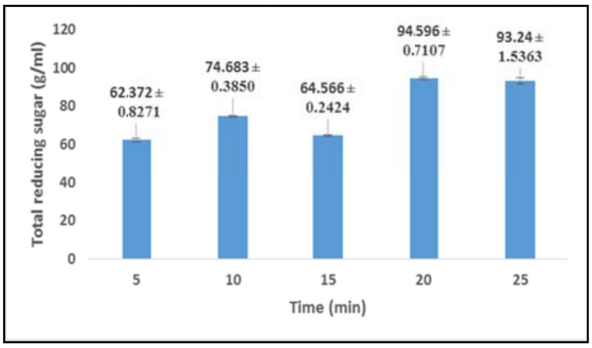

Fig. 5: Effect of time on the total reducing sugar (TRS)

Amplitude was affected by ultrasonic power used. By increasing the amplitude, the power input will increase too. Increased of amplitude of sonication has been related with the enhancement of sugar yield in other lignocellulose biomass, however there are also different findings reported. By lowering the amplitude of sonication, the protein and sugar yield on a per unit basis is greater compared to sonicate at higher amplitude. It is proposed that the sonication duration to be the main concern than the sonication amplitude (Rehman et al., 2013).

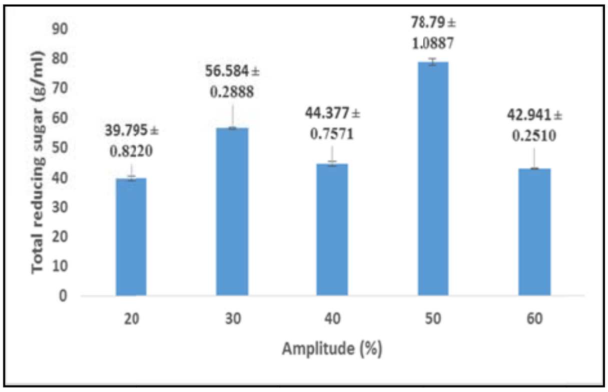

Fig. 6: Effect of amplitude on the total reducing sugar (TRS)

3.7. Characterization of Treated and Untreated Nipah Fruit Husks

Fig. 7 represents the SEM images of untreated nipah fruit husks. It can be observed that the structure of the tested sample was still intact and tightly bonded to each other. This due to the untreated sample consist higher amount of lignin that protects cellulose and hemicellulose from degradation. Thus, the chemical pretreatment steps were required for lignin removal in sugar recovery.

On the other hand, when nipah husks were pretreated, changes in the structure took place which can be seen in Fig. 8, 9, 10, 11 and 12. From the illustrated figures, it was shown that acid and alkali pretreatment together with ultrasound wave had altered the structure of the lignocellulose biomass. Fig. 8 displayed nipah fruit husk been significantly destroyed by the combination effect of H_2SO_4 and ultrasonic wave. Hemicellulose was hydrolyzed as the sample was mixed with dilute H_2SO_4 to form xylose and other sugars. Then, xylose was broken down to form furfural. Hemicellulose is removed when H_2SO_4 is added and this enhances digestibility of cellulose in the residual solids (Mosier et al., 2005).

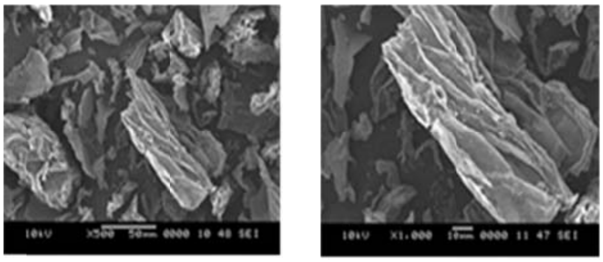

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Fig. 7: SEM images of untreated nipah fruit husks (a) Magnification 500X, (b) Magnification 1000X

However, when the nipah husks were pretreated with $Ca(OH)_2$, insignificant changes in structure was observed (Fig. 9). The lignin was not much being removed, resulting very low total reducing sugar produced as hemicellulose and cellulose were protected by lignin. Thus, indicates that crystallinity structure of nipah husks were not reduced much by the combined action of $Ca(OH)_2$ and ultrasonic wave.

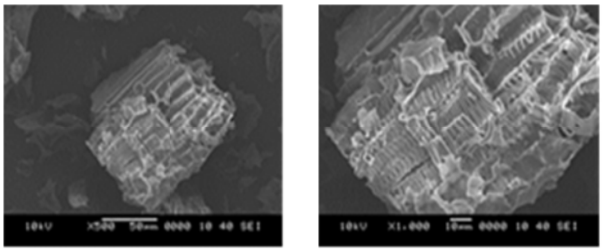

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Fig. 8: SEM images of nipah fruit husks pretreated with H_2SO_4 (a) Magnification 500X, (b) Magnification 1000X

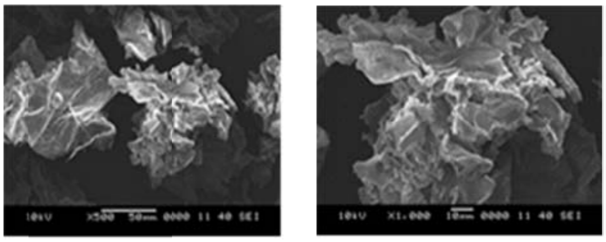

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Fig. 9: SEM images of nipah fruit husks pretreated with $Ca(OH)_2$ (a) Magnification 500X, (b) Magnification 1000X

As for HCl and $C_6H_8O_7$-pretreated nipah fruit husks, the porosity of the lignocellulose biomass were significant as shown by the presence of small holes on the surface of nipah husks (Fig. 10 and 11).

Nipah fruit husks pretreated with NaOH shown the greatest disruption in the structure among other samples (Fig. 12). NaOH solubilized and removed the lignin on the husk resulting the formation of pores structure (Iberahim et al., 2013).

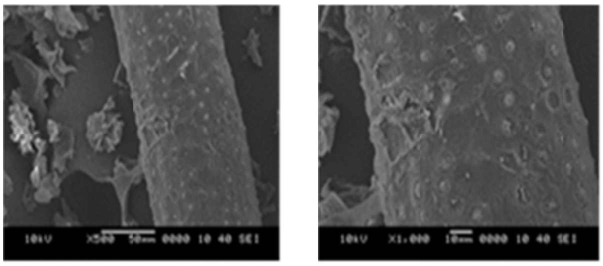

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Fig. 10: SEM images of nipah fruit husks pretreated with HCl (a) Magnification 500X, (b) Magnification 1000X

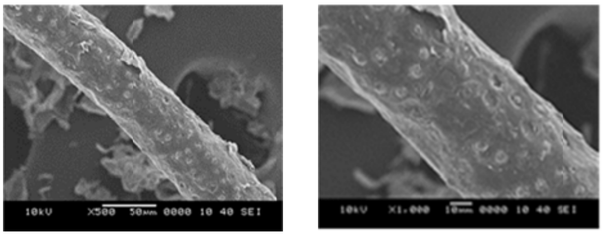

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Fig. 11: SEM images of nipah fruit husks pretreated with $C_6H_8O_7$ (a) Magnification 500X, (b) Magnification 1000X

Previous study mentioned that alkali-assisted ultrasonication resulted in defibration and fibrillation (Gabhane et al., 2014). Defibration is mainly caused by alkali action that removed lignin from lignocellulose and the ultrasonic wave that split cellulose fibers into fine fibrils (Fibrillation). In fact, Tang and Liang, (2000), stated that ultrasonication can crack the cell wall, dislocating the secondary wall of the middle layer and resulting in fibrillation.

Lignocellulosic materials such as nipah fruit husks have external and internal surface area. The external surface area is related to the size and shape of the particles, while the internal surface area depends on the capillary structure of cellulosic fiber. Swelling of lignocelluloses with water and polar solvents creates large internal surface area (Fan et al., 1980). Good swelling media such as water and NaOH will not only break the H-bonding, but also expose the fiber structure to increase the accessible surface area.

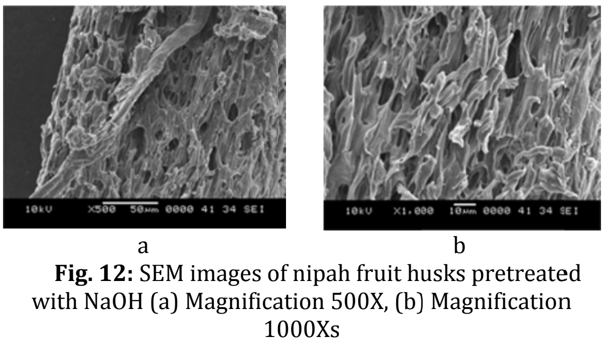

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Fig. 12: SEM images of nipah fruit husks pretreated with NaOH (a) Magnification 500X, (b) Magnification 1000Xs

Greater surface area allowed higher rate of reaction thus producing higher reducing sugars for bioethanol production (Parikh et al., 2007).

4. Conclusion

It can be concluded that pretreatment of nipah fruit husks with 40% w/v NaOH, LSR 10:1, sonication amplitude 50% at 80°C for 20 minutes produced the highest total reducing sugar (TRS), 78.79±1.0887 g/L. Alkali pretreatment is the most effective method to release fermentable sugars from the lignocellulose biomass with low lignin content. NaOH is one of the preferable solvent that can be used for alkaline pretreatment as compared to $Ca(OH)_2$. Besides that, screening results shown that concentration of solvent, liquid-to-solid ratio (LSR), temperature and time greatly influenced the pretreatment of nipah husks. SEM observations shown that different types of solvent gave different effect on the structure of lignocellulose biomass and sample pretreated with sodium hydroxide showed the greatest affect towards cell disruption of nipah fruit husks as compared to the other solvents.

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