Comparison of Milling Modes as a Pretreatment Method for Cellulosic Biofuel Production

Hyeon Jeong Kim, Jeong Ho Chang, Bong-Yong Jeong, and Jin Hyung Lee

Abstract—**Here we proposed clean and acceptable pretreatment methods for the pretreatment of lignlicellulosic biomass. To prove the feasibility of it, we compared three modes of mills such as ball, attrition and planetary mills. Among the mill modes, attrition and planetary mills are more effective to reduce biomass size when compared to ball mill. In enzymatic saccharification process, planetary mill treated crystalline cellulose produced higher amount of glucose and galactose than attrition mill treated. The mill pretreatments did not cause toxic compounds, such as hydroxymethylfurfuraldehyde (HMF) and levulinic acid. It was performed in enzymatic process buffer. Hence, it is not needed to exchange buffer for further process. It is convenient and economic process for cellulosic biomass pretreatment and can be applied to mass biofuel production.**

Index Terms—**Attrition mill, biofuel, cellulosic biomass, planetary mill, pretreatment.**

I. Introduction

Lignocellulosic biomass is a renewable resource that stored sunlight and carbon dioxide. It consists of cellulose, hemicelluloses and lignin. Lignocellulosic biomass contains 50~80% carbohydrates but the structural compositions of lignocelluloses are recalcitrant to direct use of the carbohydrates. Therefore, pretreatment step is necessary to exposure the cellulose to enzyme for hydrolysis. The aim of pretreatment is to remove lignin, reduce the crystallinity of cellulose. It was reported that pretreatment of lignocellulosic biomass has been viewed as one of the most expensive steps within biofuel production [1]. Various techniques have been developed for the pretreatment of lignocellulosic biomass using physical, chemical and biological [2]. One of most widely used method is soaking in aqueous ammonia (SAA) method [3]. This method is needed for high energy input due to high temperature. It causes almost half of xylan loss along with the lignin. Acid pretreatment uses the concentrated acid such as concentrated H_2SO_4 and HCl [4]. Even though concentrated acid method is effective for enhancing sugar production, the reagents used is strongly toxic and hazardous. Alternative dilute acid pretreatment was proposed by using dilute sulfuric acid [5], dilute phosphoric acid [6] and peracetic acid [7]. The diluted acids dissolve hemicelluloses and lignin, which enhanced enzymatic digestibility of cellulose. It would hydrolyze the oligomeric hemicellulosic saccharide into monosaccharides. However, it also causes the sugar degradation. The diluted acids are also corrosive, which is necessary expensive material container. It is also needed that the biomass samples should be neutralized after the pretreatment, which costs a lot of waters and produces toxic chemicals such as vanillin, ferulic acid and coumaric acid [8]. Alkaline pretreatment showed an excellent lignin removal of 87.5 % using dilute NaOH solution [9]. Alkaline pretreatment leads decreased crystallinity, increased internal surface area, disruption of the lignin structure and separation of lignin and carbohydrates [10]. Even though alkaline pretreatment uses lower temperatures and pressures, this process produces a large amount of salts during the pretreatment process, which is interrupt subsequent saccharification and fermentation processes.

Physical pretreatment does not cause toxic by-products during treatment process. However, most of physical pretreatments, for example steam-explosion, hydrothermal method, microwave heating, ultraviolet radiation and ultrasound, require high energy consumption [2]. Previously, ball mill was used to reduce the crystallinity of cellulose [11]. However, ball mill did not solely used for cellulose pretreatment but with dilute H_2SO_4 in this study.

Here, we proposed environmentally friendly pretreatment method that do not involve strong chemical and produce toxic compounds. This method was effective for enzymatic saccarification. Ball milling has a high energy requirement and is not economically feasible in general. To eliminate these disadvantages, we investigated the applicability of planetary and attrition mills to lignocellulosic biomass pretreatment. Those mills required low energy consumption and treatment time. Preliminary comparison of three mill modes was performed.

II. Materials and Methods

A. Materials and Experiment

Microcrystalline cellulose, monosaccharide standards (i.e., glucose, cellobiose, xylose, galactose and mannose) and acetic acid were purchased from Sigma-Aldrich. Cellulase cocktail purchased from Worthington Biochemical Co. Rice straw grown and harvested in KyeongBuk, Rep. Korea in 2011, was used in this study.

The mill treated samples were prepared using a laboratory ball mill (Nano in tech Co., Rep. Korea), attrition mill (Dea Wha Tech, Rep. Korea) and planetary mill (Fritsch Co., Germany). To prepare each sample, different weights of rice straw or cellulose were mixed with 200 mL of acetic acid

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buffer (pH 5.0) in the grinding cell and the mill operated in the operating condition according to the mill modes. A ZrO2 ball (mass of 1 kg and diameter of 3 mm or 5 mm) were loaded into a grinding cell.

Saccahrifications of samples were performed by adding 3mg/ml of cellulase for 72 hours at 50 ℃ as recommended by National Renewable Energy Laboratory on USA [12]. Before performing tests, rice straw was grinded by using home blender.

B. Characterization of Samples

Cellulose crystallinity was characterized by using X-ray Diffraction Method (XRD). XRD measurements were performed on a Rigaku D/max-RB powder diffractometer (Japan). The diffracted intensity of Cu K α radiation was measured in a 2θ range between 0˚ and 100˚.

Morphology observation was performed using microscope (Olympus, USA). Microstructures of cellulose or rice straw were observed using Fe-SEM (JSM-6700F, JEOL, Japan). The samples were coated with Pt on a Cressington Scientific Instruments 108 Auto Sputter Coater (Cranberry Tep., USA). The accelerating voltage for SEM images was 15 kV.

Production of monosaccharides was measured by High Performance Liquid Chromatography (Waters Co. , USA). For HPLC analysis, a Bio-Rad Aminex HPX-97H column and refractive index detector were used.

C. Data Analysis

Each test was performed at least twice and the results were similar in both tests. Shown are the typical responses seen.

III. Results and Discussion

A. Comparison of Three Mill Modes in Operating Principle

A ball mill is a typical grinder and cylindrical device used in grinding or mixing materials such as chemicals, ceramic raw materials and paints. The principle of ball mill function is that the ball mill rotates around a horizontal axis, partially filled with the material to be ground plus the grinding medium. When the ball moves, it break down materials (Fig 1 (a)). As increasing the speed of revolution per minute (rpm), theoretically the efficiency of grinding should be increased. However, the balls do not fall down after reaching to equilibrium of force between gravity of the balls and centrifugal force (Fig. 1 (a)).

Attrition mill is used for ultra-fine grinding. It comprises a grinding chamber and an axial impeller having a series of mainly radially directed grinding elements (Fig. 2 (b)). The grinding elements are approximately equally spaced along the impeller by a distance chosen to permit adequate circulation between the opposed faces of adjacent grinding elements. During the attrition mill, the balls are impacted with each other, causing fracturing the biomass to yield fines.

A planetary mill consists of at least one grinding jar which is arranged eccentrically on a so-called sun wheel. The direction of movement of the sun wheel is opposite to that of the grinding jars. The grinding balls in the grinding jars are subjected to superimposed rotational movements. The difference in speeds between the balls and grinding jars produces an interaction between frictional and impact forces which release high dynamic energies. These energies produce the effective degree of size reduction.

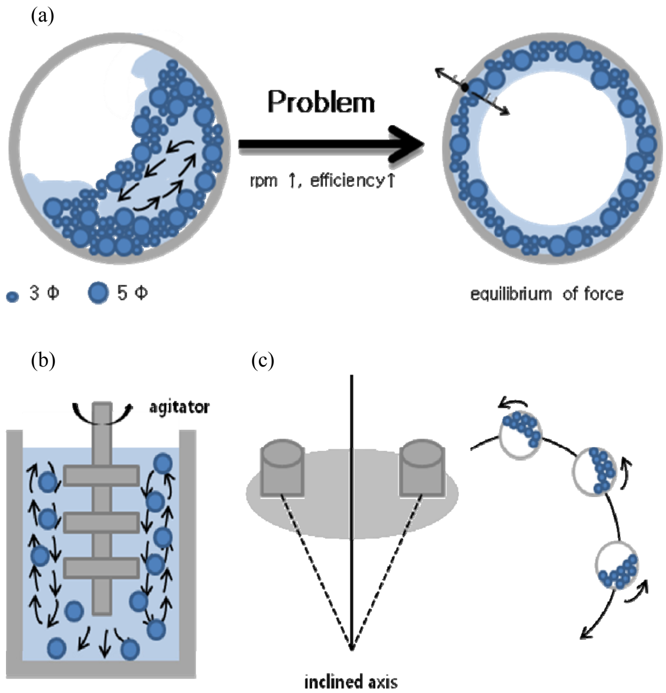


Fig. 1. Principles of three mill modes

B. Morphological Change

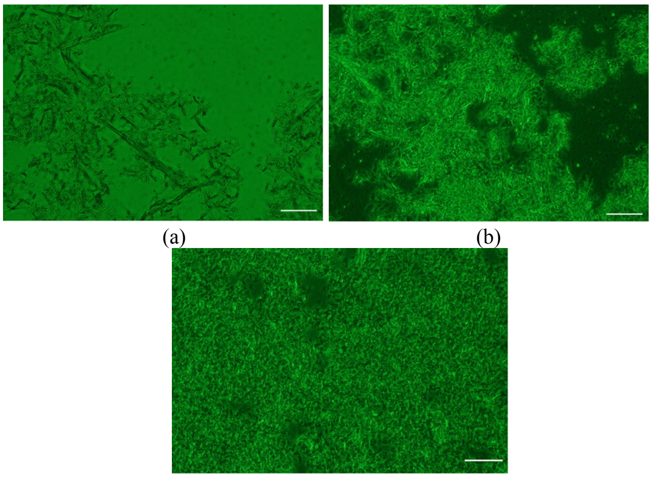
Fig. 2 represents the change of rice straw after planetary mill.

(a) (b)

Fig. 2. Rice straw before (a) and after planetary mill (b)

Rice straw was grinded by using home blender (Fig 2 (a)). Mill pretreatments were performed after adding the rice straw into enzymatic reaction buffer. Hence, it is not necessary to exchange the buffer for further processes. After the planetary mill pretreatment, the rice straw was completely dissolved into the buffer as shown in Fig. 2 (b).

Microscope images showed that attrition and planetary mills are more efficient to size reduction than ball mill (Fig 3). After ball mill treatment, there are still long shape particles found. However, the rice straws were broken into small piece of particles after attrition or planetary mills (Fig. 3 (b),(c)). Most of particles represent micron sizes. This result clear presents that the attrition and planetary mills are more effective to reduce size, which ultimately increase the accessibility of enzyme to biomass.



(a) (b)

(c)

Fig. 3. Microscope image after treating ball mill (a), attrition mill (b) and planetary mill (c). Scale bar indicates 50 μm.

C. Production of Monosaccharides

To evaluate the reactivity of pretreated biomass, crystalline cellulose hydrolysis was carried out with three mill modes. Previous study reported that the crystallinity and structure of the crystalline cellulose are not altered without pretreatment [11]. Diluted sulfuric acid (0.05 M) effectively decreases the crystallinity of cellulose. However, Hydrolysis occurs to only a small extent and produces hydroxymethylfurfuraldehyde (HMF) and levulinic acid, presumably via glucose formation followed by glucose degradation.

Here, we initially pretreated crystalline cellulose by ball, attrition and planetary mills. As shown in figure 4, attrition and planetary mills are more efficient to produce monosaccharide, typically glucose and galactose. When compared between attrition and planetary mills, the planetary mill showed a little bit higher sugar production. Glucose is metabolic energy source for most of microorganisms. It will be energy source for further fermentation process. Galactose also could be utilized in fermentation process as carbon source. Mannose and cellobiose is not directly utilized in fermentation source. Hence, it is recommended to reduce the production of mannose and cellobiose, which could be performed in further studies.

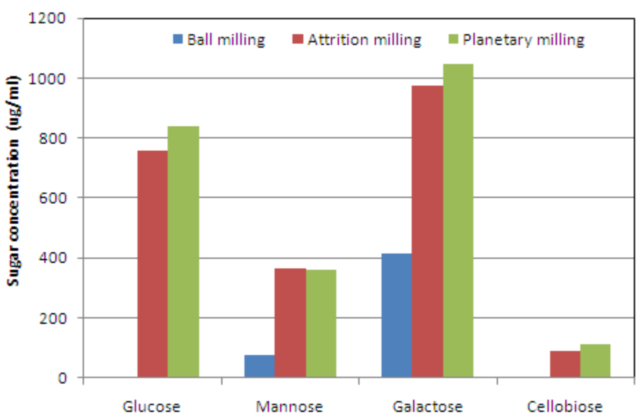


Fig. 4. Production of monosaccharides after enzymatic saccahrfication depending on pretreatment modes

IV. Conclusion

Mechanical mill techniques have several benefits prior to chemical pretreatment for biofuel production. First of all, it does not produce toxic compounds which improve saccharification and fermentation processes. It also uses same buffer with enzymatic process. Hence, it can reduce process time. Pretreatment methods currently used, such as chemical pretreatment, are not acceptable to mass biofuel production. However, the method proposed in this study can be applied to mass biofuel production since it is a clean and green process.

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References

[1] N. S. Mosier, C. Wyman, and B. Dale, "Features of promising technologies for pretreatment of lignocellulosic biomass," *Bioresour Technol*, vol. 96, pp. 673-686, 2005.

[2] Y. Zheng, Z. Pan, and R. Zhang, "Overview of biomass pretreatment for cellulosic ethanol production," *Int J Agric & Biol Eng*, vol. 2, pp. 51-68, 2009.

[3] T. H. Kim, F. Taylor, and K. B. Hicks, "Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment," *Bioresour Technol*, vol. 99, pp. 5694-5702, 2008.

[4] I. R. Goldstein, H. Pereira, and L. J. Pittman, "The hydrolysis of cellulose with superconcentrated hydrochloric acid," *Biotech Bioeng Symp*, vol. 13, pp. 17-25, 1983.

[5] D. J. Schell, J. Farmer, M. Newman, and J. D. McMillan, "Dilute-sulfuric acid pretreatment of corn stover in pilot-scale reactor," *Appl Biochem Biotechn*, vol. 105-108, pp. 69-85.

[6] D. L. Brink, "Method of treating biomass material," U.S. Patent vol. 5, no. 366, pp. 558, Nov 22, 1994.

[7] X. B. Zhao, L. Wang, and D. Liu, "Effect of several factors on peracetic acid pretreatment of sugarcane for enzymatic hydrolysis," *J Chem Technol Biotechnol,* vol. 82, pp. 1115-1121, 2007.

[8] S. Lee, A. K. Monnappa, and R. J. Mitchell, "Biological activities of lignin hydrolysate-related compounds," *BMB reports*, vol 45, pp. 265-274, 2012.

[9] S. Kim, M. T. Holtzapple, "Lime pretreatment and enzymatic hydrolysis of corn stover", *Bioresour Technol* vol 96, pp1994-2006, 2005.

[10] L. T. Fan, M. M. Gharpuray, and Y-H. Lee, *Cellulose Hydrolysis Biotechnology Monographs*, Berlin, Springer, pp. 57, 1987.

[11] H. Zhao, J. H. Kwak, Y. Wang, J. A. Franz, and J. M. White, J. E. Holladay, "Effects of crystallinity on dilute acid hydrolysis of cellulose by cellulose ball-milling study," *Energy & Fuels*, vol. 20, pp. 807-811, 2006.

[12] M. Selig, N. Weiss, and Y. Ji, "Enzymatic saccharification of lignocellulosic biomass," Technical Report NREL/TP-510-42629, Mar. 2008.

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