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Enzyme feeding strategies for better fed-batch enzymatic hydrolysis of empty fruit bunch

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HIGHLIGHTS

- It is important to decide enzyme feeding method for enzymatic hydrolysis.
- If enzymatic hydrolysis is run less than 40 h, whole enzyme feeding is preferable.
- If enzymatic hydrolysis is run more than 40 h, proportional enzyme feeding is preferable.

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ABSTRACT

Lignin inhibitory becomes a major obstacle for enzymatic hydrolysis of empty fruit bunch conducted in high solid loading. Since current technology required high enzyme loading, surfactant application could not effectively used since it is only efficient in low enzyme loading. In addition, it will increase final operation cost. Hence, another method namely "proportional enzyme feeding" was investigated in this paper. In this method, enzyme was added to reactor proportionally to substrate addition, different from conventional method ("whole enzyme feeding") where whole enzyme was added prior to hydrolysis process started. Proportional enzyme feeding could increase enzymatic digestibility and glucose concentration up to 26% and 12% respectively, compared to whole enzyme feeding for hydrolysis duration more than 40 h. If enzymatic hydrolysis was run less than 40 h (25% solid loading), whole enzyme feeding is preferable.

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1. Introduction

Renewable energy development has been attracting in the moment due to limitability of fossil fuels resources. One of them is lignocellulose based bioethanol that could substitute liquid fuels, principally gasoline, without reducing its quality (Park et al., 2013; Hoyer et al., 2010; Kristensen et al., 2009). Lignocellulose based biomass are complex material of cellulose, hemicellulose, and lignin. One of them was empty fruit bunch (EFB) that is not only abundant and cheap (Park et al., 2013; Han et al., 2011) but also does not compete with food supply.

Lignocellulose based bioethanol technology is potential today, however it is still facing several obstacles for commercialization. Enzymatic hydrolysis has been reported to be the bottleneck of this technology (Eriksson et al., 2002). The major obstacles on this process are high price of enzymes (Lozano et al., 2014; Xue et al., 2012; Gnansounou and Dauriat, 2010; Qing et al., 2010), high consumption of enzymes (Lu et al., 2002) long time incubation (Lu et al., 2002), inhibitions of lignin (Qi et al., 2011; Qing et al., 2010; Lu et al., 2002; Mes-Hartree et al., 1986) and inhibition of glucose/product (Wanderley et al., 2013; Xue et al., 2012; Mes-Hartree et al., 1986). However, many ideas had been employed to solve these problems, like fed-batch operation (Wanderley et al., 2013; Zhao et al., 2013; Modenbach and Nokes, 2013; Gupta et al., 2012), simultaneous saccharification and fermentation/SSF (Paulova et al., 2014; Park et al., 2013), enzyme recycling (Xue et al., 2012; Qi et al., 2011; Lu et al., 2002), and membrane separation (Lozano et al., 2014; Yang et al., 2009).

Low sugar concentration in hydrolysis product is undesired (Zhao et al., 2013; Gupta et al., 2012). Therefore, high solid loading operation is favored for higher concentration of glucose (Cui et al., 2014; Pihlajaniemi et al., 2014; Modenbach and Nokes, 2013; Gupta et al., 2012). High solid loading operation refers to enzymatic hydrolysis process that operated with solid loading

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exceeds 16% (Pihlajaniemi et al., 2014). Consequently, lignin and other solid residue accumulation in the reactor became major challenge in this system, as been reported by Gupta et al. (2012). Fed-batch methods has been conducted to solve this problem but lignin inhibitory effect still could not be neglected. Usage of surfactant was proposed by several preceding researchers to prevent unproductive binding between enzyme and lignin through adsorption of surfactant on lignin surfaces. Eriksson et al. (2002) conducted enzymatic hydrolysis for steam pretreated spruce with application of various kinds of surfactant and found that Tween type surfactant is the most suitable for the process because it is non-toxic. Positive effect of polyethylene glycol for enzymatic hydrolysis of steam pretreated spruce was reported by Sipos et al. (2010). Same phenomena were also observed on enzymatic hydrolysis of acid treated corn stover (Qing et al., 2010) and softwood pulp (Xue et al., 2012) with the addition of Tween 80. Unfortunately, the significant effect of surfactant was only found at low dosage of enzyme (Eriksson et al., 2002). Qing et al. (2010) stated that it was only effective while enzyme dosage did not exceed 10 FPU/gram biomass. In contrast, current hydrolysis technology required enzymes dosage more than 10 FPU/gram. Han et al. (2011) found that the best enzyme dosage for empty fruit bunch enzymatic hydrolysis process in batch mode was 50 FPU/gram cellulose. However, Park et al. (2013) proved that 15 FPU/gram biomass gave the optimum performance for fed batch simultaneous saccharification and fermentation process. So, surfactant application was not quite suitable for recent enzymatic hydrolysis.

Enzyme feeding strategy could improve the performance of enzymatic hydrolysis by reducing the deactivation of enzyme. Adding extra enzyme proportional to the substrate fed (namely "proportional enzyme feeding") could replace "whole enzyme feeding" where all required enzyme was initially added. The term proportional and whole enzyme feeding was proposed by Modenbach and Nokes (2013). Proportional enzyme feeding was conducted by Hoyer et al. (2010) for simultaneous saccharification and fermentation of spruce. This proportional enzyme feeding gave slightly higher ethanol produced. Better hydrolysis process for corn cobs through fed-batch substrate and proportional enzymes feeding was also reported by Cui et al. (2014).

The aim of this paper is evaluate the performance of both proportional and whole enzyme feeding for enzymatic hydrolysis of empty fruit bunch.

2. Materials and methods

2.1. Empty fruit bunch

Empty fruit bunch (EFB) was obtained from palm plantation in Sumatra Island, Indonesia. Based on NREL analysis, composition of this substrate (% w/w) is 32.47% cellulose, 29% hemicellulose, and 21.19% lignin. Prior to processing, the empty fruit bunch was milled and sieved.

2.2. Steam explosion pre-treatment

Pretreatment process conducted in these experiments was combination of alkali pretreatment and steam explosion. The empty fruit bunch was pretreated with 1.5 M sodium hydroxide for a half of hour and temperature of 180 °C followed by steam explosion. The pretreatment process was held in a metal reactor with volume of 5 L.

2.3. Enzymatic hydrolysis

Enzyme used in these experiments was Cellic CTec 2 with 185 FPU/ml activity which purchased from Novozymes. Enzymatic hydrolysis process was held in 100 mL Schott-Duran bottle with 30 mL working volume, while temperature of reaction was maintain at 50 °C inside shaker incubator. The experiments were conducted with 70 FPU/gram biomass of enzyme and 150 rpm mixing speed. Total solid loading of EFB for this system was 45% w/w. These substrates were fed into the system in fed-batch mode and the last feeding process took place in the 54th hour.

The enzyme feeding in this experiment was conducted in two different methods, namely whole enzyme feeding and proportional enzyme feeding. In whole enzyme feeding, all enzymes required for entire substrate was loaded initially while in proportional enzyme feeding, enzyme was added proportionally with the substrate feeding.

2.4. Analysis method

2.4.1. Lignocellulose composition analysis

Lignocellulose composition (cellulose, hemicellulose, and lignin) was analyzed by NREL method. The reference of this method was Technical Report "NREL/TP-510-42618 Revised August 2012". This method used acid hydrolysis with sulfuric acid (H_2SO_4). First, empty fruit bunch was soaked in concentrated acid for one hour. Then, the acid solution was diluted into 4% v/v. After dilution, sample was autoclaved with pressure and temperature of 2 bar and 121 °C respectively for two hours. Autoclaved sample was filtered to separate solid and hydrolysis liquid. Solid sample was used to analyze acid insoluble lignin by gravimetric analysis and ashing method while liquid one used to analyze acid soluble lignin (through spectrophotometric method), cellulose, and hemicellulose (through chromatographic method).

2.4.2. Glucose concentration analysis

Product of the hydrolysis process was analyzed using High Performance Liquid Chromatography (HPLC) series Waters 1515 with refractive index detector to determine glucose concentration. Column used in this HPLC was HPX87H. Details of operation method of this HPLC were: 5 mM H_2SO_4 as the carrier and 0.6 ml/min dilution rate. Running time of each sample was 30 min.

2.4.3. Enzymatic digestibility calculation

Enzymatic digestibility (ED) calculation is calculated using following equation:

$$\text{Enzymatic digestibility } (\%) = \frac{C_{\text{glucose}} \cdot V_{\text{hydrolysis}} \cdot 0.9}{M_{\text{cellulose}}} * 100\% \quad (1)$$

where C_{glucose} is glucose concentration on calculation hour (gram/liter), $V_{\text{hydrolysis}}$ is remaining hydrolysis volume on the calculation hour (liter), $M_{\text{cellulose}}$ is initial cellulose mass (gram).

3. Results and discussion

3.1. Steam explosion treated empty fruit bunch

The goal of pretreatment process is to alter the structure of biomass so it more accessible for enzyme. Pretreatment process would break down lignin structure and destruct the crystalline structure of cellulose (Mosier et al., 2005). In case of agricultural waste, such as EFB, alkali pre-treatment is more effective (Han et al., 2011). Fig. 1 showed the composition of both fresh and alkali treated EFB.

From Fig. 1, hemicellulose loss and lignin removal were observed in alkali treated EFB samples, as indicated by decreasing composition for both compounds. In addition, cellulose composition in treated EFB was increased to 68% of solid content. Lignin removal which was desired from this process, was also reported

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