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Improving Saccharification of Oil Palm Shell by Acetic Acid Pretreatment for Biofuel Production

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Abstract

Lignocellulosic biomass is utilized as raw materials for biofuel and electricity production in different ways. The major bottleneck of biofuel production from lignocellulosic biomass is the saccharification to produce small molecules of sugars that subsequently converted to value added compounds. Pretreatment is performed before saccharification to enhance conversion rate. In this study, acetic acid was selected to pretreat oil palm shell to enhance the production of reducing sugars in enzymatic saccharification reaction. To obtain the maximum sugars, Response Surface Methodology (RSM) with Box-Behnken design was applied in the pretreatment. Three variable factors, including reaction time, reaction temperature and acetic acid concentration were varied with three levels. The generated model has high correlation coefficient (R^2) at 0.9725 indicating the high confident level. Based on the model, the maximum reducing sugars at 40.408 mg/g-biomass could be obtained at optimum condition (107.30 ^{o}C reaction temperature, 30 min reaction time and 8.24% acid concentration). The results of this study suggested the benefit of using acetic acid for improvement of saccharification of lignocellulosic biomass to biofuel.

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Keywords: Acetic acid pretreatment ; Lignocellulosic biomass ; Saccharification ; Oil palm shell ; Response Surface Methodology ; Biofuel

1. Introduction

Lignocellulosic biomass has been utilized as raw materials for biofuel production worldwide due to its availability. Oil palm shells were obtained in large amounts in Thailand as agricultural residues from biodiesel production industries and currently they were mainly burned in the industrial boiler for electricity production creating pollution to environment. Oil palm shell is lignocellulose that consists of cellulose, hemicellulose and lignin with different proportion depending on the types of plants [1]. Hydrolysis of lignocellulose biomass creates small

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sugar molecules that, subsequently, were converted to various forms of products including biofuels. However, the major limiting problem of biofuel production from lignocelluloses is the resistance to hydrolysis [2].

Different types of pretreatments including chemical, physical, biological or combined methods were developed for decades to modify biomass to be more susceptible to enzymatic or microbial degradation [2-5]. Considering to the cost, inorganic acid and alkaline, for example, sodium hydroxide, sulfuric acid and hydrochloric acid are the most common chemicals for pretreatment. However, using these strong acids can cause corrosion to the equipments, and sometime hydrolysis inhibitors are generated as by-products [6]. Organic acid pretreatment, as weak acid, could be selected as an alternative option, although its efficiency was demonstrated to be relatively less [7-8], however, it generates lower amounts of inhibitors than inorganic acid pretreatment [9].

This study aimed to study the effect of acetic acid pretreatment to improve enzymatic saccharification of oil palm shell using Response Surface Methodology (RSM). The generated model from RSM created predicted optimum pretreatment condition that allows to achieve the maximum amounts of reducing sugars from biomass and to demonstrate the impact of each pretreatment factor on the pretreatment efficiency.

2. Materials and methods

2.1. Pretreatment experiment with response surface methodology

Oil palm shell collected from a local farm in Ayutthaya province, Thailand was dried in hot air oven at 80 ^{o}C until the constant weight was achieved. The experiments were designed based on RSM with Box-Behnken experimental design using Design-Expert software (version 7.0.0 Stat-Ease, USA). The pretreatment parameters were (i) pretreatment temperature (X_1: 100 – 140 °C), (ii) reaction time (X_2: 30–60 min) and (iii) acetic acid concentration (X_3: 5–15% (w/w)). Each tested parameter was varied with three levels (max, mid, min). The amounts of reducing sugars were measured as the response parameters (Y).

Each pretreatment experiment was performed according to the designed Table 1. Dried oil palm shell was cut and ground to smaller pieces and sieved through a 20-mesh aluminium sieve. The 10% (w/w) of oil palm shell was mixed with acetic acid solution in a screw-capped bottle with a total volume of 50 ml. The samples were then heated in a hot air oven with the set parameters of temperature and time based on RSM experimental design. After pretreatment, solid residue was separated by filtration using Whatman No.1 filter paper and washed with deionized water for three times. Samples were dried at 60 °C until constant weight was achieved, and kept in the sealed bags until used in subsequent enzymatic hydrolysis.

2.2. Enzymatic saccharification

Pretreated oil palm shell was enzymatic hydrolyzed by mixing 0.5 g sample with 20 FPU of Celluclast 1.5 L (20 FPU) (Sigma, USA, *Trichoderma reesei* ATCC 26921) and 100 CMU of Cellobiase (Megazyme, *Aspergillus niger*) in a screw-capped plastic tube containing 20 ml of 50 mM sodium citrate buffer (pH 4.7). The reaction was incubated at 45 °C, 200 rpm for 72 h in a shaking incubator. The amount of reducing sugars of hydrolysate was determined by using the 3,5-dinitrosalisylic acid (DNS) method [10]. Pretreatment efficiency of each condition was determined based on the amounts of reducing sugars obtained from enzymatic hydrolysis reaction.

3. Results and discussion

In this study, total three pretreatment factors, including reaction temperature (X_1), reaction time (X_2), and acetic acid concentration (X_3) were varied to three different levels, based on RSM with Box-Behnken design. Dried oil palm shell sample was pretreated as designed Table 1 and the pretreated sample was hydrolyzed. The reducing sugar in each hydrolysate was quantitated and collected as a response factor (Y). Then the correlation of pretreament factor and response factor was analyzed to fit with the polynomial regression model. The significant pretreatment factors were identified by ANOVA analysis and the insignificant factors were removed with P-value less than 0.1 Table 2. In this study the factors X_1X_2 and X_1^2 were significantly affect the Y value as they all have P-value less than 0.1 (Table 2). The final mathematical models (Equation 1) representing the relationship between each pretreatment parameters on concentration of reducing sugars were generated with high confidence of statistical analysis (P-value = 0.0882, R^2 =0.9725).