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Optimization of alkaline peroxide pretreatment of rice straw

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Abstract

Lignocellulose represents a promising starting material for conversion to fuels and chemicals in biorefinery; however, its efficient conversion to sugar requires a prerequisite pretreatment step. In the present research, the pretreatment of rice straw by alkaline peroxide pretreatment was studied aiming to enhance enzymatic digestibility of the cellulose-enriched solid. The alkaline hydrogen peroxide (AHP) pretreatment of rice straw using different concentration of hydrogen peroxide, reaction time, temperature, and solid loading were evaluated based on enzymatic digestibility and sugar recovery. The highest reducing sugar recovery was in the range of 499±6 mg/g native biomass after pretreatment using 2.5% H_2O_2 (v/v) at 35 ºC for 24 h and 7.5% biomass loading (w/v) after enzymatic hydrolysis using 20 FPU/g Accellerase® 1500. The work demonstrates the potential of the developed process for low temperature pretreatment of biomass for further conversion to value-added products.

Keywords: rice straw, biorefinery, alkaline peroxide pretreatment

1. Introduction

Nowadays, world faces the energy and environmental crisis due to the depletion of petroleum resources which results in the increasing oil price. This leads to public concerns on adequacy of long term energy supply and also environmental problems due to greenhouse gas release. Biofuel, particular bioethanol is a widely used alternative fuel in many countries which can be produced from renewable resources. Due to controversy on food vs. fuel issue, research on bioethanol production from alternative underused biomass is thus of great interest [1].

Utilization of renewable lignocellulosic materials as feedstock in biorefineries is considered a sustainable approach for production of biofuel and commodity chemicals. Moreover, the uses of agricultural by-products also has benefits on disposal of problematic solid wastes and provides opportunity to improve energy security, reduce the trade deficit, and decrease greenhouse gas emissions, which in overall improve value of agricultural by-products [2]. Lignocellulose materials consist of three primary chemical components: (a) cellulose, a linear homopolymer of glucose which forms into organized microfibril structure, (b) hemicellulose, amorphous heterogenouspolymer of pentoses, hexoses, and sugar acids, and (c) lignin, a complex polymer of phenolic compounds which provides shields to external physical, chemical and biological stresses, in addition to other minor components such as ash, and a small amount of proteins.

Conversion of lignocellulosic materials into fermentable sugars by enzymatic hydrolysis is an important step for further conversion to biorefinery products by fermentation or catalytic routes. Lignocellulosic materials is highly recalcitrant due to its rigid structure, particular by lignin which acts as physical barrier against hydrolytic enzyme penetration to the biomass microstructure. An effective pretreatment step is required in order to release the carbohydrates from lignin association which will lead to the increasing of fermentable sugars from the enzymatic hydrolysis step [3]. Different pretreatment methods, including physical, chemical, thermal and biological approaches are used to improve the availability of cellulose for enzymatic hydrolysis. Each pretreatment has its own effects on the cellulose, hemicellulose, and lignin fractions, which result in increased digestibility of plant biomass [4]. These include removal of hemicellulose and lignin, inacrese in biomass accessiable surface area, and decrytallization of cellulose.

Alkaline peroxide provides an effective method for delignification and is considered a promising approach for low-energy pretreatment of lignocelluloses under mild conditions [5]. Principle of this method relies on formation of hydroperoxide anion formed in alkaline media which acts as active species in hydrogen peroxide bleaching systems. Decomposition products such as hydroxyl radicals and superoxide anion radicals cause

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oxidation of lignin structures, which leads to the introduction of carbonyl group, cleavage of some interunit bonds and eventually, the dissolution of lignin and hemicelluloses [6].

In order to develop an effective low-energy pretreatment process, the work in this study focuses on optimization of alkaline hydrogen peroxide pretreatment on rice straw, one of the most potent feedstocks for biorefinery in Thailand and many tropical countries. Effects of hydrogen peroxide concentration, reaction time, pretreatment temperature, and biomass loading were evaluated. The work will provide an alternative method for pretreatment of rice straw for its further conversion to biorefinery products in industry.

2. Material and methods

2.1 Materials

Rice straw (*Oryza sativa*) was obtained from a paddy field in Pathumthani province. It was milled by cutting mill and sieved through a 0.5 mm size screen. The physically processed rice straw was dried in a hot air oven at 70 ^{0}C for overnight and stored in plastic bag at room temperature before use.

2.2 Alkaline peroxide pretreatment

The milled rice straw was pretreated by alkaline hydrogen peroxide. The effects of the following parameters on the pretreatment of rice straw were evaluated: hydrogen peroxide concentration (2.5-7.5% v/v), rice straw loading (5-10% w/v), pretreatment temperature (25-45 ^{0}C) and incubation time (6-24 h). The hydrogen peroxide solution was adjusted to pH 11.5 using NaOH. The pretreatment reaction with a total volume 10 ml was conducted in an incubator with no shaking. After the pretreatment, the solid and liquid fractions were separated by filtration. The solid fractions were washed with distilled water until neutral and dried in an oven at 70 ^{0}C overnight before use as the substrate for subsequent study.

2.3 Enzymatic saccharification

The pretreated biomass was hydrolyzed by Accellerase®1500 (Danisco, Rochester, NY) at 20 FPU/g at the initial sample loading of 5% (w/v) in 50 mM sodium citrate buffer pH 4.8 containing 50 µl of 5% sodium azide. The 1 ml slurries of hydrolysis reactions were incubated at 50 ^{0}C and shaking at 30 rpm for 72 hours. The amount of reducing sugars released was analyzed by dinitrosalicylic acid (DNS) method [Miller et al. 1959].

2.4 Physical analysis of pretreated biomass

The morphology and the physical structure of the untreated rice straw and the rice straw pretreated by alkaline peroxide were observed by scanning electron microscopy. The structure and morphology of the pretreated biomass was analyzed by scanning electron microscope (SEM) using a JSM-6301F Scanning Electron Microscope (JEOL, Tokyo, Japan). An electron beam energy of 5 kV was used for analysis.

3. Results and discussion

Rice straw used in this study containd 38.1±0.4% cellulose, 21.4±0.4% hemicellulose and 26.7± 0.4% lignin which made up the total carbohydrate content of 59.5±1.0%. This make a unique characteristic of rice straw for bioprocessing in sugar platform biorefinery.

Pretreatment of rice straw using hydrogen peroxide in the presence of alkaline catalyst led to increasing reducing sugar yield from enzymatic hydrolysis compared to the native rice straw that is highly recalcitrant toenzymatic attack. The biomass loss physical and morphological integrity due to delignification reaction and disintegrated into small, highly dispersed fibers owing to the attack of hydroxyl (HO•) and superoxide radical anions (O_2-), by decomposition of the hydrogen peroxide, which can oxidize and degrade lignin. The oxidized lignin fragments was then dissolved in the liquid phase and resulted in increased digestibility of the solid fraction the effects of parameters in alkaline peroxide pretreatment (oxidant concentration, temperature, residence time, and solid loading) was evaluated by determining the amounts of released reducing sugars from enzymatic hydrolysis of the solid residues (Table 1). The increase inreducing sugar yield from pretreated rice straw was observed markedly increased compared to reducing sugar from the untreated rice straw (155 mg per g native rice straw). The highest reducing sugar yield of 499 mg/g native biomass was obtained using 7.5% biomass loading (w/v) treated with 2.5% H_2O_2 (v/v) at 35^o C for 24 h and hydrolysed with Accellerase® 1500. This was equivalent to 69.1% recovery of available glucan in the native rice straw. This pretreatment condition was used as the initial optimal pretreatment conditions for subsequent study on the effects

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Table 1. Effects of alkaline peroxide pretreatment of rice straw at different hydrohen peroxide concentration, pretreatment temperature, residence time, and solid loading.

Run	Condition				Weight loss (%)	Reducing sugar (mg/g pretreated rice straw)	Reducing sugar (mg/g native rice straw)
	Concentration of H_2O_2 (%v/v)	Temp (^oC)	Time (h)	Solid loading (%w/v)			
1	2.5	25	18	7.5	56	799	461
2	2.5	35	6	7.5	44	873	410
3	2.5	35	18	5	53	881	468
4	2.5	35	18	10	53	662	436
5	2.5	35	24	7.5	49	855	499
6	2.5	45	18	7.5	50	849	463
7	5	25	6	7.5	47	821	435
8	5	25	18	5	47	884	444
9	5	25	18	10	49	888	417
10	5	25	24	7.5	61	848	424
11	5	35	6	5	50	846	474
12	5	35	6	10	49	905	471
13	5	35	18	7.5	51	903	439
14	5	35	18	7.5	55	849	425
15	5	35	18	7.5	34	916	471
16	5	35	18	7.5	51	879	426
17	5	35	18	7.5	50	914	453
18	5	35	24	5	48	903	442
19	5	35	24	10	51	912	462
20	5	45	6	7.5	51	840	432
21	5	45	18	5	58	987	465
22	5	45	18	10	53	978	482
23	5	45	24	7.5	52	931	459
24	7.5	25	18	7.5	42	889	414
25	7.5	35	6	7.5	42	769	472
26	7.5	35	18	5	46	984	416
27	7.5	35	18	10	50	933	443
28	7.5	35	24	7.5	53	927	410
29	7.5	45	18	7.5	51	933	420
Untreated	-	-	-	-	-	155	155

The effects of pretreatment parameters which are hydrogen peroxide concentration (1.25, 2.5, 5, and 7.5%, v/v), pretreatment temperature (25, 35, 45, and 55^{o}C), reaction time (6, 12, 18, and 24 h), and solid loading (2.5, 5, 7.5, and 10%, w/v) were then evaluated by varying one factor at a time while other parameters were fixed under the initial optimal conditions. Increasing hydrogen peroxide concentration led to increasing sugar yield (Fig. 1A). Approximately > 85% of the maximal sugar yield was achieved using 2.5% w/w H_2O_2. The highest reducing sugar of 527 mg/g native rice straw at 55^{o}C; however no significant effect of temperature was observed (Fig. 1B). This could be due to the exothermal nature of the pretreatment reaction. Pretreatment time led to slight increasing trend of reducing sugar yield. Majority of the reducing sugar was released during the first 6 h (Fig. 1C). The maximal reducing sugar yield of 470 mg/g was obtained after 24 h pretreatment. Decreasing reducing sugar yield was found with increasing solid loading from 2.5-10% (w/v) with the highest sugar yield of 517 mg/g using the solid loading of 2.5% (Fig. 1D). The reducing sugar yield achieved in this study was comparable to those obtained using different pretreatment methods.

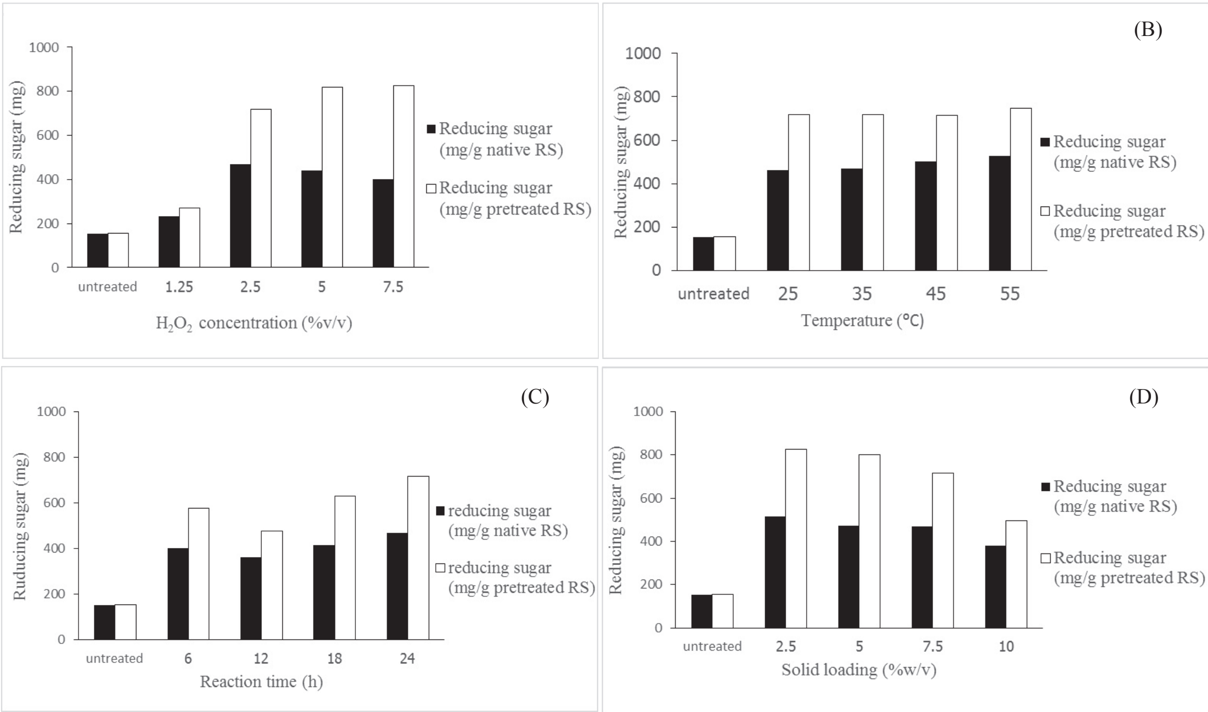

Figure 1. Effects of reaction parameters on alkaline hydrogen peroxide pretreatment of rice straw. (A) H2O2 concentration; (B) tempearure; (C) reaction time; (D) solid loading. The reaction parameter of interest was varied while other reaction parameters were fixed under the initial optimal conditions (biomass was pretreated under the optimal pretreatment conditions (7.5% v/v H_2O_2, solid loading 2.5% at 35^{o}C for 24 h)

4. Conclusion

Alkaline peroxide pretreatment significantly improved the efficiency of enzyme hydrolysis of rice straw to fermentable sugars. From the results, the maximal sugar recovery of 526.9% from the native biomass was obtained under the optimal pretreatment conditions (2.5% H_2O_2 (v/v), 55 ^{o}C, 24 h and 7.5% biomass loading (w/v)). This study provides an efficient pretreatment method with high efficiency under mild operating conditions for pretreatment of rice straw in sugar platform biorefinery.

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