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Sugarcane leaves: Pretreatment and ethanol fermentation by *Saccharomyces cerevisiae*

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ABSTRACT

The susceptibility of sugarcane leaves to cellulase hydrolysis by GC220 cellulase (10 filter paper units (FPU)/gram dry weight basis (g, DS)) for 72 h was compared after pretreatment by autoclaving at 121 °C, 15 pounds per square inch (lb/in^2) in the presence of either dilute sulfuric acid or lime. The optimal (from those evaluated) conditions for each type of pretreatment of green sugarcane leaves (20–40 mesh particle) at 6% weight by volume (w/v, DS), were found to be 1.5% (w/v) sulfuric acid and 2% (w/v) calcium hydroxide for 30 and 15 min, respectively. The acid pretreated samples released more glucose than the lime treated ones (5.7% and 3.9% weight by weight (w/w, DS), respectively). Accellerase™ 1000 hydrolysis (160 FPU/g, DS) of the dilute sulfuric acid pretreated ground sugarcane leaves suspended in 0.5 M citrate buffer, or in pretreatment hydrolysate yielded 0.104 and 0.163 g glucose/g (DS) of leaves after 6 h, respectively. Fermentation of the two above obtained glucose sources by *Saccharomyces cerevisiae* for 12 and 24 h, respectively, yielded ethanol at 4.8% and 8.0% (w/w, DS), respectively.

1. Introduction

Sugarcane (*Saccharum officinarum*), an economically important plant of several countries including Thailand, is cultivated in about 10.7 thousand million square-meters (6.7 million rais) in Thailand [1]. Harvesting of sugarcane is difficult because the leaves are sharp. Therefore, removal of the leaves by burning before cutting the sugarcane shoot is a popular practice, but this causes serious air pollution. Moreover, the price of the resultant sugarcane shoot is reduced. Value addition to sugarcane leaves would be likely to stop or to reduce the practice of leaf burning as well as to improve the environmental and economic efficiency of sugarcane agriculture.

Due to the depletion of the non-renewable fossil fuel supplies, and a shortage of it in the near future, searching for renewable practical alternative energy supplies is important and necessary. Ethanol that is produced from waste agricultural products by microbial activity is one potential alternative energy source for many countries, including Thailand. Each year sugarcane cultivations generate 18 million tons of leaves [2,3], which are currently a waste product. As a lignocellulosic biomass that is composed of three major components

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(cellulose, hemicellulose and lignin), sugarcane leaves are a potential source of biofuels including production of bioethanol. Hydrolysis of the cellulose and hemicellulose components results in the formation of the fermentable monosaccharides, glucose and xylose. However, prior to the ability to saccharify the cellulose content of the sugarcane leaves, in order to use it as raw material for ethanol production, the cellulose must first be unshielded from the hemicellulose and lignin, and so made accessible to the cellulase enzyme. Such pretreatment processing of the lignocellulosic biomass can be achieved by physical methods, such as heat or pressure treatment, and by chemical methods, such as acid or alkaline treatment. Acid pretreatment improves the subsequent cellulase hydrolysis through removal of hemicellulose and lignin, but has the disadvantage of that it can lead to the formation of inhibitors of the growth and ethanol fermentation ability of the subsequently utilized ethanol-fermenting microorganism(s). These inhibitors are principally furfural, hydroxymethylfurfural, 4-hydrobenzaldehyde, syringaldehyde, organic acids and other (volatile) products. The use of dilute acid in the pretreatment generates a lower concentration of these inhibitors [4]. Alkaline pretreatment causes the swelling of the lignocellulosic structure, and so leads to an increase in the internal surface area of the cellulose, and an increase in the amorphous cellulose proportion that is more susceptible to cellulase hydrolysis than the crystalline cellulose, a disruption of the structural linkage between lignin and carbohydrates, and a disruption of the lignin structure [5]. Recently, interest in lime (calcium hydroxide) pretreatment has increased because lime is recyclable and generates a low amount of inhibitory compounds [6–8].

Sugarcane leaves have been used as a substrate for ethanol production by the simultaneous saccharification and fermentation method using *Saccharomyces cerevisiae* [9] and the thermotolerant yeast, *Kluyveromyces fragilis* [10]. The leaves were pretreated with sodium hydroxide or with alkaline hydrogen peroxide and then the pretreated residues were subject to enzymatic hydrolysis. The obtained hydrolysate was then supplemented with nutrients prior to fermentation to ethanol. Dawson and Boopathy [11] also pretreated sugarcane leaves with alkaline hydrogen peroxide or with dilute sulfuric acid, but they then fermented the pretreated sugarcane leaves to ethanol directly, without saccharification by a cellulolytic enzyme using *S. cerevisiae* ATCC 576, a yeast strain that is known for its ability to produce ethanol from cellulosic materials.

In this work, the susceptibility of ground dried green sugarcane leaf particles, derived from the green leaves and hereafter referred to simply as sugarcane leaves, pretreated by dilute sulfuric acid or lime to subsequent cellulase hydrolysis was compared. Then, sugarcane leaves that had been pretreated with dilute sulfuric acid under conditions that generated a low amount of inhibitory compounds were saccharified by cellulase. The impact of the dissolved polysaccharides in the pretreatment hydrolysate on the subsequent glucose yield of the saccharification step was evaluated by performing the saccharification in two separate methods. In the first method the pretreated sugarcane leaves were separated from the pretreatment hydrolysate, and then they were suspended in 0.5 M citrate buffer (pH 5.0) and hydrolysed by cellulase. In the second approach, the pretreated sugarcane leaves suspended in the pretreatment hydrolysate, as a pretreatment slurry, were hydrolysed by the direct addition of cellulase into the pretreatment slurry. After saccharification, the resultant released glucose was fermented to ethanol by *S. cerevisiae* without any nutrient supplementation, and the ethanol yield and fermentation period of glucose obtained from each saccharification method were compared.

2. Materials and methods

2.1. Sugarcane leaves

Green sugarcane leaves were collected from cultivation sites at Nakhon Ratchasima province, Thailand, and were then cut, dried at 60 °C, Hammer milled and then sieved to a 20–40 mesh particle size. Their major component was found to be cellulose 38.5%, hemicellulose 23% and lignin 15.6% weight for weight (w/w) on a dry weight basis (DS) (see Section 2.5). The composition result agrees well with Krishnan et al. [3] who reported that sugarcane leaves were composed of glycan 35.3%, xylan 23% and lignin 19.6% (w/w, DS).

2.2. Microorganism

S. cerevisiae TISTR 5596 was obtained from the Thailand Institute of Scientific and Technological Research (TISTR). A single colony of *S. cerevisiae* grown on yeast extract peptone dextrose (YPD) agar (yeast extract 10 g/l, peptone 20 g/l, glucose 20 g/l, agar 15 g/l, pH 4.5) at 30 °C for 24 h was inoculated into YPD broth, incubated at 30 °C with shaking (200 rpm) for 24 h, and then the culture was transferred at 1% volume by volume (v/v) to the same medium and incubated under the same conditions for either 6 or 12 h. The resulting 6- or 12-h-old yeast culture was then used as a starter inoculum at 10% (v/v). In some experiments, the culture suspension was first centrifuged (11,857× *g*, 10 min) at 4 °C to precipitate the yeast cells and then the cells were resuspended in fresh YPD broth without glucose at the original volume of the separated supernatant, and then used as the inoculum.

2.3. Pretreatment and saccharification of sugarcane leaves

2.3.1. Sulfuric acid and lime pretreatment

Ground dried sugarcane leaves were pretreated by suspending at 3% (w/v, DS) in either dilute sulfuric acid or lime solution (50 ml in 250 ml flask) and autoclaved at 121 °C, 15 lb/in^2 for 30 min. Optimization of the pretreatment condition was performed by varying the concentration of sulfuric acid (1, 1.5 or 2% (w/v)) or lime (0.1, 1, 2 or 3.0% (w/v)) solution, autoclaving period (5–60 min) and the amount of sugarcane leaves loaded (3, 6 or 8% (w/v) DS), then pretreated sugarcane leaves were examined for their susceptibility to cellulase hydrolysis.

2.3.2. Determination of pretreatment efficacy on improvement of susceptibility to cellulase

The pretreatment slurry, which contained pretreated sugarcane leaves suspended in the pretreatment hydrolysate, was