



Simultaneous saccharification and fermentation of alkali-acid pretreated sugarcane trash to ethanol

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Abstract

The simultaneous saccharification and fermentation (SSF) of alkali-acid pretreated sugarcane trash to ethanol was optimized using commercial cellulase and *Saccharomyces cerevisiae* TISTR 5596 cells. Substrate concentration (12.5, 15, 17.5, and 20%w/v), enzyme loading (25, 50, and 75 FPU/g dry substrate (DS)), and temperature (30, 35, and 40°C) were evaluated. The SSF optimal conditions for alkali-acid pretreated sugarcane trash were 20%w/v of substrate concentration, enzyme loading 50 FPU/ g DS, temperature 35°C, initial pH 5.0 and yeast inoculum 10^7 cells/mL. Under the above optimal conditions, ethanol concentration was possible to reach in the range of 50.14 and 55.08 g/L at 96 and 144 hrs, respectively. This study could establish the effective utilization of sugarcane trash for bioethanol production using optimized fermentation parameters.

Keywords: cellulosic biomass, simultaneous saccharification and fermentation, sugarcane trash, ethanol production.

Introduction

Currently, there is growing interest in the use of lignocelluloses bioresources, including agro-industrial residues, such as sugarcane trash (Carrilo, 2005). Simultaneous saccharification and fermentation (SSF) is a process scheme for integrating enzymatic hydrolysis into the overall cellulose to ethanol bioconversion process. The SSF is a more efficient process than separate hydrolysis and fermentation (SHF), since it reduces the accumulation of sugar and minimizes end-product inhibition (Brethauer and Wyman, 2010). Moreover, simultaneous saccharification and fermentation (SSF) technique provides the possibility of decreasing the production cost (Kádár et al., 2004) and to reduce the risk of contamination (Wyman et al., 1992). The main microorganisms used for industrial ethanol production are yeasts. *S. cerevisiae*, the yeast traditionally used for ethanol production, cannot metabolise xylose, the second most abundant sugar in lignocellulosic hydrolysates (Hahn-Hägerdal et al., 2001). *Saccharomyces* strains require temperature lower than 35°C (Kádár et al., 2004). On the other hand, cellulases, which are frequently applied in the cellulose hydrolysis, have 50 °C as the optimal temperature. At lower temperatures, the substantially lower hydrolysis rates would be unfavorable in terms of increased processing time (Kádár et al., 2004). The optimal temperature for the yeast and the enzymes used differ, which means that the conditions used in SSF cannot be optimal for both the enzymes and the yeast (Öhgren et al., 2007). In this study, enzymatic saccharification and fermentation was studied using alkali-acid pretreated sugarcane trash as substrate. The optimum condition of ethanol production was investigated using commercial cellulase enzyme and yeast, *S. cerevisiae* TISTR 5596.

Methods

Preparing of materials and composition analysis

Sugarcane trash was collected from Khamphang-Phet province, Thailand. It was sundried and milled in Hammer mill, subsequently passed through 2 mm sieve. The milled sugarcane trash was stored at room temperature and used as substrate in the experiments.

Alkali-acid pretreatment

Milled sugarcane trash 15%w/v were pretreated with 2%w/v of NaOH in an autoclave at 121°C, 15 lb/in² for 15 min followed by 2%w/v of H₂SO₄, autoclave at 121°C, 15 lb/in² for 15 min. After the mixtures were cooled down, they were then washed with water and adjusted to pH 5.0. The solid residue was then separated from the liquid fraction by filtering through muslin cloth. The solid residue was subsequently used as substrate for enzymatic hydrolysis.

SSF process

The SSF experiments were performed in 250 mL duran bottle. The solid fraction from the pretreatment stage was used as substrate by adding water to adjust the concentration. A commercial cellulase from Novozymes, Denmark was used as enzyme hydrolysis. *S. cerevisiae* TISTR 5596 was used for fermentation in this study with the concentration of 10⁷ cells/mL. The SSF process was done in shaking incubator. Samples were periodically withdrawn for reducing sugar, cell number, and ethanol analyses.

The optimization experiments were carried out by varying substrate concentrations (12.5, 15, 17.5, and 20%w/v), enzyme loading (25, 50, and 75 FPU/g DS, and temperature (30, 35, and 40°C) to optimize the most ideal conditions for maximizing ethanol production.

Analytical method

The contents of cellulose, hemicellulose and lignin were determined according to the TAPPI standard test method (TAPPI, 2002). Cell number was determined using haemocytometer. The reducing sugars were estimated by DNS method (Miller, 1959). Ethanol concentration was analyzed using gas chromatography, Agilent 6890 series (Agilent GC system, USA) using 19091N-133 Innowax column and flame ionization detector. The activity of cellulase was measured according to the reference of Ghose (Ghose, 1987).

Results and Discussion

Chemical composition of sugarcane trash

The moisture content of sugarcane trash (Figure 1A) was found to be 9.18%. Sugarcane trash on dry weight basis contained 35.2% cellulose, 23.4% hemicellulose, 12.6% lignin, and 6.59% ash. In this study, alkali-acid pretreated sugarcane trash (Figure 1B) was taken as substrate for SSF process.



Figure 1: Sugarcane trash (A) and alkali-acid pretreated sugarcane trash (B)

Optimization on temperature of SSF process

The results in Figure 2 indicate that maximum ethanol concentration of 40.43 g/L was produced at temperature of 35°C. The ethanol concentration was decreased when the temperature increased from 35 to 45°C up to 120 hrs of incubation. Comparing to the optimal temperature, ethanol production capability of the yeast strain significantly decreased at 40°C and 45°C giving ethanol concentration of 21.65 and 2.29 g/L, respectively.

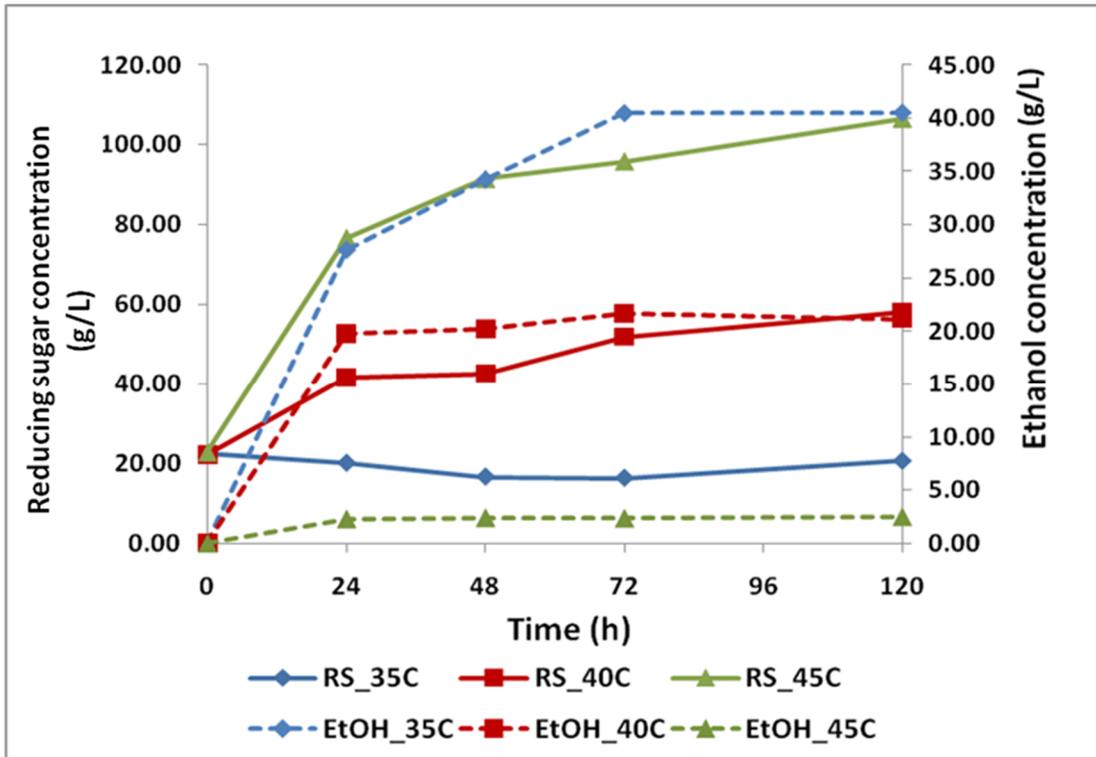


Figure 2 : Ethanol production in SSF process with different temperatures

In SSF the concentration of monomeric sugars is constantly kept low since the microorganism ferments them to ethanol as soon as they are liberated from the polymers (Holtzapfle et al., 1990). Even though cellulases are inhibited by glucose as well, conversion of monomeric glucose to ethanol reduces the inhibitory effect. Ethanol has also a noticeable inhibitory effect on *S. cerevisiae* at concentrations above 15 g/L but the ethanol producing capability is not completely inhibited until the ethanol concentration reaches 105 g/L (Luong, 1984), which was not the case in this study. The main affecting factors of the SSF process are temperature, enzyme loading, and substrate concentration. SSF processes combine enzymatic hydrolysis of cellulose with simultaneous fermentation of glucose obtained to ethanol. Thus, the presence of yeast together with cellulase reduces the accumulation of cellulose, thereby increasing saccharification rate and ethanol yield (Saha et al., 2005).

Optimization of sugarcane trash concentration and enzyme loading for SSF process

According to the economic evaluation of ethanol production from dilute sugar solutions, the ethanol concentration in the mixture has a major effect on the energy demand, and the cost of distillation step will increase significantly when the ethanol concentrations below 50.0 g/L (Zacchi and Axelsson, 1989; Galbe and Zacchi, 2002). To improve the economy of the ethanol process, the ethanol concentration above 50.0 g/L in the fermentation mixture is generally required (Wingren et al., 2003).

To increase the ethanol concentration, SSF at high dry matter content is generally conducted to obtain high cellulose. However, SSF at high dry matter content resulted in high viscosity and uneven slurry distribution of the reaction, so the actual ethanol yield was far less than the theoretical yield. In the most SSF processes, the cellulose content of substrate is low, therefore high glucose concentration after the hydrolysis cannot be obtained (Wingren, 2003). In this study, alkali-acid pretreated sugarcane trash was taken as substrate for SSF process,

different substrate concentrations (12.5, 15, 17.5, and 20 %w/v) and enzyme loadings (25, 50, and 75 FPU/g DS) were varied. After 96 hrs of incubation, the ethanol concentrations of 52.53, 50.14 and 59.68 g/L were obtained by using 17.5%w/v substrate with enzyme loading 75 FPU/g DS, 20%w/v substrate with enzyme loading 50 FPU/g DS, and 20%w/v substrate with enzyme loading 75 FPU/g DS, respectively. At the same concentration of 20%w/v substrate, an increase in the enzyme loading from 50 to 75 FPU/g DS would increase ethanol concentration only 9.54 g/L or 19.03% as the use of such high enzyme concentrations may not be justified economically. Considering the cost effectiveness of ethanol production, the optimum substrate and enzyme loading were 20% substrate with enzyme loading 50 FPU/g DS, as the final ethanol concentration reached 50.14 and 55.08 g/L in 96 and 144 hrs, respectively. The requirement of industrial ethanol concentrations in the fermentation mixture is therefore obtained (Table 1, Figure 3).

Table 1: Ethanol concentration at 96 hrs of SSF with different substrate concentrations and enzyme loadings with a total incubation time of 168 hrs

Substrate (%w/v)	Enzyme (FPU/g DS)	Ethanol concentration (g/L)
12.5	25	29.740
12.5	50	35.860
12.5	75	42.135
15	25	35.505
15	50	44.710
15	75	44.895
17.5	25	38.485
17.5	50	44.860
17.5	75	52.530
20	25	36.010
20	50	50.140
20	75	59.680

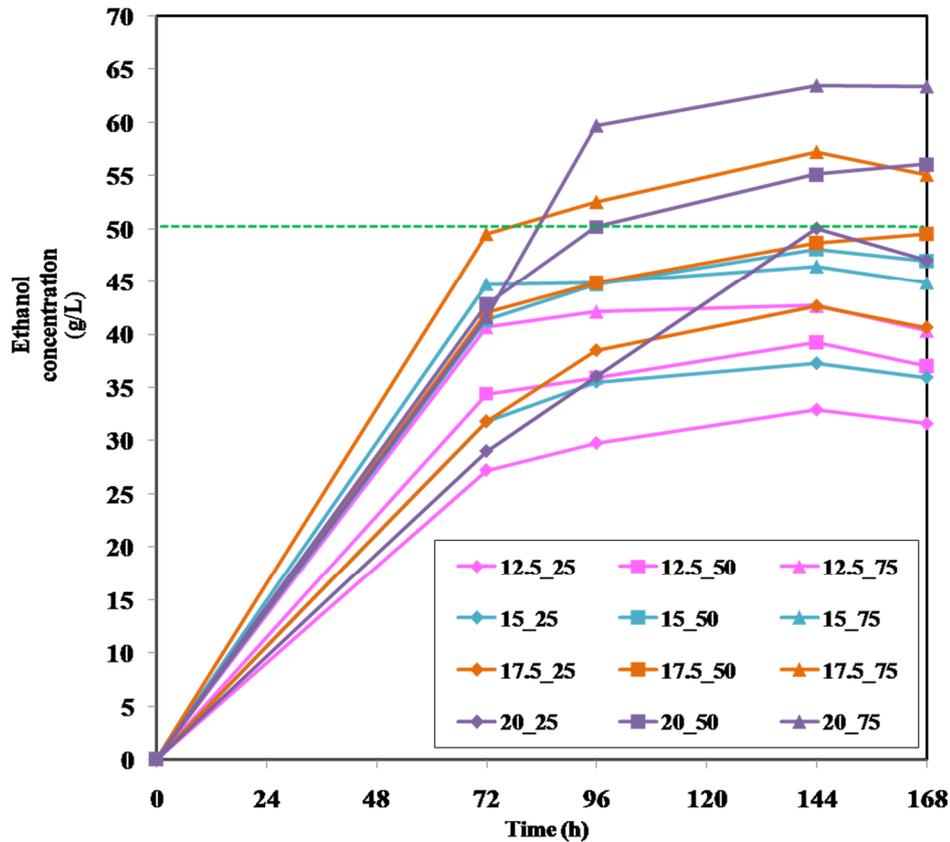


Fig 3. Ethanol production in SSF process with different substrate concentrations and enzyme loadings

Conclusion

The optimum condition of SSF process, 20%w/v of pretreated sugarcane trash with enzyme loading 50 FPU/ g DS, and temperature (35°C) was a successful method to ethanol production by using *S. cerevisiae* TISTR 5596 in SSF of alkali-acid pretreated sugarcane trash. Under the optimum conditions, the ethanol concentration reached 50.14 g/L (6.35%v/v) and 55.08 g/L (6.97%v/v) at 96 and 144 hrs, respectively. The process with optimized fermentation parameters described in the paper could be used for scaling up to a fermenter level thereby making the process more cost effective. This study could establish that sugarcane trash which has not been commercially exploited for any industrial application and is poorly disposed could effectively be used for ethanol production through the process of simultaneous saccharification and fermentation.

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