KKU Res.j. 2016; 21(2) : 229-235
http://resjournal.kku.ac.th

# Comparison of SHF and SSF Processes for Ethanol Production from Alkali-Acid Pretreated Sugarcane trash

*Suthkamol Suttikul[1,*], Teerapatr Srinorakutara[1], Ekarat Butivate[2] and Kitti Orasoon[1]*

*[1]Department of Energy Technology, Thailand Institute of Scientific and Technological Research (TISTR), 35 Mu 3, Technopolis, Khlong 5, KhlongLuang, PathumThani 12120, Thailand.*
*[2]Department of Agricultural Technology, Thailand Institute of Scientific and Technological Research (TISTR), 35 Mu 3, Technopolis, Khlong 5, KhlongLuang, PathumThani 12120, Thailand.*
*[*]Corresponding author:suthkamol@tistr.or.th*

## Abstract

Sugarcane trash, an agricultural by-product, contained 16.21% neutral detergent soluble (NDS), 38.43% hemicellulose, 34.06% cellulose, 5.51% lignin and 5.79% ash on dry solid (DS) basis. After it was pretreated with 2%w/v NaOH followed by 2%w/v $H_2SO_4$ in autoclave (121$^o$C, 15 min), the content of cellulose, hemicellulose and lignin were 73.17%, 7.21% and 3.41%, respectively. The pretreated sugarcane trash was used as substrate for ethanol production in separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) processes, using commercial cellulase and *Saccharomyces cerevisiae* TISTR 5596 cells. The optimum conditions in a flask scale of SHF process (15% w/v substrate loading hydrolyzed with cellulase 50 FPU/g DS at 50$^o$C, pH 5.0 and fermentation at 30$^o$C), and SSF process (20%w/v substrate loading, cellulase 50 FPU/g DS, hydrolysis and fermentation at 35$^o$C,pH 5.0) were applied to compare the ethanol production in a fermenter. In the SSF process, the highest level of ethanol production was 57.75 g/L, which was 16.26% higher than that of SHF process (48.36 g/L). The SSF process was therefore provided a more efficient method for the utilization of sugarcane trash.

**Keywords :** *cellulosic ethanol production, alkali-acid pretreatment, separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), sugarcane trash.*

## 1. Introduction

Currently, ethanol is increasingly used as an alternative to fossil fuels in the transportation sector [1]. Traditional fuel ethanol is produced from sugar cane, corn, and other kinds of starch, which are high in cost and lead to shortages and increases in the prices of food and feed. Value-added bio-products, such as fuel ethanol derived from efficient utilization of agro-industrial by-products, have attracted increased attention because of their use of abundant, renewable materials. The employment of proper bio-processing for the production of

fuel ethanol is a propitious method to reduce dependence on fossil fuel and decrease environmental problems. Ethanol production from lignocellulosic biomass involves three core steps: i) pretreatment ii) enzymatic hydrolysis or saccharification and iii) fermentation. Hydrolysis of sugars followed by fermentation step is called separate hydrolysis and fermentation (SHF). As an alternative these hydrolysis and fermentation steps can be merged together in one process known as simultaneous saccharification and fermentation (SSF). An advantage of SHF is that enzymes and yeast can each operate at their optimal conditions, e.g. with respect to temperature, However, SHF has the disadvantage that inhibitory hydrolysis products accumulate, decreasing reaction rates [2]. In SSF, temperature is not optimal for cellulases and, therefore, the rate of hydrolysis is slow, but hydrolysis products can be consumed as they are formed due to fermentation, thus avoiding the inhibition seen with SHF [2]. Furthermore, ethanol in the fermentation broth prevents significant microbial contamination. Other advantage of SSF is that the process integration of hydrolysis and fermentation in one reactor reduces the overall capital cost. There are a number of studies available related to SSF of pretreated lignocellulosic biomass. Most of these studies used *S. cerevisiae* as the fermentative organism at 30-35$^{o}$C [2].

To determine better process for the ethanol production, SHF and SSF were performed on alkali-acid pretreated sugarcane trash using commercial cellulase and *S. cerevisiae* TISTR 5596 in a fermenter under the optimum condition from a flask scale.

## 2. Materials and Methods

### 2.1 Raw material preparation and composition analysis

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

### 2.2 Enzyme

A commercial blended cellulase enzyme, derived from *Trichoderma reesei*. The enzyme containing of cellulase, β-glucosidase and hemicellulase, was used in enzymatic hydrolysis step.

### 2.3 Pretreatment

Milled sugarcane trash 15%w/v were pretreated with 2%w/v of NaOH in an autoclave at 121°C, 15 lb/in$^2$ for 15 min followed by 2%w/v of $H_2SO_4$, autoclaved at 121°C, 15 lb/in$^2$ 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.

### 2.4 SHF process

The separate hydrolysis and ethanol fermentation (SHF) was conducted in a 5-L fermenter under the optimal condition from the previous study in the flask scale [3]. Enzymatic hydrolysis was done using wet pretreated solid residue, washed with water and adjusted to pH 5.0. Commercial cellulase (50 FPU/g DS) was then added to the pretreated solid residue fraction and cultivated at 50°C, 160 rpm for 48 hrs. Sugar hydrolysate from enzymatic

hydrolysis of pretreated sugarcane trash was used as substrate for ethanol production. The inoculum of *S. cerevisiae* TISTR 5596 ($10^7$ cells/mL) grown in malt extract-glucose-yeast extract-peptone (MGYP) medium, was then inoculated into the sugar hydrolysate and cultivated at 30°C, 160 rpm [3]. Aliquots of samples were withdrawn and assayed for ethanol concentration, reducing sugar and cell number.

**2.5 SSF process**

The optimal condition of SSF process from flask scale (20%w/v solid loading, 50 FPU/g DS enzyme loading, $10^7$ cells/mL of *S. cerevisiae* TISTR 5596 and temperature 35°C) [4] was also used in the 5-L fermenter. Samples were periodically withdrawn for reducing sugar, cell number, and ethanol analyses.

**2.6 Analytical methods**

The contents of NDS, cellulose, hemicelluloses, lignin and ash were determined according to the method of Van Soest et al.[5]. Cell number was determined using haemacytometer. The reducing sugars were estimated by DNS method [6]. 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 [7].

## 3. Results and Discussion

### 3.1 Chemical composition of raw material before and after pretreatment

**Figure 1.** Sugarcane trash

Sugarcane trash (Figure 1) on dry weight basis contained 16.21% NDS, 34.06% cellulose, 38.43% hemicellulose, 5.51% lignin, and 5.79% ash (Table 1).

**Table 1.** The compositions of sugarcane trash before and after alkali-acid pretreatment.

| Chemical composition | % dry weight | |
|---|---|---|
| | Before | After |
| NDS* | 16.21 | 15.69 |
| Cellulose | 34.06 | 73.17 |
| Hemicellulose | 38.43 | 7.21 |
| Lignin | 5.51 | 3.41 |
| Ash | 5.79 | 0.49 |

The data presented are mean for triplicate assays.
*Neutral detergent solid.

The average chemical composition of pretreated sugarcane trash by alkali followed by acid was 15.69% NDS, 73.17% cellulose, 7.21% hemicellulose, 3.41% lignin, and 0.49% ash (Table 1). In this study, alkali-acid pretreated sugarcane trash (Figure 2) was taken as the substrate for SHF and SSF processes.

**Figure 2.** alkali-acid pretreated sugarcane trash

### 3.2 SHF process

In previous study, the optimal conditions for enzymatic hydrolysis and ethanol fermentation (SHF) process in flask scale, to obtain high glucose and ethanol concentration, was determined as substrate concentration 15% w/v, enzyme loading 50 FPU/g substrate at 50°C and pH 5 for 48 hrs. Under these conditions, the reducing sugar concentration of 117.16 g/L and ethanol concentration of 48.17 g/L or yield 0.509 g/g sugar which was equal to 99.81% of theoretical yield were obtained by using $10^7$ cells/mL of *S. cerevisiae* TISTR 5596 as inoculum [3]. The optimal condition of SHF process from the flask scale was used in this study to production of ethanol in 5L fermenter (Figure 3). The results showed that the reducing sugar concentration of 128 g/L after enzymatic hydrolysis and ethanol concentration of 48.36 g/L, productivity 0.50 g/l/h which was equal to 99.39% of

theoretical yield were obtained from the SHF process (Figure 4). We found that no significant difference in the overall ethanol yield of SHF process between the flask scale and the 5-L fermenter.

**Figure 3.** Ethanol production in a 5-L fermenter

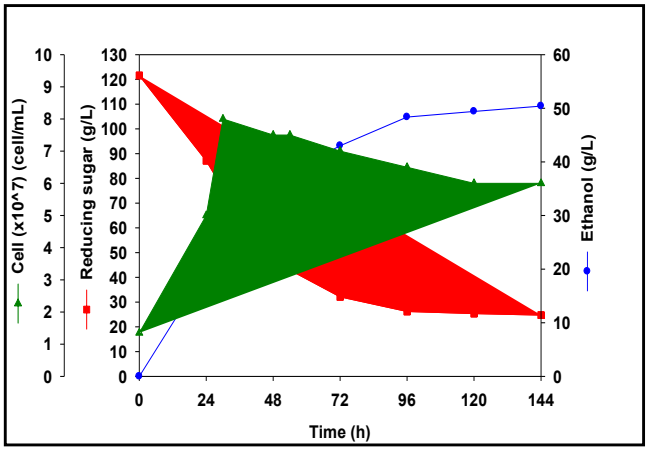


**Figure 4.** Ethanol production from sugarcane trash of SHF process in a 5-L fermenter.

### 3.3 SSF process

Also in the previous study, the simultaneous saccharification and fermentation (SSF) process in flask scale to obtain high ethanol concentration, was determined as substrate concentration 20%w/v, enzyme loading 50 FPU/g substrate at 35°C and pH 5 for 72-96 hrs. Under these conditions, ethanol concentration of 50.14 g/L, productivity 0.50 g/l/h were obtained by using $10^7$ cells/mL of *S. cerevisiae* TISTR 5596 as inoculum [4].

The optimal condition of SSF process from flask scale was used to production of ethanol in 5-L fermenter. The results showed that the ethanol concentration of 57.75 g/L, productivity 0.60 g/l/h were obtained from the SSF process in the 5-L fermenter (Figure 5).

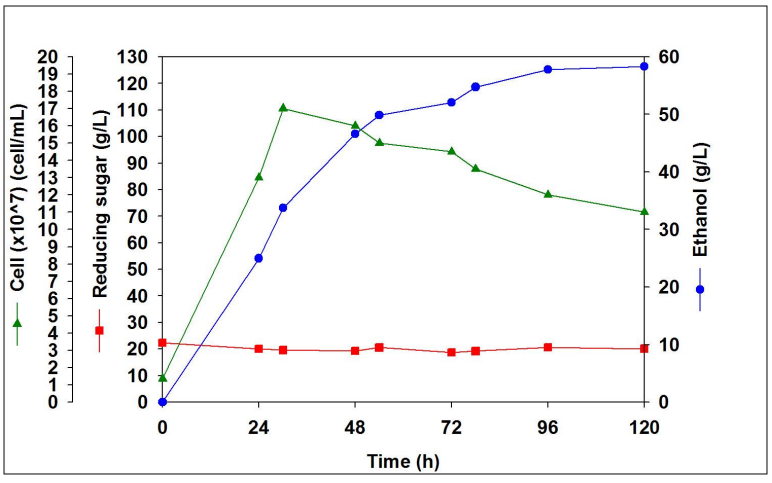


**Figure 5.** Ethanol production from sugarcane trash of SSF process in a 5-L fermenter.

In order to solve the problem of mixing due to high solids level, the residual solid of the pretreated sugarcane trash was divided into three portions. The SSF experiment was started with inclusion of one third of solid in the starting fermentation broth and the remaining two portion were add at 12 and 24 hrs. This approach helped with the mixing problem.

## 4. Conclusions

Comparison between separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) found that SSF was more efficient for ethanol production than SHF, despite using a lower reaction temperature which was suboptimal for the enzyme hydrolysis reaction. The highest ethanol concentration 57.75 g/L or 7.32%v/v was achieved when alkali-acid pretreated sugarcane trash was hydrolyzed with 50 FPU/g DS at 20% solid loading through a SSF process, which was 16.26% higher than that of SHF process (48.36 g/L).

## 5. Acknowledgements

The authors are grateful thanks to Thai government and Thailand Institute of Scientific and Technological Research for supporting the research fund, equipment and laboratory supports.

## 6. References

[1] Zhu M, Li P, Gong X, Wang J. A comparison of the production of ethanol between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using unpretreated cassava pulp and enzyme cocktail. Biosci. Biotechnol. Biochem. 2012; 76(4) : 671-8.

[2] Rana V, Eckard AD, Ahring BK. Comparison of SHF and SSF of wet exploded corn stover and loblolly pine using in-house enzymes produced from *T. reesei* RUT C30 and *A. saccharolyticus*. SpringerPlus. 2014; 3:516.

[3] Srinorakutara T, Suttikul S, Butivate E, Panphan V, Boonvitthya N. Optimization on Pretreatment and Enzymatic Hydrolysis of Sugarcane Trash for Ethanol Production. J. Food Sci. Eng. 2014; 4: 148-154.

[4] Srinorakutara T, Suttikul S, Kanhanont T, Siriniwatkul J. Simultaneous Saccharification and Fermentation of Alkali-Acid Pretreated Sugarcane trash to Ethanol. Proceedings of The 26th Annual Meeting of the Thai Society for Biotechnology and International Conference. 2014; 26-29 November; Thailand *(conference paper)*

[5] Van Soest PJ, Robertson JB, Lewis BA. Method of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 1991; 74:3583-3597.

[6] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 1959; 31:426-8.

[7] Ghose TK. Measurement of cellulose activity. Pure Appl. Chem. 1987; 59 : 257-68.