CHAPTER V

CONCLUSIONS AND DISCUSSIONS

Panicum maximum cv TD 53 (purple guinea grass) composed of 41.7% (w/w) cellulose, 27.1% (w/w) hemicellulose and 10.4% (w/w) lignin was cut, dried and grinded to 20-40 mesh, then pretreated by dilute sulfuric acid or calcium hydroxide (lime) at 121 $^{\circ}C$, 15 lb/inc^2. After pretreatment, it was hydrolyzed by cellulase.

Pretreatment condition of dilute sulfuric acid and lime which maximized cellulose susceptibility of pretreated purple guinea grass residue were 3.0 %(w/v) H_2SO_4, 6%(w/v) substrate loading, 30 min autoclaving and 2%(w/v) $Ca(OH)_2$ or 1.5 g(DS)/g $Ca(OH)_2$, 6%(w/v) substrate loading, 5 min autoclaving, respectively. Autoclaving period for $Ca(OH)_2$ pretreatment longer than 15 min resulted in lower cellulose saccharification due to $Ca(OH)_2$ pretreatment dissolved lignin and lignin form complex led to less accessibility of cellulase to cellulose. Purple guinea grass pretreated with H_2SO_4 or $Ca(OH)_2$ at the above condition, and hydrolyzed by Accellerase™ 1000 (265 FPU/ml, 2355.6 pNPG units of β-glucosidase/ml, at dose of 53 FPU/g (DS) or 471.1 pNPG units of β-glucosidase/g (DS)) at 50 $^{\circ}C$, pH 5.0 for 6 hours released reducing sugar 261 mg/g (DS) and 254 mg/g (DS), respectively. But glucose released was 168.3 and 198.3 mg/g (DS), respectively. From this results indicated that $Ca(OH)_2$ pretreatment (5 min) which was faster than H_2SO_4 pretreatment (30min) made purple guineas grass more susceptible to cellulase than H_2SO_4 pretreatment.

Analysis of hydrolysate obtained after pretreatment of purple guinea grass at the both optimized pretreatment condition, revealed that concentration of all pretreatment byproducts (furfural, hydroxymethylfurfural, 4-hydroxybenzaldehyde, syringaldehyde , vanillin) was much lower than *S. cerevisiae* effective inhibitory concentration for growth and fermentation of *S. cerevisiae* (Olsson and Hahn-Hagerdal, 1996; Palmqvist *et. al.*, 2000a; Delgenes *et. al.*, 1996). Xylose released from the dilute sulfuric acid pretreatment was 71.51 mg/g (DS), which xylose released in lime pretreatment hydrolysate was undetectable.

Cellulase hydrolysis of the $Ca(OH)_2$ pretreated purple guinea grass residue by various dose (53, 106, 159, 212 FPU/g (DS) or β-glucosidase 471, 942, 1413, 1884 pNPG U /g DS of

substrate) of Accellerase ™ 1000 at 50 °C, pH 5.0 for 6 hours, maximum glucose 218.3 mg/g (DS) was released by 159 FPU/ g (DS) or 1413 units of β-glucosidase/g DS of substrate. But maximum glucose liberation efficiency of the enzyme (11.23 mg glucose/ FPU) occurred at the enzyme dose of 53 FPU/g (DS) or 471 units of β-glucosidase/g DS of substrate. Hydrolysis of the $Ca(OH)_2$ pretreated purple guinea grass residue by the 53 FPU/g (DS)(471 units of β-glucosidase/g DS of substrate) enzyme dose for 6, 12, 18, 24 hours. Maximum glucose (213.3 mg/g (DS)) was obtained at 12 hours, but glucose liberation rate was maximum (99.17 mg glucose/h) during the first 6 hours of hydrolysis. To reduce a risk of glucose lost due to contamination, the $Ca(OH)_2$ pretreated purple guinea grass residue was saccharified by 53 FPU/g (DS)(471 units of β-glucosidase/g DS of substrate) of Accellerase ™ 1000 for 6 hours.

Separate hydrolysis and ethanol fermentation (SHF) was performed, hydrolysate containing 11.9 g/l glucose obtained after cellulase hydrolysis of the lime pretreated purple guinea grass residue was fermented to ethanol by *Saccharomyces cerevisiae* TISTR 5596 at 30 °C, pH 4.5 under oxygen limit condition. At the optimized condition, maximum ethanol (5.24 g/l or 0.44 g ethanol/g glucose or 0.21 g ethanol/g cellulose was obtained after 48 hours.

Simultaneous hydrolysis of lime pretreated purple guinea grass residue by cellulase and fermentation to ethanol (SSF) by *S. cerevisiae* was performed. After 96 hours, at the optimized condition, maximum ethanol (4.45 g/l or 0.18 g ethanol/g cellulose) was produced. This result indicated that the SHF ethanol production process gave higher ethanol yield (g ethanol/ g cellulose) than the SSF. Lower ethanol yield of the SSF was due to a lack of complete mixing of the substrate and the enzyme in cellulase hydrolytic reaction under oxygen limit condition of the ethanol fermentation. Cellulase hydrolysis and ethanol fermentation were not performed at its optimal condition leading to a lower ethanol yield (Saha *et. al.*, 2008).

Scaling up of the SHF from 40 ml in 50 ml flask to 3L in 5L fermentor (100 rpm agitation without aeration) resulted in an increase of ethanol yield from 0.44 to 0.49 g ethanol/g glucose or 0.21 to 0.24 g ethanol/g cellulose after 9 hours.

Theoretical ethanol production yield of glucose consumption was 0.51 g ethanol/g glucose. This value depending on feedstock and process and an actual yield could be varied from 60% to 90% of the theoretical yield (Onsoy *et. al.*, 2007). An ethanol production yield (g

ethanol/g glucose) of our experiment was 0.44 (flask scale) and 0.49 (5L fermentor). It was 86% (flask scale) and 96% (5L fermentor) of the theoretical, respectively.

The ethanol production yield of purple guinea grass (0.49 g/g glucose) were higher than those of sweet sorghum (0.40 g/g glucose) (Mamma *et. al.*, 1995), corncob (0.48 g/g glucose) (Chen *et. al.*, 2007) and the same as those of *Prosopis juliflora* (0.49 g/g glucose) (Gupta *et. al.*, 2009). However, pretreatment process of the *P. juliflora*, a hardwood substrate, is more complicate. Ethanol yield of each step in our experiments are shown in Figure 31.

The ethanol production yield (0.49 g/g glucose) in our experiment could be improved by optimization of cellulase and β-glucosidase ratio in the Accellerase ™ 1000, are commercial mixed cellulase enzymes.

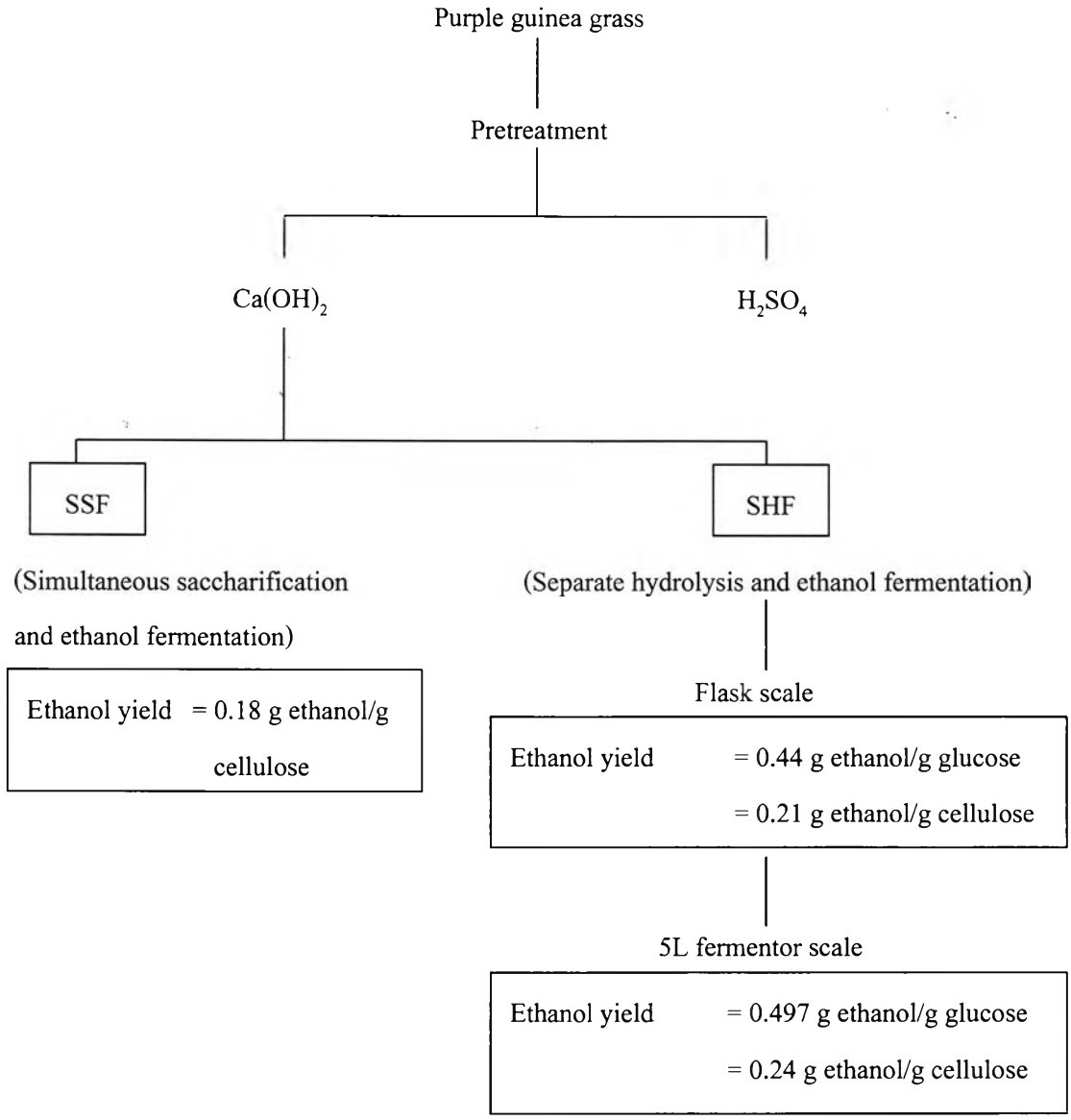

Figure 32 Ethanol production yield of each steps in the experiments