CHAPTER VII

EFFICIENT PROCESS FOR ETHANOL PRODUCTION FROM THAI MISSION GRASS (*PENNISETUM POLYSTACHION*)

7.1 Abstract

Mission grass (*Pennisetum polystachion*) obtained from Tak Province, Thailand, possesses the potential to become a lignocellulosic biomass for bioethanol production. After the grass underwent milling and alkaline pretreatments, it was subjected to acid and enzymatic hydrolysis. The glucose hydrolyzate from the grass was detoxified to remove inhibitory compounds and degradation products such as furfural and 5- hydroxymethylfurfural. Overliming at pH 10 produced the highest ethanol yield. Among various strains of baker's yeasts, *Saccharomyces cerevisiae* TISTR 5596 with a yeast concentration of 10 % v/v produced the maximum ethanol yield at 16 g/L within 24 h, which is among one of the fastest ethanol producing microorganisms compared to other strains of *S. cerevisiae* as well as other ethanol-producing microorganisms.

(**Keywords**: Bioethanol production; Detoxification; Overliming; *Pennisetum polystachion*; *Saccharomyces cerevisiae*)

7.2 Introduction

Ethanol has become the subject of interest due to its potential to substitute energy from fossil fuels. Presently, food crops such as corn, sugarcane, rice, wheat, and sugar beet are the major sources of ethanol production (Balat, 2011). However, continuous utilization of food crops could jeopardize the food demand for the world population; moreover, frequent consecutive cultivation of corn and sugarcane could result in nutrient depletion and soil erosion (Xu *et al.*, 2011). A potential solution to answer the issue is to utilize lignocellulosic materials to produce ethanol. Lignocellulosic materials originated from biomass are comprised mainly of cellulose, hemicellulose, and lignin. Some lignocellulosic materials include wood, agricultural residues, grasses, and newspaper (Sun and Cheng, 2002).

With a high cellulose and hemicellulose content, Mission grass (*Pennisetum polystachion*, denoted as MG) is one suitable lignocellulosic biomass to produce bioethanol (Tatijarern *et al.*, 2013). In addition to it being ubiquitous in Africa, Asia, and Australia, its ability to seed during dry season, and its low requirement of energy, fertilizers, and soil moisture allow MG to be a good candidate for bioethanol production. Mission grass can grow to be 3 min height, and thus a high quantity of cellulose and hemicelluloses could be obtained within a small plot. Currently, no paper has reported on the conversion of MG to produce ethanol. This paper presents a novel study of using MG lignocellulosic biomass to produce bioethanol. The production of ethanol from Mission grass could be beneficial to both weed management and bioethanol production (Tatijarern *et al.*, 2013).

A variety of microorganisms ranging from fungi, bacteria, and yeasts could be utilized for producing ethanol from grass hydrolyzate. Baker's yeast, *Saccharomyces cerevisiae*, is one of the most popular candidates for ethanol fermentation. The yeast *S. cerevisiae* has produced ethanol as its main fermentation product and also high tolerance to inhibiting compounds compared to other microorganisms (Almeida *et al.*, 2007). One disadvantage of employing *S. cerevisiae* as an ethanol fermenter is its inability to produce ethanol from other sugars beside glucose.

Generally, to obtain bioethanol from lignocellulosic biomass, the lignocellulosic biomass initially undergoes a pretreatment to remove lignin and decrease cellulose crystallinity to make cellulose become more susceptible to hydrolysis (McMillan, 1994). The pretreated biomass is then hydrolyzed to break down polysaccharides into sugars, mainly glucose. However, various compounds other than sugars are simultaneously released during the hydrolysis process, which could have detrimental effects to the microorganisms and thus the overall ethanol yield (Palmqvist and Hahn-Hagerdal, 2000). Consequently, the hydrolyzate is gone through a process called overliming to detoxify the toxic compounds (Leonard and Hajny, 1945; Palmqvist and Hahn-Hagerdal, 2000). Finally, the sugar is utilized by microorganisms to produce ethanol.

Since MG is widely distributed throughout Thailand, this study focused on the production of bioethanol from Thai MG via a two stage microwave/chemical pretreatment, as described by our group (Tatijarern *et al.*, 2013). Various strains of Baker's yeast (*S. cerevisiae*) at different pHs were studied due to their robustness and capability to withstand inhibitory compounds from grass hydrolysis and produce a high yield of ethanol (Palmqvist and Hahn-Hagerdal, 2000).

7.3 Experimental

7.3.1 Materials and Chemicals

MG was collected from Tak Province, Thailand. The grass was air- and sun dried before being cut into small pieces. The dried grass was milled with a 60 mesh-sized sieve, and then the powdered grass was stored in a sealed plastic bag at room temperature. Sodium hydroxide (NaOH, Labscan Asia Co., Thailand) was utilized in the pretreatment process of MG. Sulfuric acid (H_2SO_4, Merck Co., Germany) and cellulase from *Trichoderma reesei* ATCC 26921 (Sigma Aldrich Chemical Co., USA) were used to hydrolyze cellulose and hemicellulose of Mission grass. Calcium hydroxide ($Ca(OH)_2$, Sigma Aldrich Chemical Co., USA) and sodium sulfite (Na_2SO_3, Labscan Asia Co, Thailand) were employed during the detoxification process of MG hydrolyzate. D-(+)-Glucose (G5400) standard (Sigma Aldrich Chemicals Co. Inc., USA) was used for sugar quantitative analysis. Yeast

extract (3 g/L), malt extract (5 g/L), peptone (5 g/L) were obtained from Himedia Laboratories and utilized for the inoculating yeast medium.

7.3.2 Pretreatment of MG

MG was pretreated using the grass optimization method according to Boonmanumsin *et al.* (2012) and Tatijarern *et al.* (2013). The milled grass was mixed with 3 % w/v NaOH using a liquid-to-solid ratio (LSR) of 15 mL of NaOH: 1 g of grass (Boonmanumsin *et al.*, 2012; Tatijarern *et al.*, 2013). The mixture was stirred thoroughly until it was homogenous. Then, it was subjected to microwave treatment (300 W, Ethos Series, Milestone Inc.) at 120 °C for 10 min. After the alkaline pretreatment, the mixture was washed until the pH was neutral. The solid residue was oven-dried for 24 h. The dry solid was stored in a dry container at room temperature.

7.3.3 Hydrolysis of MG

The dry solid from the alkaline pretreatment was mixed with 1 % v/v H_2SO_4 using a LSR of 15 mL of H_2SO_4: 1 g of pretreated grass. The mixture was stirred thoroughly, and then subjected to microwave treatment (300 W) at 200 °C for 5 min (Tatijarern *et al.*,2013). The mixture was left to cool at room temperature. The pH of the hydrolyzate was adjusted to 4.8 using 40 % w/v NaOH. Cellulase from *T. reesei* ATCC 26921 (160 µL/1 g of grass) was added into the liquid hydrolyzate. The hydrolyzate was incubated for 60 h at 50 °C (Qureshi *et al.*, 2008).

7.3.4 Detoxification of MG Hydrolyzate

A combined detoxification method of evaporation, overliming, addition of sodium sulfite, and heating was carried out to remove inhibitory products from the MG hydrolyzate. Approximately 60 % of the hydrolyzate was evaporated using a rotary evaporator. The evaporated hydrolyzate was treated with $Ca(OH)_2$ to increase the pH to 8–12. Sodium sulfite (1 g/L) was then added to the solution. The overlimed solution was heated at 90 °C for 30 min. The condensate in the mixture was filtered, and the pH was adjusted to 6 using 95 % v/v H_2SO_4 at room

temperature. The liquid was stored in a closed bottle at ambient temperature for fermentation.

7.3.5 Inoculation of Baker's Yeast (*Saccharomyces cerevisiae*)

Yeast extract, malt extract, peptone, and glucose were mixed together to make a yeast medium. The medium was autoclaved at 121 °C for 15 min and was left to cool at room temperature. One inoculation loop of *S. cerevisiae* was transferred to the sterile medium and incubated for 24 h at 30 °C. The standard deviation obtained is in triplicates.

7.3.6 Fermentation of MG Hydrolyzate

Yeast extract, malt extract, and peptone were added to the overlimed grass hydrolyzate. No additional glucose was added. The mixture was sterilized at 121 °C for 15 min and allowed to cool at room temperature. *S. cerevisiae* (2–10 % v/v) from the previous step was added to the hydrolyzate, and the solution was incubated in a shaker at 30 °C for 96 h. Samples from the solution were taken out every 24 h for glucose and ethanol detection.

7.3.7 Characterization

Physical pretreatment is a necessary method for reducing cellulose crystallinity (Ghosh and Ghose, 2003). The particle size of the MG after physical pretreatment was detected by a particle size analyzer (Malvern/Mastersizer X) with a 300 mm lens size in a sample detection unit. The physical structure of untreated and pretreated MG were obtained using a scanning electron microscope (SEM, Hitachi/S-4800) at an accelerating voltage of 2 kV. The monomeric sugars and others chemicals were detected by High-Performance Liquid Chromatography (HPLC, RID-10A, Shimadzu Corp., Kyoto, Japan) equipped with a refractive index detector, and Aminex-HPX 87H column (300 x 78 mm, Bio-Rad Lab, USA) under the conditions of a 0.005 M H_2SO_4 mobile phase and 0.60 ml/min flow rate. Ethanol production was detected by gas chromatography (GC, Agilent Technologies, USA) equipped with TCD Detector, using 0.5 µL injection volume, 55 kPa of helium as a

carrier gas, 200 °C oven and injector temperatures. Both glucose and ethanol concentrations were detected every 24 h of incubation.

7.4 Results and Discussion

Dry MG underwent a physical pretreatment through milling. Milling decreases the particle size and the crystalline structure of MG, which results in an enhancement of surface area. The increase of surface area allows MG to be further pretreated and digested more easily (Quintero *et al.*, 2011). After milling with a 60 mesh sieving size, the size of the powdered MG was determined using a particle size analyzer. According to Sluiter, the optimal size for lignocellulosic biomass hydrolysis for ethanol production should stay in between -20 and +80 mesh particle size. A larger mesh size would cause inefficient hydrolysis of carbohydrates, and lead to lower amount of sugar released. Alternatively, over-hydrolysis of carbohydrates would occur if a smaller mesh size is used, resulting in an increase of sugar degradation products (Sluiter and Sluiter, 2010). These degradation products may potentially cause detrimental effects on fermenting yeasts as well as ethanol yield.

The average size of the milled MG obtained from Tak Province is approximately 300 μm. The milled size of Tak MG is comparable to size of the milled grass in a study by Tatijarern, where his MG from Nakhon Ratchasima Province had a particle size of 330 μm after using a 60 mesh sieving size (Tatijarern *et al.*, 2013).

7.4.1 Chemical Composition of MG

The chemical composition of milled MG was found using the method from the National Renewable Energy Laboratory (NREL) (Sluiter and Sluiter, 2010). The chemical composition of MG in this study (obtained from Tak Province, Thailand) is compared to that in a previous study (obtained from Nakhon Ratchasima Province, Thailand) in Table 7.1 (Tatijarern *et al.*, 2013). The MG from Tak Province presents a better candidate for ethanol production due to its higher percentage of cellulose, which is more preferable as cellulose is the main source of

sugar for fermentation. However, Tak MG is comprised of a higher percentage of lignin, which could become degraded during the pretreatment process to produce inhibitory compounds and hinder ethanol fermentation (Palmqvist and Hahn-Hagerdal, 2000). The chemical composition of MG from Tak and Nakhon Ratchasima province differ considerably due to a few factors, such as location, climate, and agricultural practices, for the MG to grow (Malherbe and Cloete, 2002). Tak province is located in the northern part of Thailand's mountainous landscape whereas Nakhon Ratchasima province is located in northeastern plateau where drought often occurs. MG from Tak province thus contains more preferable cellulose which is the main source of glucose. However, Tak MG is also comprised of a higher percentage of lignin, which could degrade during the pretreatment process to produce inhibitory compounds and hinder the ethanol fermentation. Thus, further research could be done on optimizing the location of MG and pretreatment method of Tak MG.

7.4.2 Chemical Pretreatment

Milled MG was chemically pretreated with 3 % w/v NaOH, and hydrolyzed using 1 % v/v H_2SO_4 with a 15:1 LSR according to the optimized grass pretreatment method (Boonmanumsin *et al.*, 2012; Tatijarern *et al.*, 2013). Then, it was further hydrolyzed by cellulase from *T. reesei* to ensure complete saccharification.

The SEM image of milled raw MG shows a highly fibrillar structure covered by thin waxy layer on its surface, which is frequently found in herbaceous biomass (Hu and Wen, 2008). In comparison to the SEM image of untreated raw switchgrass, milled MG possesses a more ordered structure with less waxy film. After treating the grass in dilute alkaline, the crystalline structure of MG fibrils is noticeably disrupted. The waxy layer on the surface is partially removed, which indicates the breaking down of the lignin (Hu and Wen, 2008).

The breakdown of fibrils in MG after dilute acid hydrolysis assisted by microwave can easily be observed. The dilute acid primarily solubilizes and ruptures hemicellulose. Although acid hydrolysis causes chemical changes in hemicellulose which disrupts covalent bonds, hydrogen bonds, and van der Waals

forces (Li *et al.*, 2010), the treatment allows cellulose and hemicelluloses to become more susceptible to enzymatic hydrolysis. In addition to having a higher cellulose content than switchgrass (47 % of cellulose in MG, and 34 % in switchgrass (Hu and Wen, 2008)), the lower lignin content further ensures that MG would be a better candidate for ethanol production. The presence of lignin localized on the grass' surface is less in MG than that in switchgrass. The image of residual lignin condensing on the surface of the cellulose confirms that acid treatment possesses only minimal effect on lignin removal (Yu *et al.*, 2011).

7.4.3 Enzymatic Hydrolysis

The SEM image of MG after being treated with cellulase shows a thorough destruction of cellulose fibrils; the cellulose crystalline structure can no longer be observed after enzymatic hydrolysis. Compared to enzymatic hydrolysis of pretreated eucalyptus, sorghum bagasse, and sugarcane bagasse, MG was better hydrolyzed when treating with enzyme (Wang *et al.*, 2012), as also indicated by the SEM image showing cellulose fibrils destruction. Cellulase liberates twice the amount of glucose in comparison to the glucose released by acid hydrolysis alone, indicating that the glucose concentration increases by twofold after enzymatic hydrolysis using cellulase.

7.4.3.1 Optimization of Detoxification Process on MG Hydrolyzate

Common physical detoxification methods, such as evaporation and membrane separation (Chandel *et al.*, 2011), involves no addition of other chemicals into the lignocellulosic hydrolyzate. Evaporation was chosen as the method of physical detoxification in this study due to its low cost and ease of operation.

Advantages of using the evaporation are not only to remove inhibiting volatile compounds, such as furfural, and acetic acid, but also that glucose concentration in the hydrolyzate can be regulated (Taherzadeh and Karimi, 2011). (Dawson and Boopathy, 2007) showed that the production of ethanol from post-harvest sugarcane residue could take up to 12 days for the fermenting yeast to produce the maximum ethanol if the evaporation was not used. Moreover, the

maximum concentration of ethanol produced from this study is only 336 mg/L probably due to extreme dilution of the obtained hydrolyzate.

In this study, to increase the concentration of glucose approximately 60 % of water was evaporated. According to Larsson *et al.* (1999) 10 % of hydrolyzate evaporation could remove more than 40 % of furfural, one of the major glucose degradation products and inhibiting compounds in the fermentation process (Larsson *et al.*, 1999). Further evaporation could remove more toxic compounds, including acetic acid, formic acid, and other lignin degradation products. However, if water is scarce, they would produce glycerol to regulate their cells, and the production of glycerol could hinder the ethanol production because the carbon source utilized to produce ethanol is redirected into the glycerol production (Pagliardini *et al.*, 2013). On the other hand, as described previously, too much water dilutes glucose concentration, resulting in lower ethanol production.

Larsson *et al.* (1999) compared various effective detoxification techniques, namely, conditioning with calcium hydroxide called overliming, treatment with laccase, addition of sulfite at pH 10, and anion exchange at pH 10. Each method possesses various advantages and disadvantages in regards to amount of sugar loss, time, and cost. Moreover, a study by Telli-Okur and Eken-Saraçoğlu (2008) also shows an increase in ethanol yield when the lignocellulosic hydrolyzate is detoxified by combining overliming and sodium sulfite methods compared to overliming alone (Telli-Okur and Eken-Saracoglu, 2008). Therefore, combined detoxification was performed in this study. Overliming was chosen as one of the detoxification techniques for MG hydrolyzate since more degradation products can be removed as the overliming pH increases although monomeric sugar loss also occurs (Millati *et al.*, 2002; Mohagheghi *et al.*, 2006). After overliming, sodium sulfite was subsequently added into the hydrolyzate, and the solution was filtered and pH-adjusted for fermentation (Leonard and Hajny, 1945; Millati *et al.*, 2002). Figure 7.1 shows the amount of sugar loss at various overliming pH.

Mohagheghi *et al.* (2006) claimed that about 7 %, 12 % and 14 % of monomeric sugar was lost during conditioning at pH 9, 10, and 11, respectively (Mohagheghi *et al.*, 2006). The results of this study share the same trend as that of Mohagheghi *et al.* (2006) where glucose loss is proportional to overliming pH.

However, as little as 2 % and 7 % of the glucose concentration is lost at overliming pH 9 and 10, respectively, while up to 53 % is lost at pH 11. The results are similar to that of Millati *et al.* (2002) where the glucose concentration was not reduced significantly when overliming was performed at pH 10, but was halved at pH 12. The sugars that are lost during overliming could be converted into lactic acid (Millati *et al.*, 2002).

In this study, however, the concentration of acetic acid increased after overliming. One possible explanation for this increase is that during overliming with calcium hydroxide, acetic acid reacts with calcium to form soluble calcium acetate in the solution. Furfural concentration decreased (0.19 %) and untraceable quantities of 5 (hydroxymethyl)furfural were detected after over overliming (Table 7.2), consistent to Martinez *et al.* (2000) concentration of acetic acid does not decrease with overliming treatment while the concentrations of furfural and 5-(hydroxymethyl)furfural were dramatically reduced by overliming treatment (Martinez *et al.*, 2000).

7.4.3.2 Fermentative Microorganisms

A variety of microorganisms ranging from fungi and bacteria to yeast could be utilized to produce ethanol from grass hydrolyzate. Baker's yeast, *S. cerevisiae*, is one of the most popular candidates for ethanol fermentation. In addition to producing ethanol as its main fermentation products, *S. cerevisiae* possesses a high tolerance for inhibiting compounds compared to other microorganisms (Almeida *et al.*, 2007). One disadvantage of employing *S. cerevisiae* as an ethanol fermenter is its inability to produce ethanol from sugars beside glucose. Figure 7.2a shows the growth of Baker's yeast (*S. cerevisiae* TISTR 5049) in MG hydrolyzate at various overliming pHs.

The number of *S. cerevisiae* in Figure 7.2a begins to rise rapidly within 24 h. After 48 h, the growth of *S. cerevisiae* remains relatively stable. This may be due to exhaustion of sugar as the population of yeast increases. Overliming at pH 10 produces the highest colony forming units of yeast, which implies that pH 10 can adequately eliminate degradation products while still maintaining a sufficient amount of glucose for the yeast's growth, as also confirmed by the SEM image of the MG hydrolyzate after overliming at pH 10, showing no

presence of lignin residues. Overliming at pH 11 produces the second highest colony forming units of yeast. This could be due to the elimination of many toxic compounds along with the loss of usable glucose. A similar explanation also applies when overliming is performed at pH 12. Even though overliming at pH 8 and 9 does not decrease the glucose concentration as severely, compared to that at higher pHs, the process does not eliminate amounts of degradation and inhibitory compounds. The remaining compounds become toxic to the fermenting yeasts, which results in a less yeast growth and potentially lower ethanol yields (Eliana *et al.*, 2014).

The consumption of glucose by baker's yeast *S. cerevisiae* at various overliming pHs is shown in Figure 7.2b. After 24 h, glucose was almost depleted when overliming was carried out at pHs 10, 11, and 12. The trend indicates that overliming at pH 10 or higher can effectively remove inhibitory products from the hydrolyzate, enabling the yeast to immediately utilize the glucose, consistent with the result in Figure 7.2a showing the highest colony forming units of yeast. In contrast, some glucose still remained within 24 h when conditioning at pH 8–9, which signifies that some glucose was not able to be utilized immediately by the yeast. This could possibly be due to residual inhibitory compounds, such as furfural and hydroxymethylfurfural in the hydrolyzate (Mohagheghi *et al.*, 2006). Furfural and hydroxymethylfurfural inhibit enzymes, such as alcohol dehydrogenase, pyruvate dehydrogenase, aldehyde dehydrogenase, and hexokinase, which are vital to glycolysis and metabolism in yeast (Taherzadeh and Karimi, 2011). Moreover, furfural causes damage in yeasts' vacuoles, mitochondria, and cell membranes (Almeida *et al.*, 2007; Taherzadeh and Karimi, 2011).

Another probable explanation of slower glucose utilization is osmotic stress caused by a large concentration of glucose and other toxic compounds that are still present when overliming at pHs 8 and 9 (Saint-Prix *et al.*, 2004). From the beginning, the steady drop of glucose concentration in pH 8 and 9 overliming hydrolyzates could be detected. The drop in glucose concentration could be due to consumption by the remaining yeast capable of naturally adapting themselves to higher concentration of toxic products (Taherzadeh and Karimi, 2011). The yeasts then consumed the glucose until the concentration reached almost 0 g/L after 48 h.

The result from Figure 7.2b gives evidence that pHs 8–12 could be a suitable overliming pH range for *S. cerevisiae*.

The production of ethanol was recorded every 24 h for 96 h. Figure 7.2c is the result of ethanol production at a various range of overliming pH. The highest amount of ethanol was produced within 48 h of incubation time. The sharp drop of ethanol concentration after 48 h of incubation suggests that ethanol may be oxidized into acetaldehyde or acetate (Zakhari, 2006). Moreover, ethanol, the product from fermentation, is also the inhibitor for ethanol production (Taherzadeh and Karimi, 2011). The rapid decrease of ethanol production after the maximum yield of ethanol was obtained could also be justified by high osmotic stress caused by the remaining glucose, ethanol, and other inhibiting compounds in the hydrolyzate (Saint-Prix *et al.*, 2004; Taherzadeh and Karimi, 2011).

At a starting glucose concentration of 53 g/L, overliming at pH 10 gave the highest concentration of ethanol followed by pH 9, 8, 11, and 12, respectively. The lowest yields of ethanol occurred at pHs 11 and 12, which suggested that much glucose was lost during the overliming process. When overliming was done at pH 8 and 9, the inhibitory compounds were not completely removed, but a higher concentration of glucose was still present in the hydrolyzate where naturally adapted yeasts could utilize the glucose to ferment ethanol. The overliming process is an effective method in removing toxic compounds from lignocellulosic hydrolyzate, but using too much lime also causes sugar loss and affects ethanol yield. Consequently, a compromise has to be made between the amount of inhibitory compounds being removed and sugar loss from the overliming process in order to obtained the highest yield of ethanol (Mohagheghi *et al.*, 2006).

7.4.4 Optimization of *Saccharomyces Cerevisiae* Strains for Ethanol Production

Baker's yeast (*S. cerevisiae*) of different strains can produce varying amount of ethanol. Despite being the same species of yeast, each strain of *S. cerevisiae* has diverse characteristics, such as glycolysis production speed, resistance against inhibitory compounds, and tolerance to osmotic stress. Four strains of *S. cerevisiae* were studied in this project: TISTR 5049, TISTR 5339, TISTR 5596, and

TISTR 5606, see Figure 7.3a showing various strains of yeast in MG hydrolyzate at pH 10 overliming. *S. cerevisiae* TISTR 5339, 5596, and 5606 have been used in ethanol production studies previously (Srinorakutara *et al.*, 2008; Jutakanoke *et al.*, 2012; Vaithanomsat *et al.*, 2013) whereas no journal has reported on the ethanol performance of *S. cerevisiae* TISTR 5049.

S. cerevisiae TISTR 5049 reproduced very quickly compared to other strains of *S. cerevisiae*. Similar to Figure 7.2a, the fastest population growth of *S. cerevisiae* TISTR 5049 occurred within 24 h. After 24 h, the cell began to stabilize which may be due to lower levels of glucose. According to Figure 7.3a, *S. cerevisiae* TISTR 5606 had the smallest yeast population due to the difficulty in counting the yeast cells under a microscope; this strain's colonies aggregated into a large group with multiple layers of cells. The yeasts *S. cerevisiae* TISTR 5339 and 5596 showed similar growth patterns, where their numbers rose rapidly within 24 h and began to stabilize.

For incubation of the MG hydrolyzate using the strains of *S. cerevisiae*, the detection of glucose concentration per incubation time is illustrated in Figure 7.3b. Glucose concentration declined very quickly within 24 h, which correlated to the rapid increase of yeast population in Figure 7.3a. *S. cerevisiae* TISTR 5049 and 5596 consumed almost all the available glucose within 24 h, while larger concentrations of glucose, 10 and 13 g/L, could still be detected in the flasks of *S. cerevisiae* TISTR 5339 and 5606, respectively, possibly due to their lower tolerance to osmotic stress compared to strains TISTR 5049 and 5596 (Navarro-Avino *et al.*, 1999), which caused some yeast cells to burst. This hypothesis explains the lower number of yeast cells for *S. cerevisiae* TISTR 5539 and 5606 after 24 h in Figure 7.3a. The remaining yeast cells that were capable of enduring the osmotic stress underwent natural adaptation and consumed the leftover glucose (Taherzadeh and Karimi, 2011). Glucose concentration dropped to almost 0 g/L after 72 h of incubation in all samples, which confirms that pH 10 is the most suitable overliming pH because all strains of *S. cerevisiae* could utilize the glucose in the hydrolyzate.

In comparison, another type of yeast, *Pichia stipitis*, takes up to 200 h to consume all sugar in the hydrolyzate when the concentration of starting sugar is approximately 45 g/L (Telli-Okur and Eken-Saracoglu, 2008). *P. stipitis* is among

one of the most common types of yeasts used to produce ethanol due to its ability to utilize variety of sugars (Lee *et al.*, 2000). However, its low tolerance to inhibitory products and slow ethanol production cause the yeast to be less preferable as an ethanol fermenter.

Ethanol production from each strain of *S. cerevisiae* at overliming pH 10 is shown in Figure 7.3c. The starting glucose concentration for the fermentation process was 45 g/L. *S. cerevisiae* TISTR 5596 produced the most ethanol at 16 g/L in 24 h. The same yeast strain also produced the maximum ethanol production within 24 h in hydrolyzate from sugarcane leaves (Jutakanoke *et al.*, 2012). The amount of ethanol produced correlates with the results from Srinorakutara *et al.*, where *S. cerevisiae* TISTR 5596's ethanol production is superior to that of *S. cerevisiae* TISTR 5606 (Srinorakutara *et al.*, 2008). The lower ethanol production of *S. cerevisiae* TISTR 5339 and 5606 could be due to their lower tolerance to osmotic stress, which is supported by the yeast population and sugar consumption results in Fig. 7.3a and b, respectively.

According to Figure 7.3c, *S. cerevisiae* TISTR 5049 produced the least ethanol out of all four strains. This could be due to its low tolerance to acetate since some of the ethanol produced gets oxidized to acetate (Verduyn *et al.*, 1990). Another explanation that could justify the difference of ethanol production performance of *S. cerevisiae* TISTR 5049 in Figures 7.2c and 7.3c is the yeast's low resistance to osmotic stress. When the yeast is under stress due to toxic compounds in the hydrolyzate, the yeast would utilize the glucose present in the hydrolyzate to produce glycerol (Pagliardini *et al.*, 2013). As a result, less glucose could be utilized for ethanol production.

7.5 Conclusions

Mission grass (*P. polystachion*) from Tak Province, Thailand, is a potential lignocellulosic biomass candidate for the production of bioethanol. After chemical pretreatment, enzymatic hydrolysis, and fermentation, the highest ethanol yield of 16 g/L was achieved within 24 h, using *S. cerevisiae* TISTR 5596 with overliming at pH 10. These obtained results are indicative of how we can make use of Mission grass.

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Table 7.1 The chemical compositions of MG obtained from Tak and Nakhon Ratchasima Provinces, Thailand

Composition (%)[a]	Mission Grass (*Pennisetum Polystachion*)	
	Tak Province	Nakhon Ratchasima Province (Tatijarern *et al.*, 2013)
Cellulose	47.2±3.1	39.8±1.5
Hemicellulose	27.3±3.4	29.2±1.0
Lignin	18.2±2.6	14.6±0.5
Ash	2.6±0.7	3.3±0.5

[a] Dry weight percentages

Table 7.2 Inhibitor concentration of Mission grass (*P. polystachion*, Tak Province) from before and after overliming treatment at pH 10

Concentration (%)	Mission Grass (*Pennisetum Polystachion*)	
	Before Overliming	After Overliming
Acetic acid	9.58±0.25	17.97±0.38
5-(Hydroxymethylfurfural)	0.67±0.09	0.00±0.02
Furfural	0.50±0.04	0.19±0.01

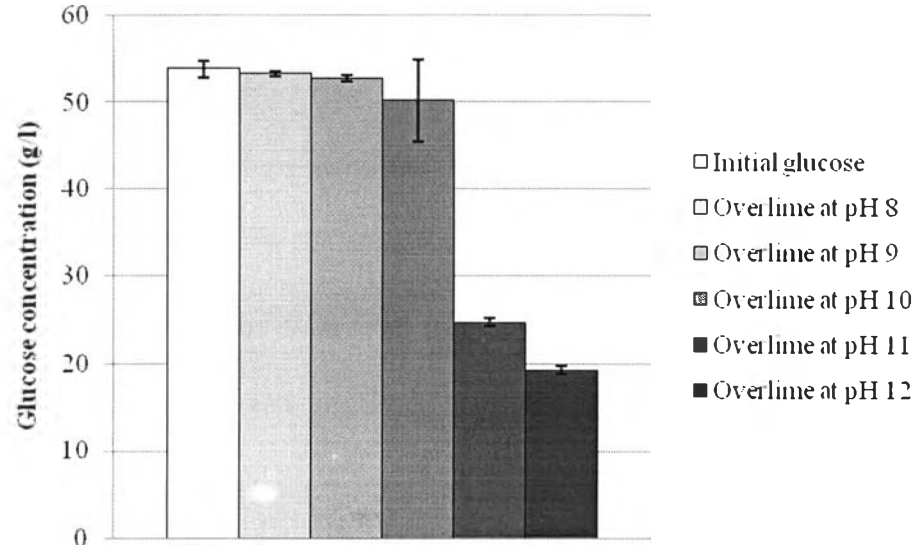
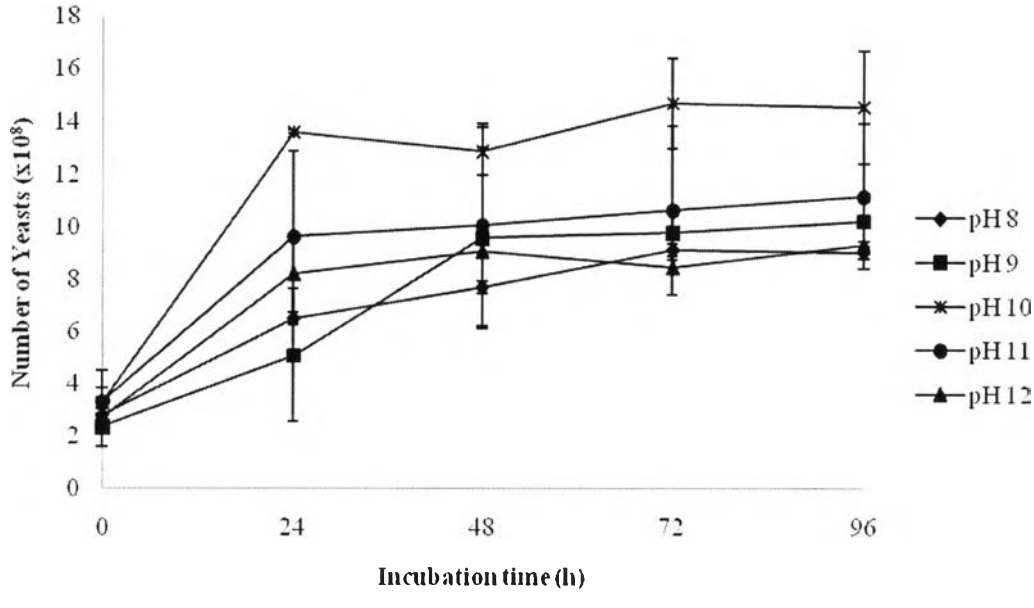
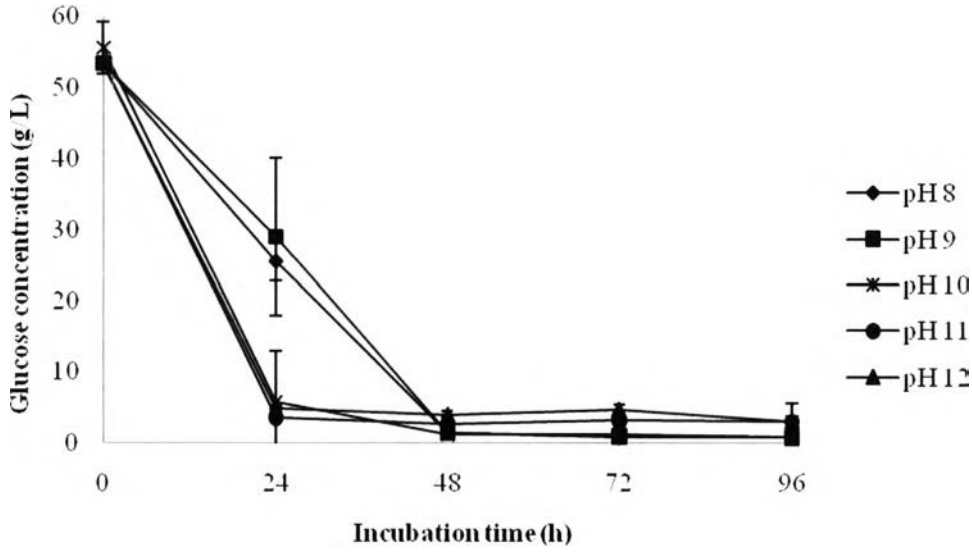


Figure 7.1 The effect of overliming in comparison to the hydrolyzate before overliming.

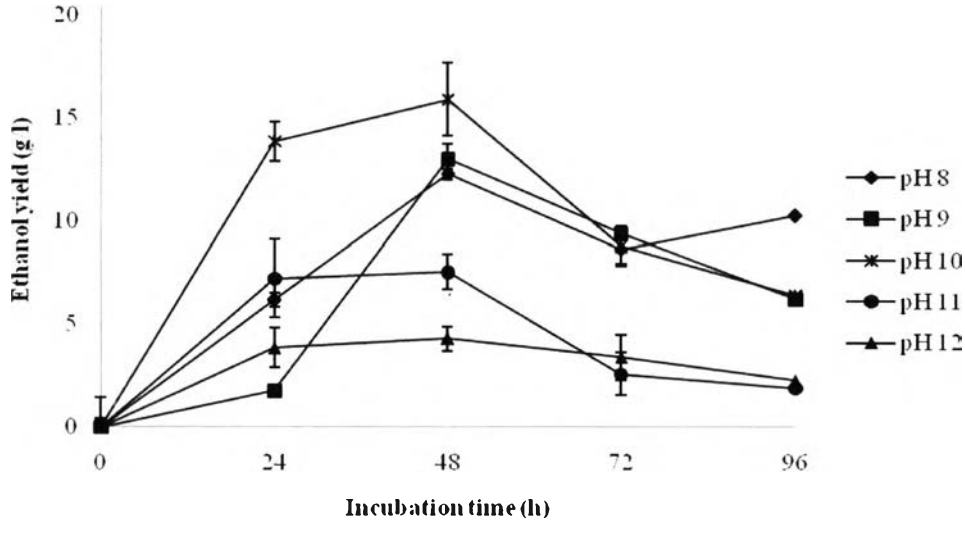


a)

Figure 7.2 a) Yeast (*S. cerevisiae* TISTR 5049) population at various overliming pHs per incubation time, b) glucose consumption of *S. cerevisiae* TISTR 5049 per incubation time, and c) ethanol production of MG hydrolyzate by *S. cerevisiae* TISTR 5049 at overliming pH 8-12.

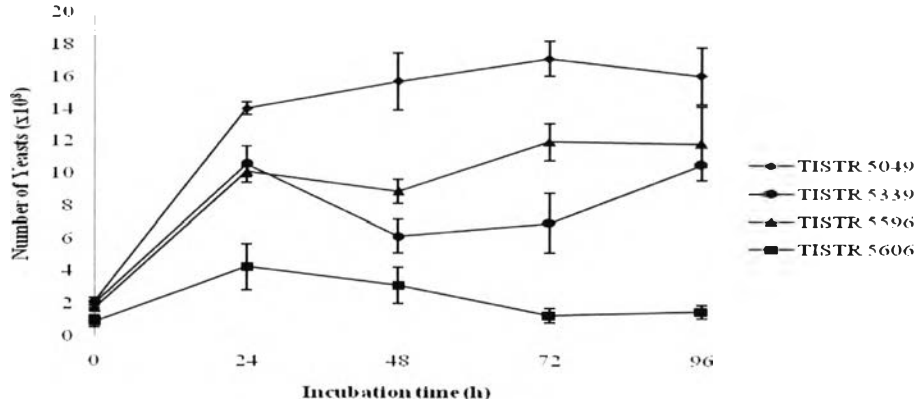


b)

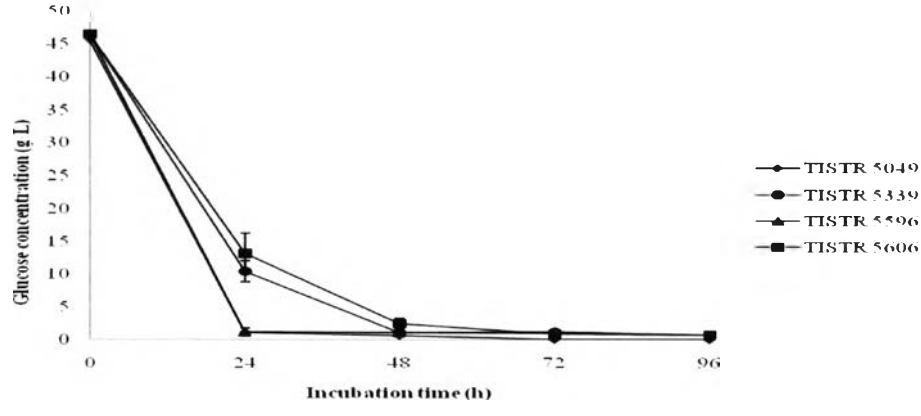


c)

Figure 7.2 a) Yeast (*S. cerevisiae* TISTR 5049) population at various overliming pHs per incubation time, b) glucose consumption of *S. cerevisiae* TISTR 5049 per incubation time, and c) ethanol production of MG hydrolyzate by *S. cerevisiae* TISTR 5049 at overliming pH 8-12. (Con't.)



a)



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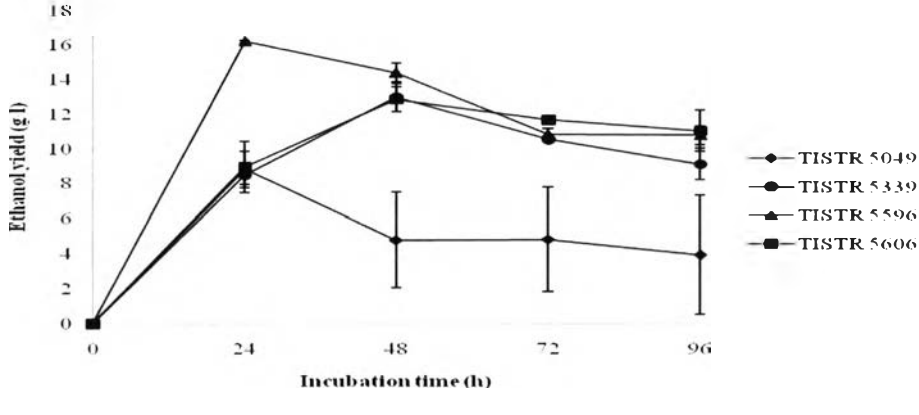

c)

Figure 7.3 a) Various strains of baker's yeast (*S. cerevisiae*) count per incubation time at pH 10 overliming, b) glucose concentration per incubation time for various strains of baker's yeast *S. cerevisiae* at pH 10 overliming, and c) the production of ethanol from various strains of *S. cerevisiae* in 96 h.