สำนักงานวิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Corncob Composition

The elemental composition of the corncob is shown in Table 4.1. Carbon was found to be the major component, followed by oxygen and hydrogen. The chemical composition of corncob is shown in Table 4.2. Cellulose was found to be the major component, followed by hemicellulose and lignin.

Table 4.1 Elemental composition of the corncob

Elemental composition	**wt%, dry basis**
Carbon	51.06
Hydrogen	7.46
Oxygen	40.02
Nitrogen	1.29
Sulfur	0.18

Table 4.2 Chemical composition of the corncob

Chemical composition of corncob	**wt%, dry basis**
Extractives	3.32
Hemicellulose	31.26
Cellulose	47.37
Lignin	17.06

The physical properties of the two corncob samples, including accessible particle size, surface area, pore volume, and pore size are shown in Table 4.3.

Table 4.3 Physical properties of the corncob

Corncob	Particle size (μm)	Surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (A^o)
40 mesh	419	4.08	$4.54x10^{-3}$	44.53
60 mesh	249	6.26	$6.08x10^{-3}$	38.83

4.2 Enzymatic Hydrolysis

The corncob was hydrolyzed for 24 h at 37 °C by two effective isolates (strain A 002 and M 018). The two isolates were obtained from Thai higher termites *Microcerotermes* sp., and found to be effective for cellulose hydrolysis (Taechapoempol, 2009).

4.2.1 Effects of Production Medium on the Produced Sugar Concentration

To investigate the effects of production medium, two sets of experiment were carried out. The first reactor was added with the production medium, whereas the second one was added with the mineral mixture. The medium and mixture compositions were given in Chapter 3. The result shows that having the product medium gave higher glucose concentration than having the mineral mixture because the production medium consists of nitrogen sources such as yeast extract and malt that are essential for bacteria to grow and hydrolyze corncob to glucose. It also confirms that the hydrolysis needs not only carbon sources from the corncob but also nitrogen sources in the yeast extract and malt.

4.2.2 Effects of Corncob Particle Size on the Produced Sugar Concentration

In order to determine the optimum particle size of corncob for the enzymatic hydrolysis, two different particle sizes of the 40 and 60 mesh were investigated at the fixed substrate concentration of 1.5-1.6 g/L. As shown in Figure

4.1(a), the results show that the amount of glucose concentration from the hydrolysis of the 60 mesh size corncob with the bacteria strain A 002 sharply increased at the beginning to the maximum, about 1.08 g/L, before gradually decreasing. However, the glucose concentration from the hydrolysis of the 40 mesh size corncob with the same condition increased at a faster rate even though it took a longer time to reach the maximum, about 0.98 g/L, and then sharply decreased, as shown in the figure. Effects of the corncob particle size on the glucose concentration produced from the hydrolysis at 37 °C with the bacterial strain M 015 are shown in Figure 4.1 (b). The glucose concentration gradually increased until it reached the maximum point at 9 h before gradually decreasing and reaching a constant value of 0.52 and 0.49 g/L, for the 60 and 40 mesh sizes, respectively. Comparison between the glucose concentration from the hydrolysis of both sample sizes with both strains at 37 °C shows that only the performance of strain A 002 was affected by the difference sizes, i.e. the smaller the corncob particle size, the higher the amount of glucose produced.

Figure 4.2 (a) shows the effect of the two corncob sizes at 30 °C with the strain A 002. It clearly indicated that the two sample sizes hardly affected the glucose concentration. After glucose reaching the maximum value, the concentration decreased at a slower rate than that from the hydrolysis at 37 °C. Figure 4.2 (b) shows that the different sizes hardly affected the hydrolysis with the strain M 015 at 30 °C similar to that at 37 °C. The glucose concentration was constant for 6 h until it increased to the maximum value at 9 h. The glucose concentration clearly shows the slower hydrolysis rate with the strain M 015 than that with the strain A 002.

In summary, the particle size clearly affected the enzymatic hydrolysis with the strain A 002. The effects were more pronounced at 37 °C. At this temperature, the smaller particles, which had a higher specific surface area than the larger particles, can be hydrolyzed faster than the larger particle sample. The obtained results are in agreement with the results from Yeh *et al.* (2010), who reported that a particle size in the submicron scale caused a significant increase in the digestibility of cellulose.

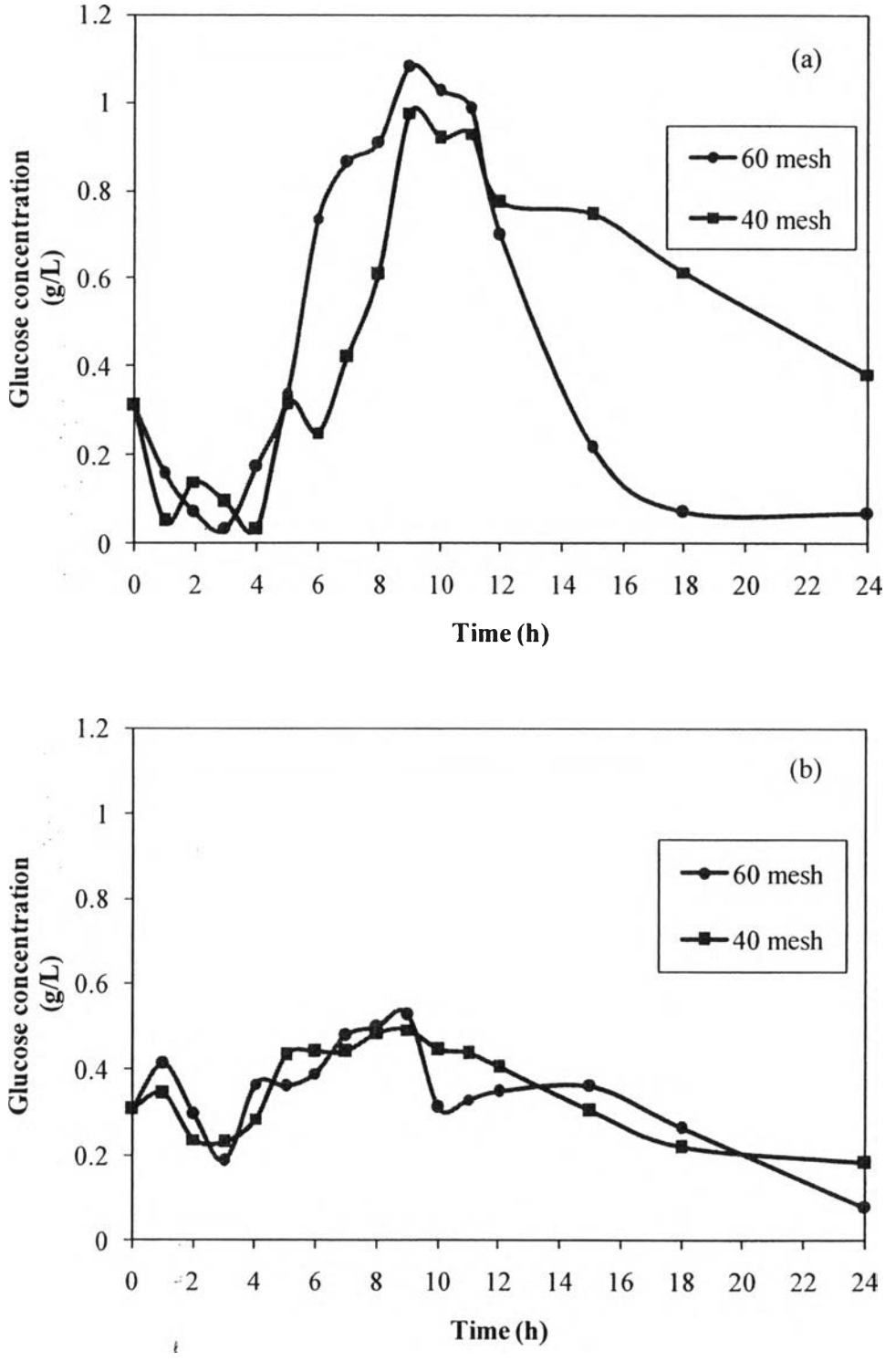

Figure 4.1 Effects of corncob particle size on the glucose concentration produced from the hydrolysis of corncob at 37 °C using the bacterial (a) strain A 002 and (b) strain M 015.

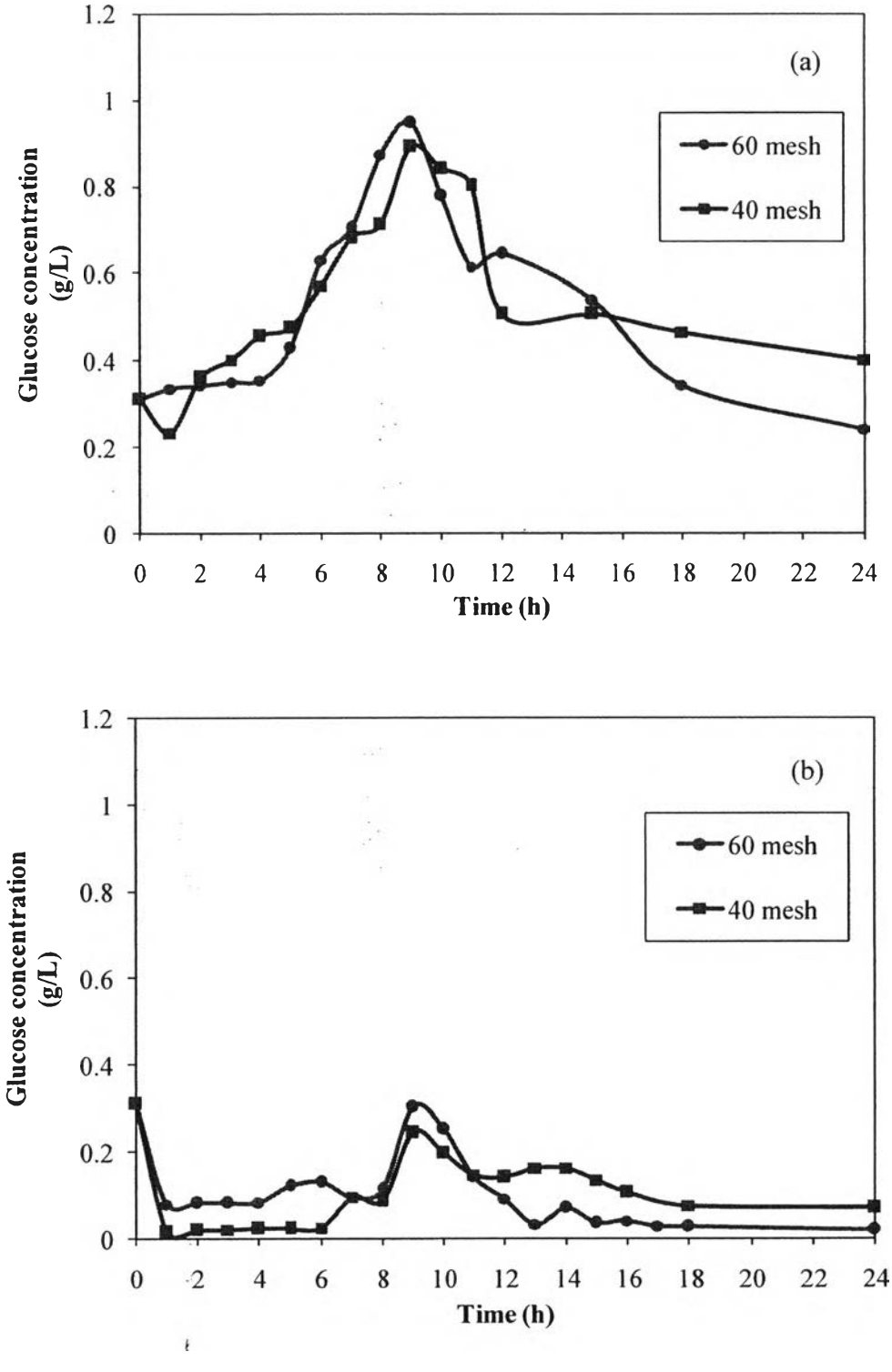

Figure 4.2 Effects of corncob particle size on the glucose concentration produced from the hydrolysis of corncob at 30 °C using the bacterial (a) strain A 002 and (b) strain M 015.

4.2.3 Effects of Hydrolysis Temperature on the Produced Sugar Concentration

Effects of temperature on the enzymatic hydrolysis of corncob were investigated at 30 and 37 °C. For the strain A 002 bacteria, the maximum amount of glucose about 1.08 g/L was produced at 9 h for the hydrolysis at 37 °C, as shown in Figure 4.3 (a). The figure also shows that the hydrolysis at 37 °C gave higher glucose concentration than that at 30 °C. The same effect of the temperature on the glucose production can be observed with the strain M 015, as shown in Figure 4.3 (b). It is worth mentioning that the strain A 002 bacteria seems not to be tolerant to the higher temperature as the glucose production rapidly decreases after reaching the higher glucose production. On the contrary, the strain M 015 bacteria possessed relatively slower hydrolysis rate over the studied time period. It is therefore important to examine the effect of temperature over a long time period for the highest cellulose conversion (Sorahi *et al.*, 2009).

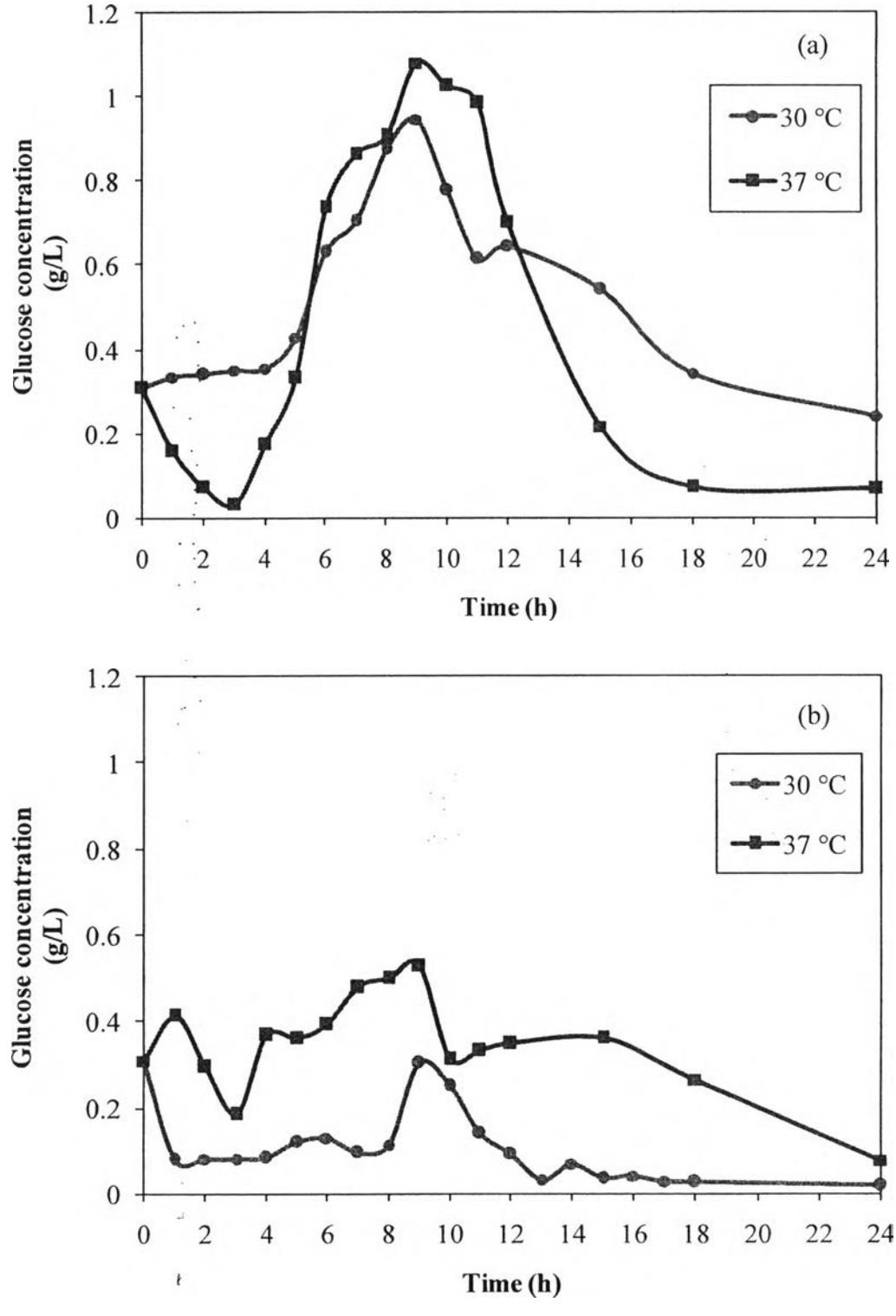

Figure 4.3 Effects of hydrolysis temperature on the glucose concentration produced from the hydrolysis of the 60 mesh particle size corncob using the bacterial (a) strain A 002 and (b) strain M 015.

4.2.4 Effects of Bacterial Strains on the Produced Glucose Concentration

4.2.4 Effects of Bacterial Strains on the Produced Glucose Concentration

Two bacterial strains, strains A 002 and M 015, were investigated for their performance on the enzymatic hydrolysis of corncob. With the strain A 002, the glucose concentration slightly increased from the beginning until reaching the maximum point, about 1.08 g/L, at 9 h from the hydrolysis of the corncob 60 mesh size at 37 °C, as shown in Figure 4.4. However, with the strain M 015, the glucose concentration was higher than that with the strain A 002 during the first five hours. The strain M 015 seems to be able to maintain the glucose concentration over 18 h albeit lower concentrations of glucose. This is because the higher specific endoglucanase activity of the strain M 015 was observed (Taechapoempol, 2009), where the endoglucanase attacks and creates more free chain-ends of cellulose. However, its β-glucosidase activity is not as high as that of the strain A 002, possibly resulting in the observed lower glucose concentration, but with a higher stability of glucose production.

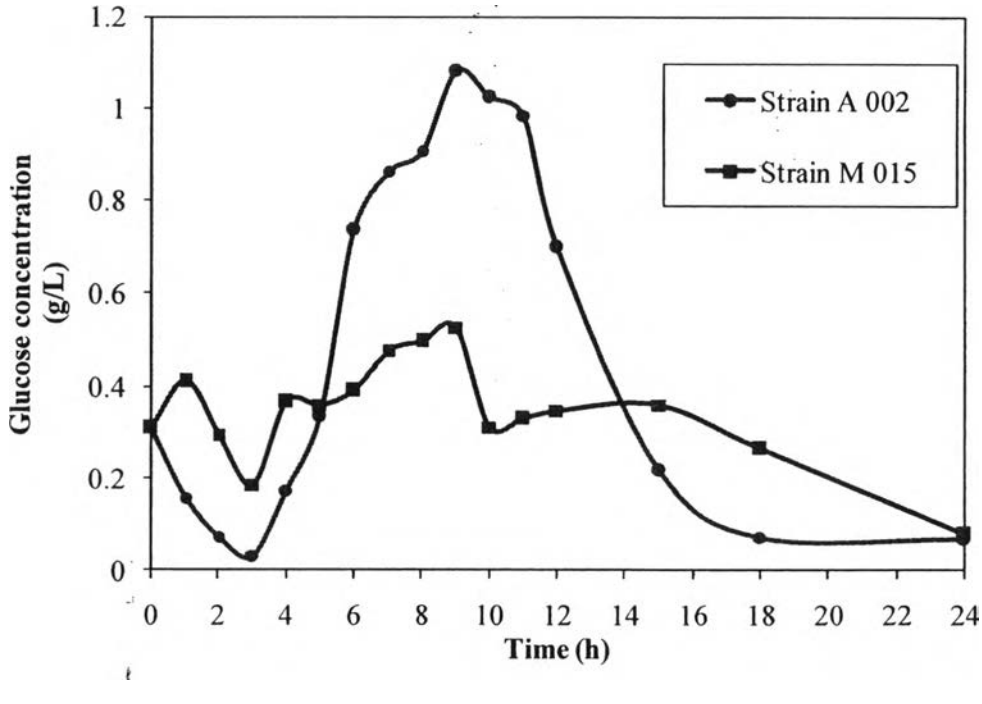

Figure 4.4 Effects of bacterial strains on the glucose concentration produced from the hydrolysis of the 60 mesh size corncob at 37 °C.

4.2.5 Glucose and Bacteria Evolution

Glucose production from the hydrolysis of the corncob with the strain A 002 slightly decreased before significantly increased until it reached the maximum at 9 h as shown in Figure 4.5. At the same time, the bacteria concentration gradually increased. After 9 h, the glucose concentration sharply dropped, while the bacteria concentration continued to increase. That may be because the produced glucose was consumed by the bacteria at a high amount after 9 h.

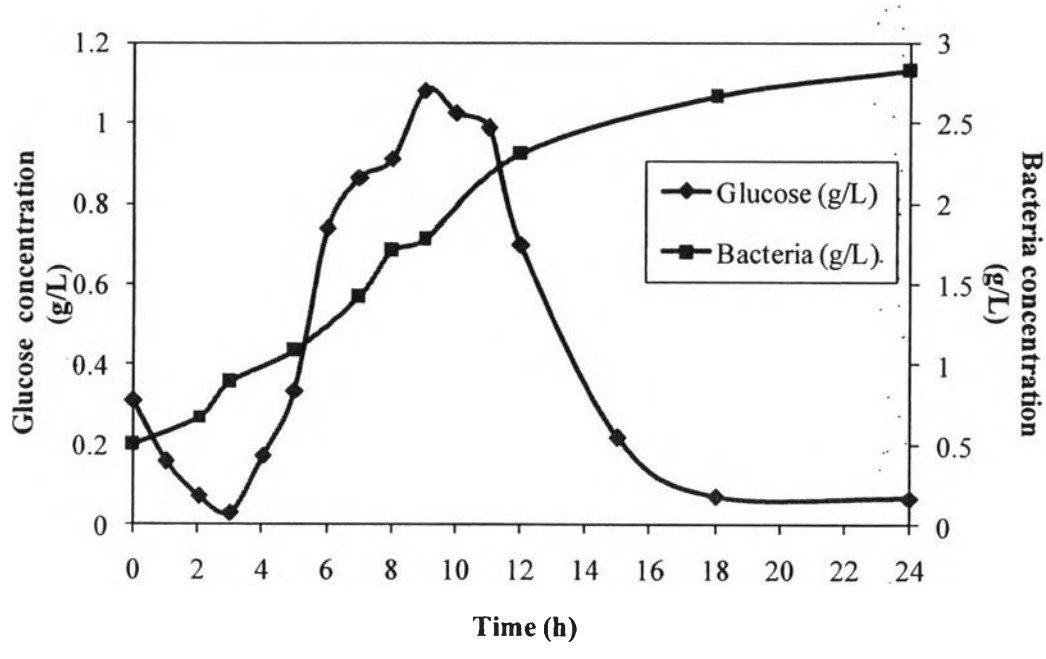

Figure 4.5 Glucose concentration and bacteria evolution from the enzymatic hydrolysis of the 60 mesh size corncob with the strain A 002 bacteria at 37 °C.

Figure 4.6 shows glucose concentration and bacteria evolution from the hydrolysis of the 40 mesh size corncob with the strain A 002 bacteria at 37 °C. Compared to Figure 4.5, it clearly indicated that the bacteria growth rate was slightly lower than that from the hydrolysis of the 60 mesh size. Despite the higher bacteria growth rate, the decrease in the glucose concentration did not drop as fast as that of the hydrolysis of corncob. It is possibly due to the bacteria grew at a slower rate during the hydrolysis with the larger corncob size.

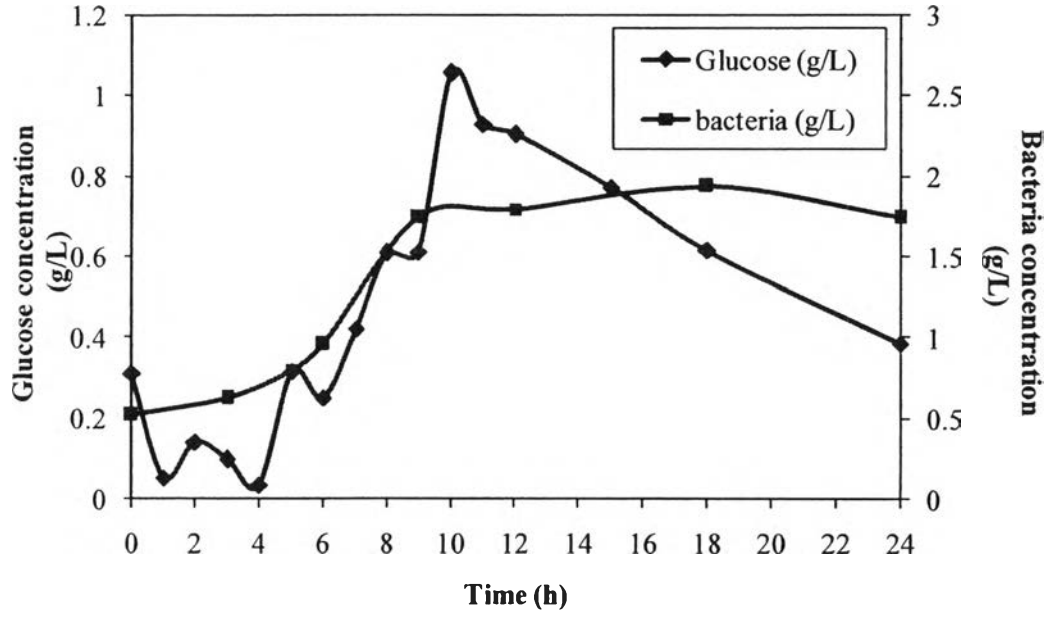

Figure 4.6 Glucose concentration and bacteria evolution from the enzymatic hydrolysis of the 40 mesh size corncob with the strain A 002 bacteria at 37 °C.

The glucose concentration and bacteria evolution from the hydrolysis of the 60 mesh size corncob with the strain A 002 at 30 °C are shown in Figure 4.7. The maximum glucose concentration of corncob about 0.95 g/L was produced at 9 h. After that, the glucose concentration decreases. Surprisingly, the bacteria growth rate at this temperature was higher during the first two hours before slowly increased and remained almost stable. The bacteria concentration after 24 h was lower than that at 37 °C.

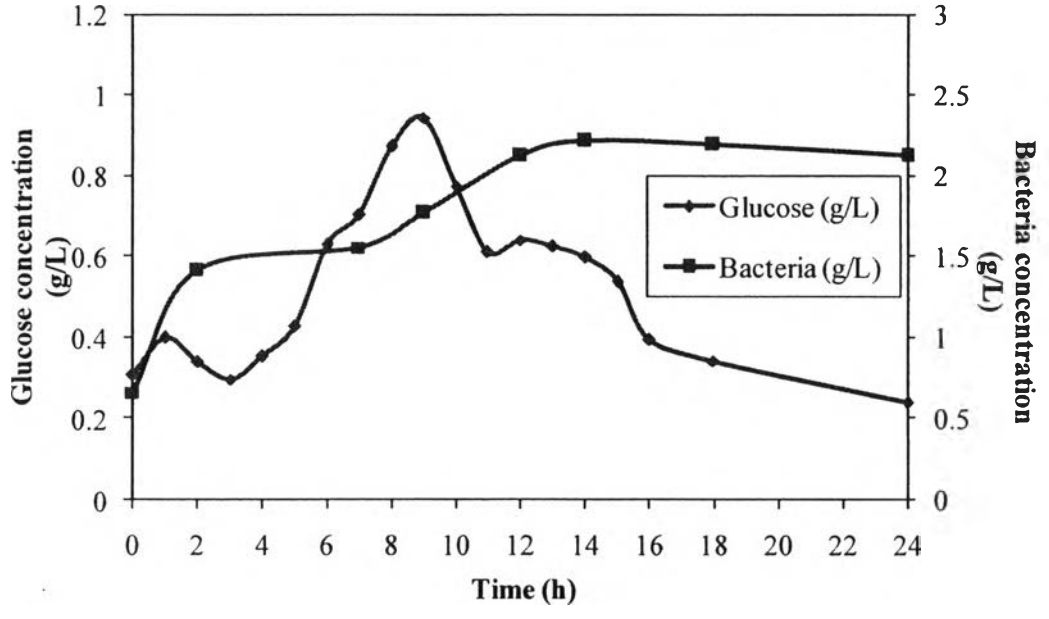

Figure 4.7 Glucose concentration and bacteria evolution from the enzymatic hydrolysis of the 60 mesh size corncob with the strain A 002 bacteria at 30 °C.

Figure 4.8 shows glucose concentration and bacteria evolution from the hydrolysis of the 40 mesh size corncob with the strain A 002 at 30 °C. The glucose concentration sharply increased and reached the highest concentration at 9 h. After that, the glucose concentration was quite stable and dropped. The bacteria concentration increased during the first few hours and then its remained steady.

Figure 4.9 shows glucose concentration and bacteria evolution from the hydrolysis of the 60 mesh size corncob with the strain M 015 at 37 °C. The glucose concentration increased and reached the highest concentration at 9 h. After that, the glucose concentration was quite stable and dropped again after 18 h. The bacteria concentration, however, increased during the first few hours and remained steady. Comparison between Figures 4.9 and 4.10 clearly indicated that the glucose concentration from the hydrolysis of the 40 mesh size corncob was more or less the same with that of the 60 mesh size. The same is also true for the bacteria concentration.

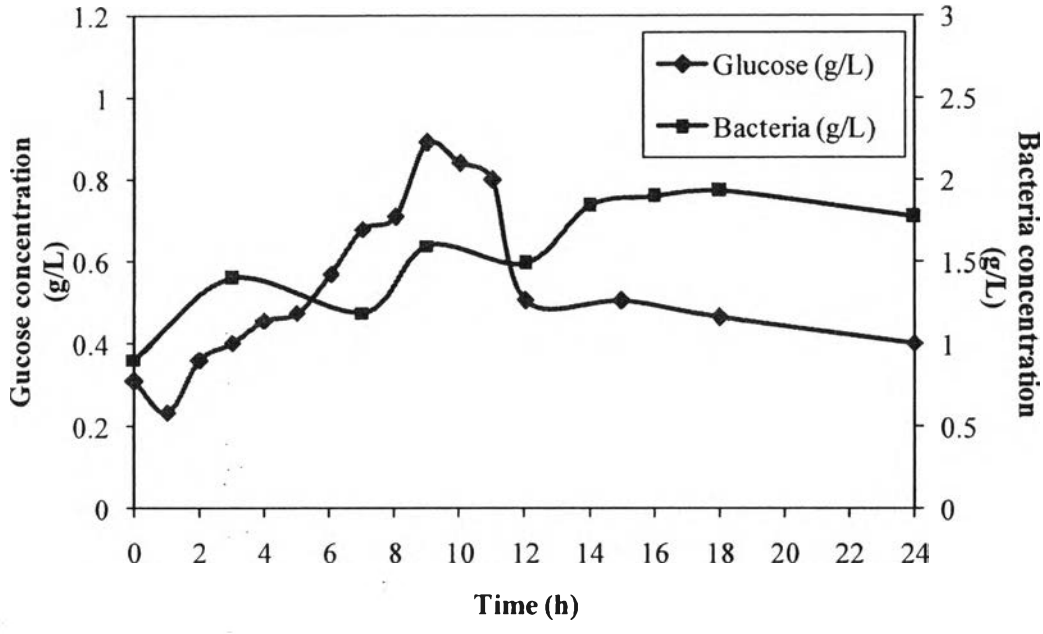

Figure 4.8 Glucose concentration and bacteria evolution from the enzymatic hydrolysis of the 40 mesh size corncob with the strain A 002 bacteria at 30 °C.

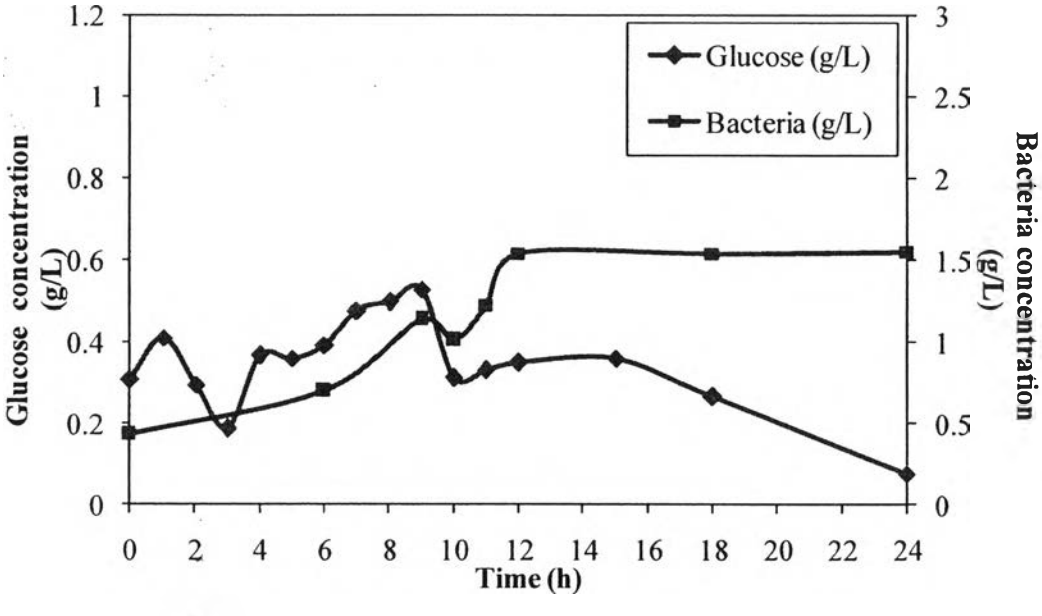

Figure 4.9 Glucose concentration and bacteria evolution from the enzymatic hydrolysis of the 60 mesh size corncob with the strain M 015 bacteria at 37 °C.

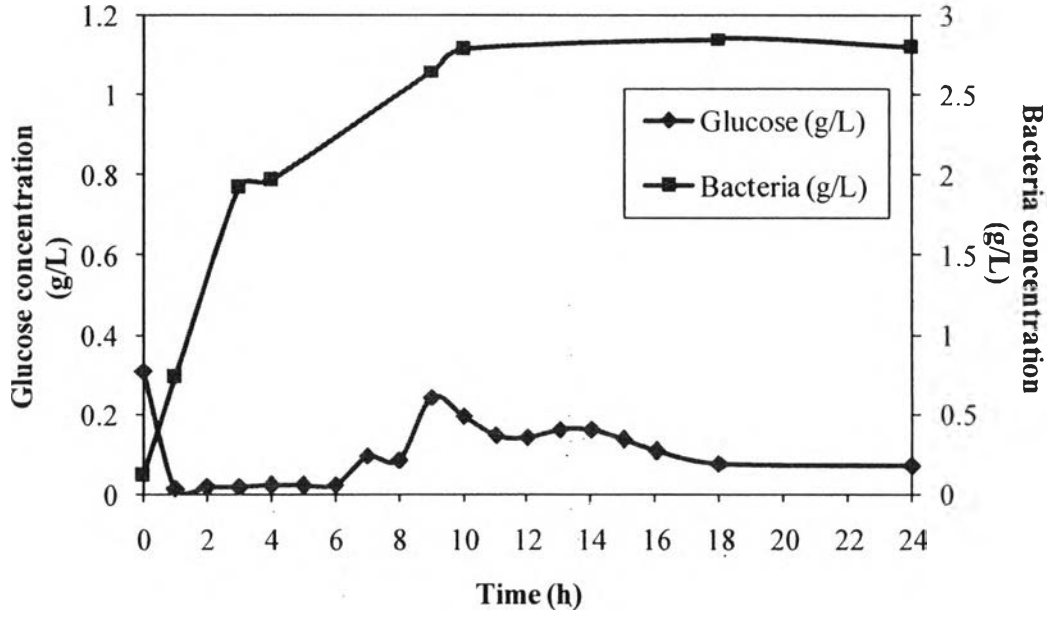

Figure 4.10 Glucose concentration and bacteria evolution from the enzymatic hydrolysis of the 40 mesh size corncob with the strain M 015 bacteria at 37 °C.

Figures 4.11 and 4.12 show results of glucose concentration and bacteria evolution from the hydrolysis of the 60 and 40 mesh size corncob samples with the strain M 015 bacteria at 30 °C. The glucose concentration in both figures was much lower than that at 37 °C. On the other hand, the bacteria growth at 30 °C was much higher than the other cases for both corncob sizes. Also, the larger corncob size resulted in the higher bacteria concentration.

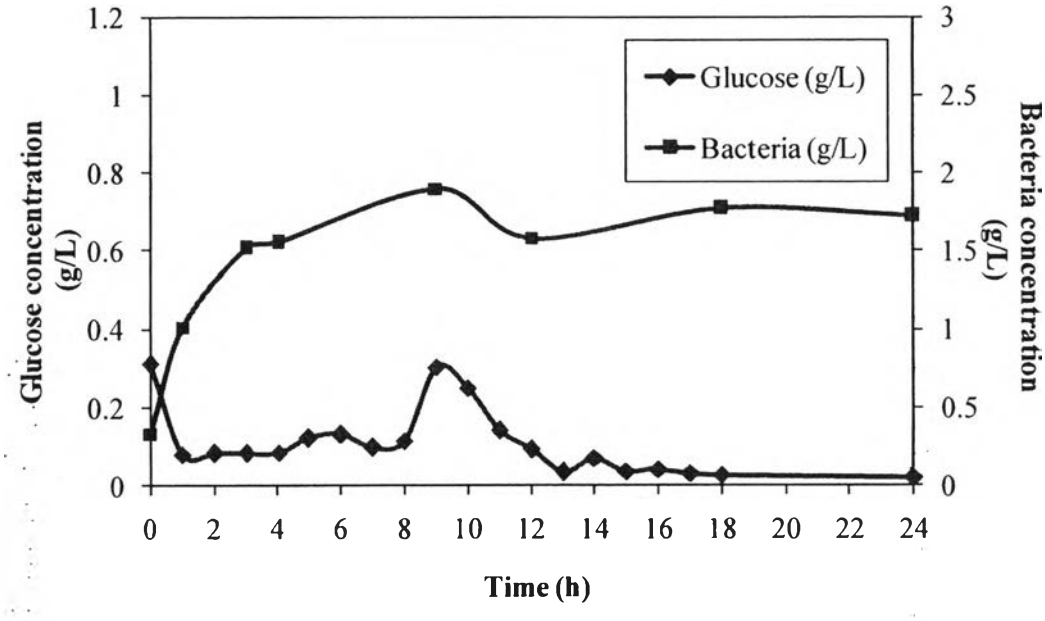

Figure 4.11 Glucose concentration and bacteria evolution from the enzymatic hydrolysis of the 60 mesh size corncob with the strain M 015 bacteria at 30 °C.

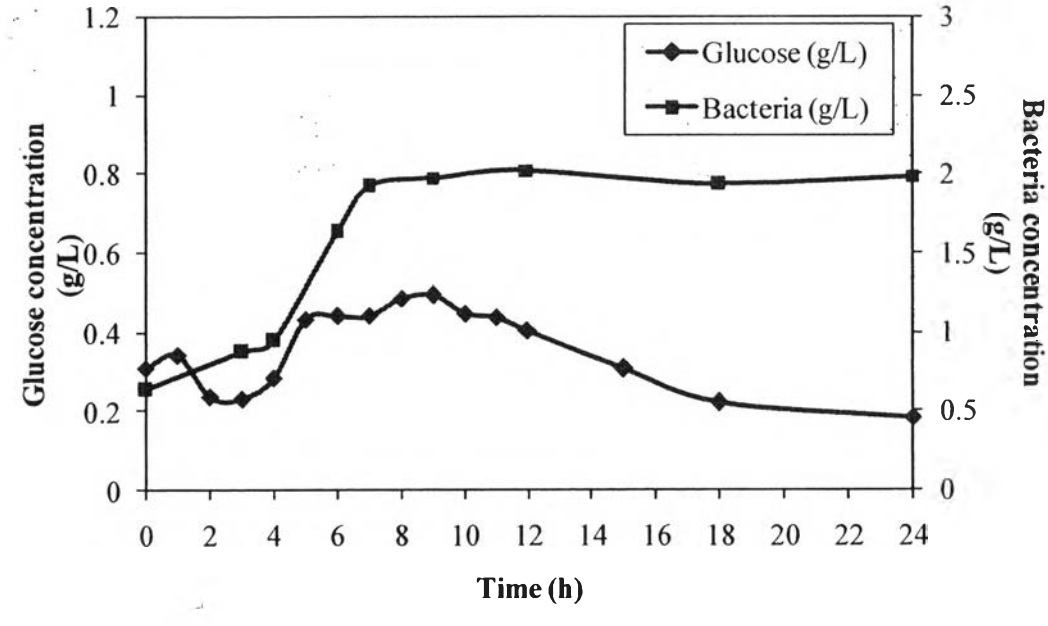

Figure 4.12 Glucose concentration and bacteria evolution from the enzymatic hydrolysis of the 40 mesh size corncob with the strain M 015 bacteria at 30 °C.

4.3 Structure of Enzymatically Hydrolyzed Corncob Sample

The morphological changes of corncob entities due to the hydrolysis process can be clearly seen from the scanning electron micrographs at 1,000 magnifications. Figure 4.13 (a) showed the original material with smooth surfaces. After the enzymatic hydrolysis at 37 °C with the strain A 002 and M 015, the morphology was significantly changed as shown in Figure 4.13 (b) and (c). This showed another indication that there was a physical transformation of the corncob sample after the hydrolysis. In addition, the micrographs after the hydrolysis can be used to identify the bacteria strain activity. As seen in Figure 4.13 (b), the morphological was changed significantly, which could probably be resulted from the higher activity of the strain A 002.

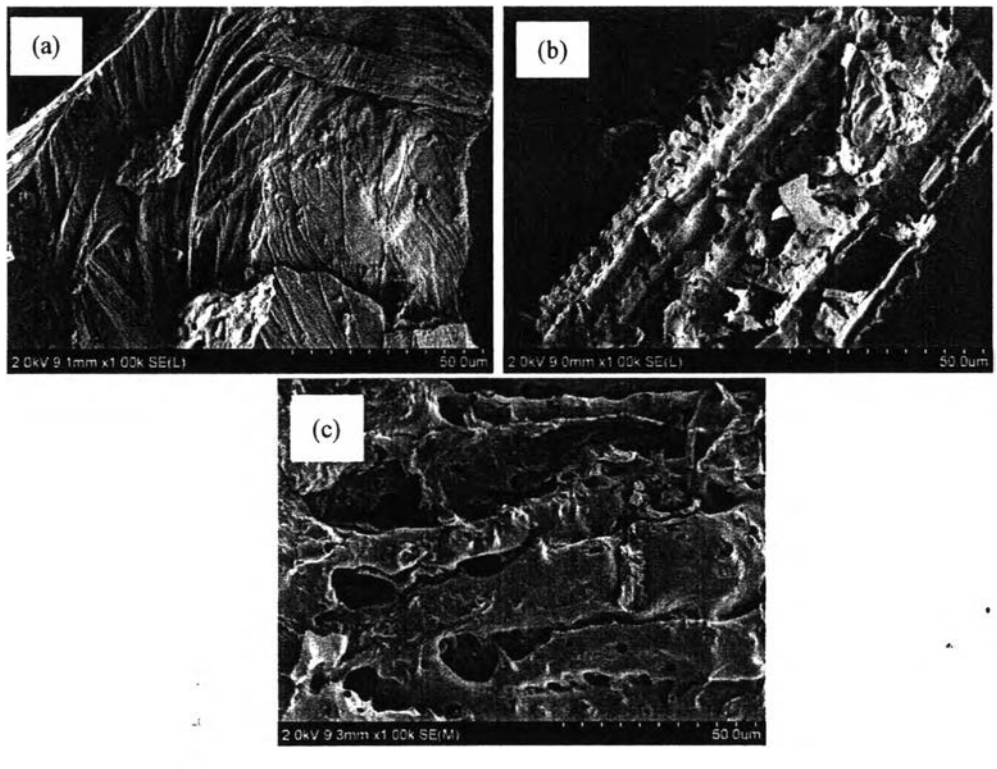

Figure 4.13 Scanning electron micrographs of the 60 mesh size corncob surface (a) before hydrolysis (b) after hydrolysis at 37 °C with the strain A 002 and (c) after hydrolysis at 37 °C with the strain M 015.

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The chemical composition of the 60 mesh size corncob after the hydrolysis at 37 °C with the strains A 002 and M 015 was determined, as shown in Table 4.4. Compared to Table 4.2, the results clearly indicated that the cellulose composition after the hydrolysis with the strains A 002 and M 015 was much less than that before the hydrolysis. This implies that cellulose is the main component of the corncob that is hydrolyzed by the cellulase-producing bacteria (A 002 and M 015) to yield glucose as the main sugar component in the liquid products.

Table 4.4 Chemical composition of the 60 mesh size corncob after the hydrolysis at 37 °C with the strains A 002 and M 015

Chemical composition of corncob	**wt%, dry basis** (Strain A 002)	**wt%, dry basis** (Strain M 015)
Extractives	6.50	6.96
Hemicellulose	33.52	21.82
Cellulose	7.95	15.26
Lignin	52.03	55.96