CHAPTER IV
RESULTS AND DISCUSSION

4.1 Corncob Composition

The results of elemental and chemical composition of corncob are shown in Tables 4.1 and 4.2, respectively. For the elemental composition of corncob, oxygen, and carbon are the major component in the corncob. For the chemical composition, cellulose is the major component, followed by hemicellulose. This corncob has quite high cellulose but low lignin content, which is consistent with previously published results for other agricultural residues (Van Dyk and Pletschke, 2012).

Table 4.1 Elemental composition of corncob

Elemental composition	wt%, dry basis
Carbon	46.16
Hydrogen	6.15
Nitrogen	0.49
Oxygen	47.13
Sulfur	0.07

Table 4.2 Chemical composition of corncob

Chemical composition of corncob	wt%, dry basis
Cellulose	43.82
Hemicellulose	39.62
Lignin	13.63
Extractive	2.93

4.2 Determination of Hydrolysis Capacity Value (HC Value)

The hydrolysis capacity (HC) value is a parameter used to determine the enzymatic activity, which governs the rate of cellulose hydrolysis in the primary step (Zhang *et al.*, 2006). The HC value is defined as the ratio of the diameter of the appeared clear-zone to the diameter of the bacterial colony. In this work, to approximately estimate the activity of any given bacterial strains, the hydrolysis capacity value measurement of strain A 002 and strain M 015 was performed. The HC values of strain A 002 and M 015 are in Table 4.3. The result showed that the HC value of strain A 002 was significantly higher than strain M 015. Hence, it was reasonable to summarize that strain A 002 provided higher efficiency for cellulose degradation compared to strain M 015. In addition, this result was in agreement with Taechapoempol *et al.* (2011).

Table 4.3 Hydrolysis capacity values of bacterial strain A 002 and strain M 015

Bacterial strain	Hydrolysis capacity value
A 002	1.79
M 015	1.52

4.3 Microbial Hydrolysis

The concentration profile of the produced glucose from a microbial hydrolysis is a crucial point for further ethanol production process. Therefore, two effective isolates (strain A 002 and M 015) obtained from the Thai higher termites *Microcerotermes* sp., were employed to hydrolyze corncob with different particle sizes (40–60, 60–80, and 80–100 mesh) and different quantity of malt extract (6, 8, 10, and 12 g/L).

4.3.1 Effect of Corncob Particle Size

The effect of corncob particle size was investigated with three different sizes—40–60, 60–80, and 80–100 mesh. The microbial hydrolysis was operated at the fixed condition of 1.0 g/L corncob loading at 37 °C.

As shown in Figure 4.1, the amount of glucose concentration from the hydrolysis of all corncob size with the bacterial strain A 002 increased with the increase in the operating time and reached the maximum glucose concentration. However, the amount of glucose concentration gradually decreased afterwards. The highest glucose production was obtained by the hydrolysis of 80–100 mesh corncob. Its evolution rapidly increased from the initial time and reached the maximum glucose concentration of 0.6 g/L at 6 h. After that, the glucose production drastically decreased and remained constantly after 16 h. Moreover, the glucose evolution from the hydrolysis of 40–60 mesh and 60–80 mesh corncob was 0.39 and 0.48 g/L, respectively, at 8 h. They show the same trend with that from the 80–100 mesh; however, the maximum glucose concentration was lower than that of 80–100 mesh.

In addition, the glucose evolution from the hydrolysis with the bacterial strain M 015 was also investigated, and the result is shown in Figure 4.2. The glucose evolutions did not change significantly over the period of hydrolysis study for all studied sizes, and the maximum glucose concentration was lower than using the bacterial strain A 002. They gradually increased and reached the maximum point and then they gradually decreased. Similar to the result of strain A 002, the highest glucose production was obtained from the hydrolysis of the smallest corncob particle size (80–100 mesh) which had the maximum glucose concentration of 0.46 g/L at 8 h. The higher corncob particle sizes had the lower maximum glucose concentration—0.42 g/L for 40–60 mesh and 0.43 g/L for 60–80 mesh at 10 h. The results suggested that for both bacterial strains—A 002 and strain M 015, the more effectiveness of glucose production can be achieved from the smaller corncob particle size. Moreover, the effect of the particle size was more pronounced on the hydrolysis with the strain A 002.

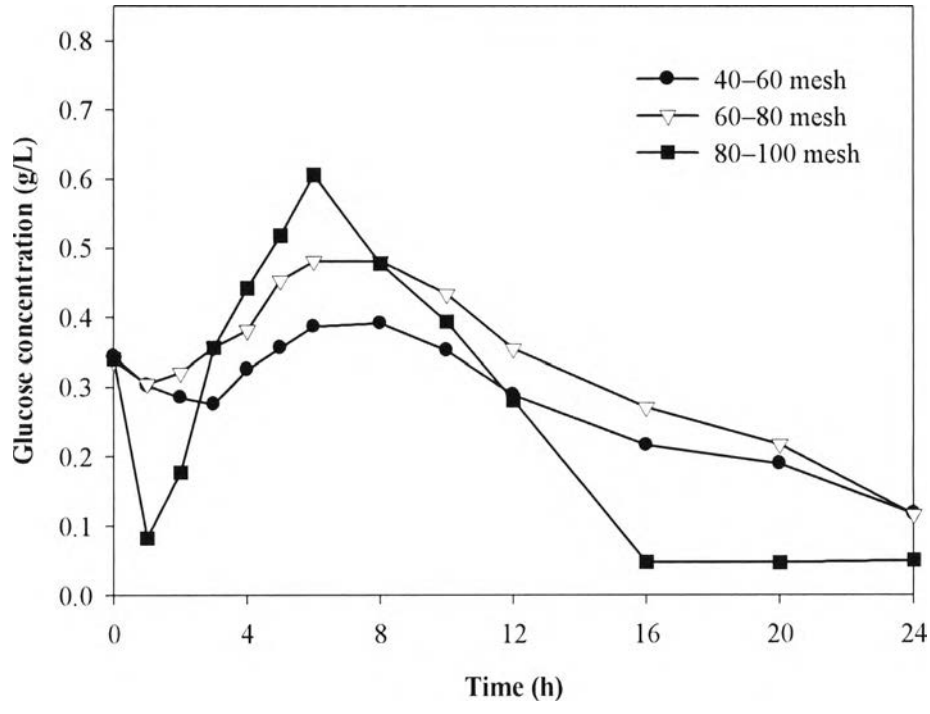

Figure 4.1 Effect of corncob particle size on the produced glucose concentration from the hydrolysis of corncob at 37 °C using the strain A 002.

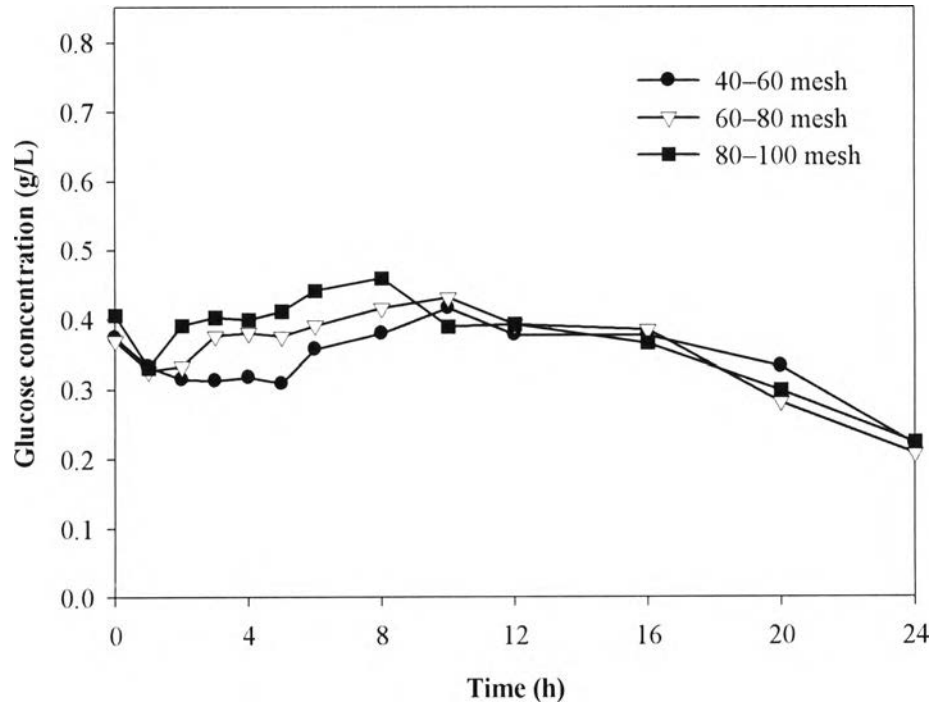

Figure 4.2 Effect of corncob particle size on the produced glucose concentration from the hydrolysis of corncob at 37 °C using the strain M 015.

The BET method was done to experimentally confirm why the smaller corncob particle size had higher glucose production efficiency in terms of amount of glucose production and optimum hydrolysis time. The BET results revealed that the smaller particle size had higher specific surface area as shown in Table 4.4. Therefore, it can be explained by the fact that the higher the specific surface area, the higher the reaction—bacterial hydrolysis efficiency That is because there are higher possibility of bacteria to attach on the surface of corncob, undergo the reaction, and produce glucose compared to the lower specific surface area. The obtained results were consistent with Yeh *et al.* (2010), who reported that the particle size in the submicron scale caused a significant increase in the glucose yield. And also, the smaller corncob particle size can be hydrolyzed faster than the larger one. Consequently, the optimum hydrolysis time of 80–100 mesh was found at 6 h with the bacterial strain A 002 and at 8 h for the bacterial strain M 015, which was shorter than that from the 60–80 mesh and 40–60 mesh for both strains. These results could be supported by Abasaeed and Mansour (1992), who also investigated the effect of particle size on the reaction time too.

Table 4.4 Specific surface area of corncob particle

Corncob	Surface area (m^2/g)
40–60 mesh	3.2
60–80 mesh	6.3
80–100 mesh	6.7

4.3.2 Effect of Bacterial Strains

The bacterial hydrolysis performance to produce glucose from corncob was investigated by using bacteria, *Bacillus subtilis*, with different strains, — A 002 and M 015, at the fixed corncob particle size of 80–100 mesh. As shown in Figure 4.3, the strain A 002 produced higher glucose concentration than the strain M 015. The glucose concentration profile from the bacterial hydrolysis using the strain A 002 show that the glucose concentration rapidly increased and reached the

maximum point having the glucose concentration of 0.6 g/L at the hydrolysis time of 6 h. On the contrary, the glucose concentration profile using the strain M 015 slightly increased with the increase in the hydrolysis time and reached the maximum point having the glucose concentration of 0.46 g/L at the hydrolysis time of 8 h. This may be resulted from the different enzymes in each strain. The strain M 015 contains higher endoglucanase than β-glucosidase, whereas the strain A 002 contains higher β-glucosidase than endoglucanase. The endoglucanase has the roles of attacking and creating free chain-ends of cellulose, while the β-glucosidase cleaves the small molecule of cellulose to be glucose. Hence, it was unquestionable to conclude that why the strain A 002 had more efficiency for producing glucose than the strain M 015, which was consistent with the hydrolysis capacity values (Taechapoempol *et al.*, 2011).

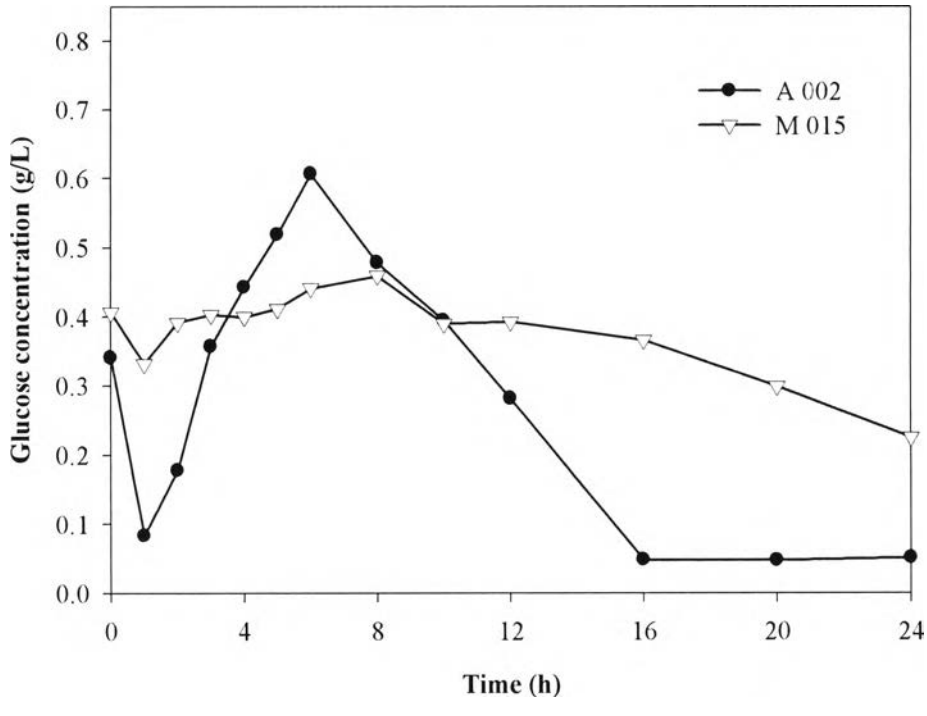

Figure 4.3 Effect of bacterial strains on the glucose concentration produced from the hydrolysis of 80–100 mesh corncob at 37 °C.

4.3.3 Effect of Concentration of Secondary Carbon Source

From the 65 modified DSMZ broth medium 2, which used 10 g/L malt extract loading. In order to optimize the malt extract loading in this formula, the concentation of the malt extract used in the microbial hydrolysis of 80–100 mesh corncob was varied. Figure 4.4 clearly shows that the maximum glucose concentration from 6, 8, 10, and 12 g/L malt extract loading in the production medium were 0.42, 0.48, 0.64, and 0.80 g/L, respectively. The results indicated that the higher the amount of the malt extract loading, the higher the glucose concentration. This can be explained that the malt extract provides the carbon, protein, and nutrient sources required for the growth of microorganisms (Altaf *et al.*, 2007). Therefore, as the higher amount of malt extract was available for growing the bacteria, the released enzyme was also high for the hydrolysis process.

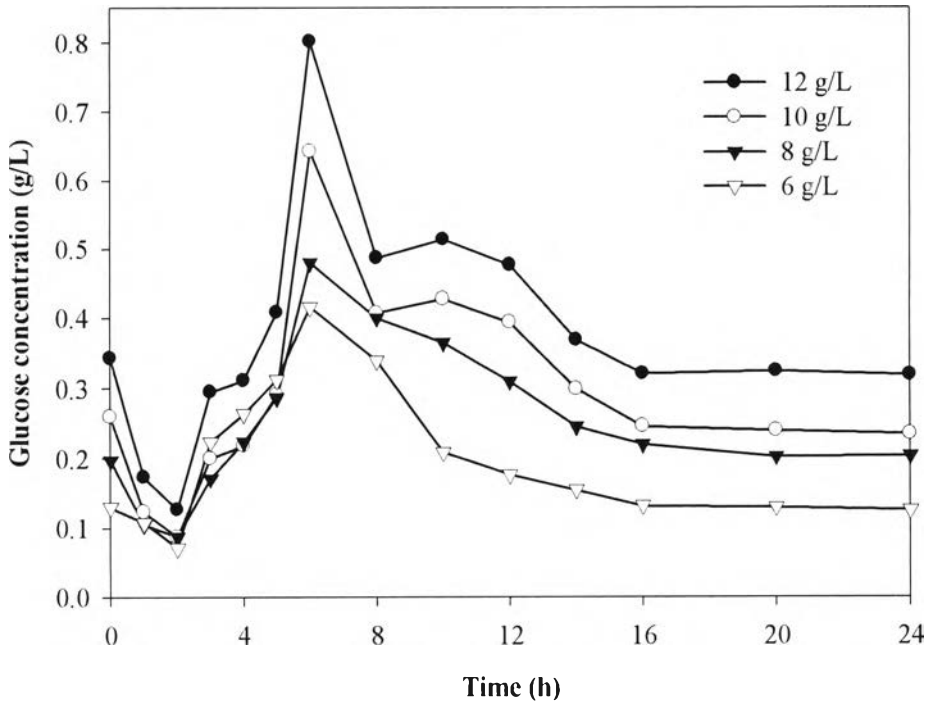

Figure 4.4 Effect of malt extract loading on the produced glucose concentration from the hydrolysis of 80–100 mesh corncob using the strain A 002 at 37 °C.

4.3.4 Glucose and Bacteria Evolutions

The optimum glucose prodution obtained from the hydrolysis of 80–100 mesh corncob with both bacterial strains at 37 °C were compared with the bacteria concentration, shown in Figures 4.5 and 4.6. Glucose significantly increased after 1 h until reaching the maximum glucose concentration and continued to decrease until 24 h, while the bacteria concentration continuously increased. It implies that the glucose is consumed by the bacteria after 6 and 8 h for the strain A 002 and M 015, respectively.

Comparison between the bacteria concentration from the hydrolysis with the strain A 002 and M 015 shows that the strain A 002 has higher growth rate than the strain M 015. This also indicates the higher activity of the strain A 002 than the strain M 015 due to its higher in cellulase activity.

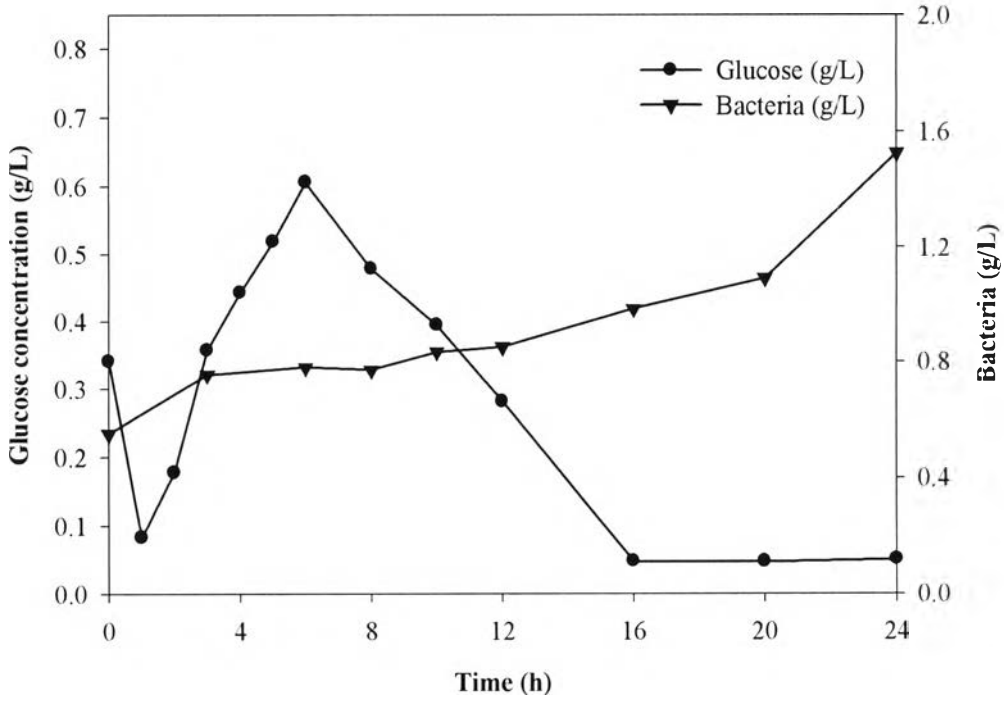

Figure 4.5 Glucose and bacteria evolutions from the hydrolysis of the 80–100 mesh corncob using the strain A 002 bacteria at 37 °C.

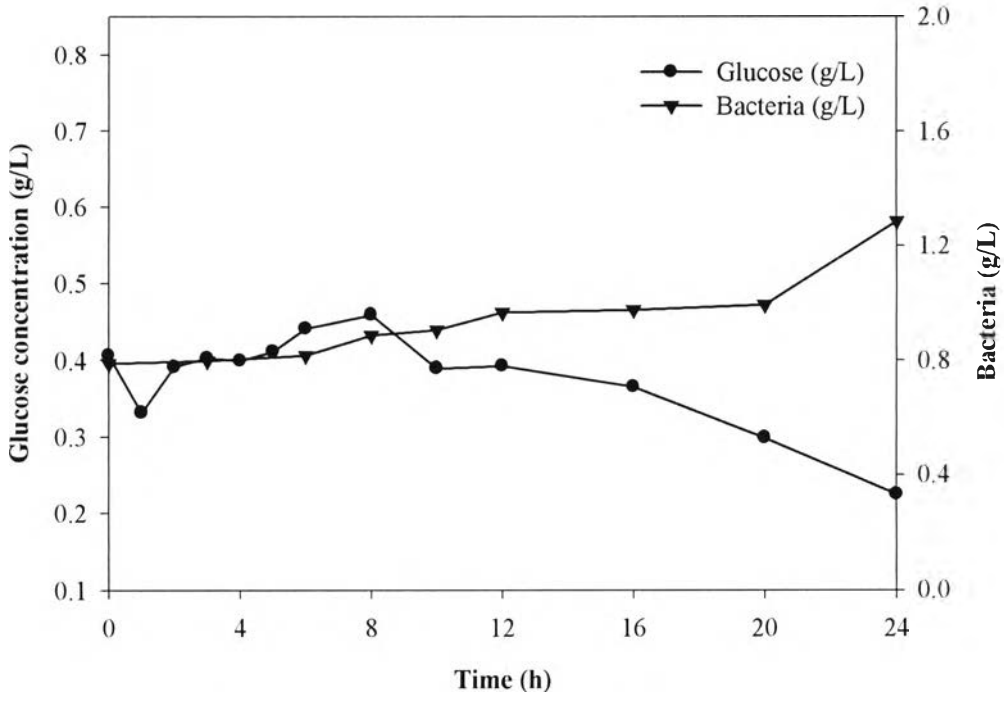

Figure 4.6 Glucose and bacteria evolutions from the hydrolysis of the 80–100 mesh corncob using the strain M 015 bacteria at 37 °C.

4.4 Enzymatic Hydrolysis

In order to compare the efficiecy of glucose production from using the bacterial enzyme with a commercial enzyme, the enzymatic hydrolysis using commercial cellulase enzyme from *Aspergillus niger* was investigated. The corncob particle size (80–100 mesh) was hydrolyzed with different commercial cellulase enzyme loadings.

4.4.1 Effects of Cellulase Enzyme Loading

The effect of commercial cellulase enzyme loading on the production of glucose was investigated by varying concentrations of cellulase loading (100, 200, 300, 400, and 500 U per gram corncob). The enzymatic hydrolysis was operated at the fixed condition of 1.0 g/L corncob loading and pH 5.5 at 37 °C. The glucose concentration profiles were examined over a 72 h period.

For any cellulase loading, the glucose production from the enzymatic hydrolysis of corncob by *Aspergillus niger* continuously increased within the first 12 h and then gradually increased as shown in Figure 4.7. The results could be explained by the fact that the enzymatic hydrolysis of corncob is a heterogonous reaction, which depends directly on the available interfacial surface area. At the beginning of the reaction, there was more possibility of the soluble enzyme to adsorb on the available surface and undergo the reaction, leading to the number of available surface was gradually decreased. Afterwards, the reaction took place with the remaining available surface resulting in the slow rate of glucose production. This result is in agreement with a report by Highina *et al.* (2011). In addition, the maximum glucose concentration of 0.80 g/L was obtained by using 500 U cellulase per gram corncob; however, beyond the cellulase loading of 300 U per gram corncob, the glucose concentration was slightly increased as shown in Figure 4.8. The results can be concluded that the higher the cellulase enzyme loading, the higher the glucose concentration.

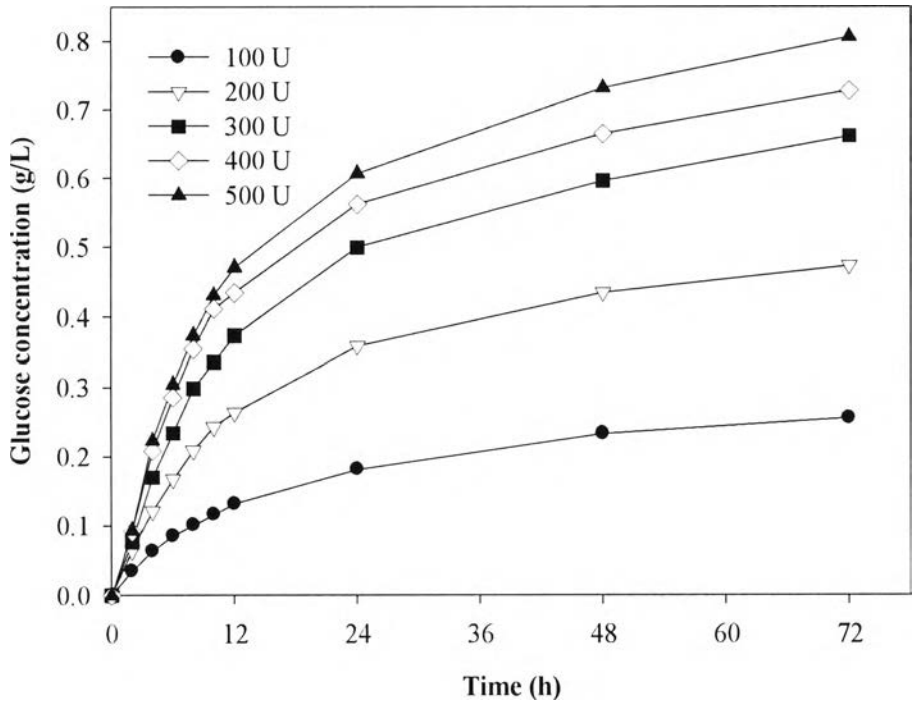

Figure 4.7 Effect of cellulase enzyme loading on the glucose concentration produced from the hydrolysis of the 80–100 mesh corncob at 37 °C.

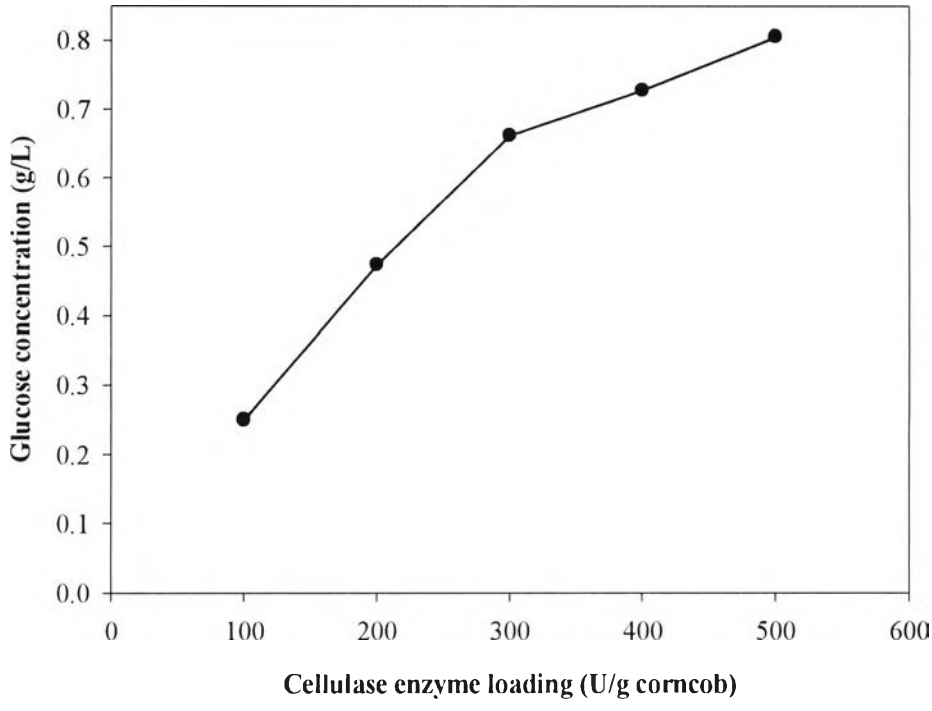

Figure 4.8 Maximum glucose produced from the hydrolysis of the 80–100 mesh corncob at 37 °C by varied cellulase enzyme loading.

The optimum glucose production from microbial hydrolysis of the 80–100 mesh corncob particle size using strain A 002 at 37 °C was compared with that from enzymatic hydrolysis using commercial cellulase enzyme loading, *Aspergillus niger*. The maximum glucose concentration from the microbial hydrolysis was 0.6 g/L, which is equivalent to the glucose concentration obtained from the enzymatic hydrolysis with using 273 U cellulase per gram corncob. As the cost of the commercial cellulase enzyme contributes significantly to the total cost of cellulose conversion process, these results indicate that the use of the microbial hydrolysis is more economical than the enzymatic hydrolysis. In addition, the results show that the microbial hydrolysis can reach the optimum condition (6 h) faster than the enzymatic hydrolysis (72 h).

4.5. Structure of Hydrolyzed Corncob Sample

The scanning electron micrographs at 1,200 magnifications revealed the corncob morphology before and after the hydrolysis. The smooth surface of corncob was observed before the hydrolysis as shown in Figure 4.9(a). However, the morphology was obviously changed to be rough and porous surface after the corncob was hydrolyzed by the bacteria either strain A 002 or M 015 and commercial cellulase enzyme, *Aspergillus niger*, at 37 °C as illustrated in Figure 4.9(b), 4.9(c), and 4.9(d), respectively.

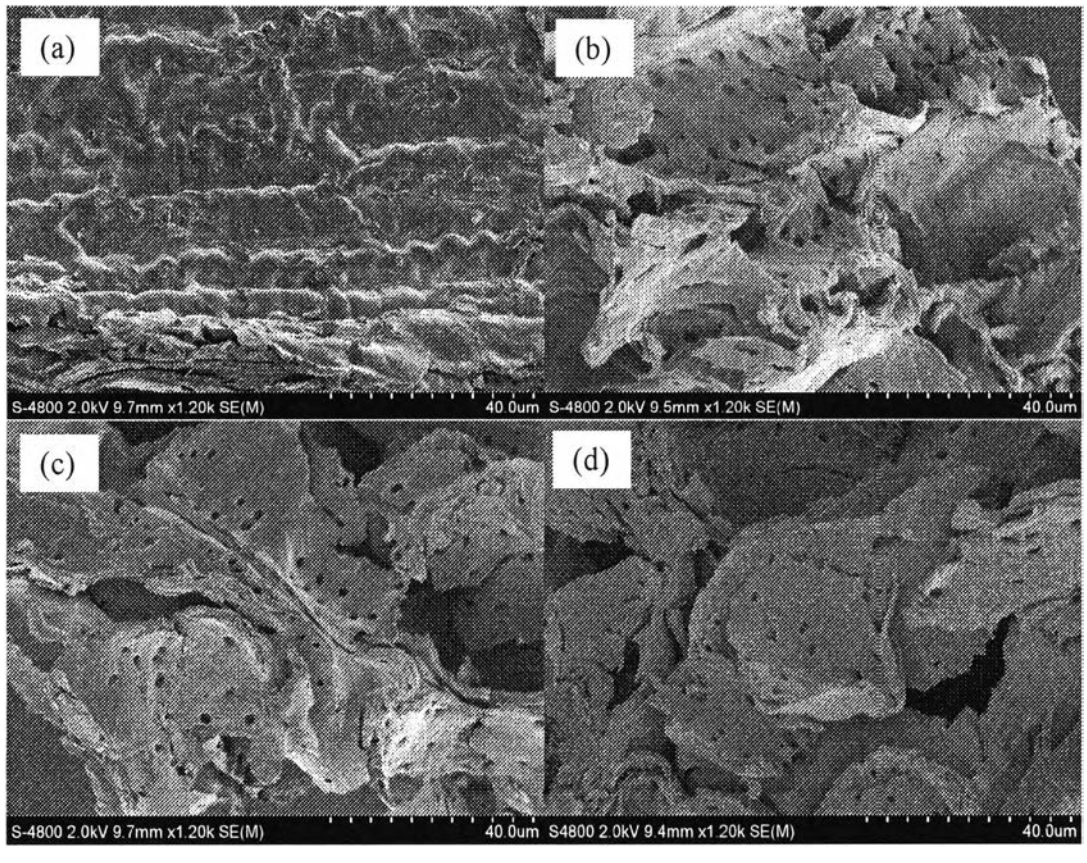

Figure 4.9 Scanning electron micrographs of the 80–100 mesh corncob surfaces (a) before hydrolysis; (b) after hydrolysis at 37 °C with the strain A 002; (c) after hydrolysis at 37 °C with the strain M 015; and (d) after hydrolysis at 37 °C with commercial cellulase enzyme.

As mentioned from the effect of bacterial strains, the strain A 002 provided higher total enzyme activity and was able to produce higher glucose concentration than the strain M 015. However, the BET analysis of the corncob after the microbial hydrolysis by using both strains shows that using the strain M 015 provided higher surface area than the strain A 002, as shown in Table 4.5. It can be described by the fact that the strain M 015 had higher endoglucanase activity which cleaves the long chains of cellulose into the short chains, resulting in high surface area obtained (Taechapoempol *et al.*, 2011). On the contrary, the strain A 002 had higher exoglucanase and β-glucosidase activity, which cleave at the ends of the short chains cellulose and release soluble sugars such as glucose leading to lower solid content and lower surface area compared with the strain M 015. Hence, it was reasonable to explain why the strain A 002 had lower surface area but still provided higher glucose concentration.

Table 4.5 Specific surface area of 80–100 mesh corncob after hydrolysis with bacterial strain A 002 and M 015

Corncob 80–100 mesh	**Surface area (m^2/g)**
Before hydrolysis	6.7
After hydrolysis using strain A 002	12.0
After hydrolysis using strain M 015	20.8