CHAPTER IV
RESULTS AND DISCUSSION

4.1 Rice Straw Composition

Table 4.1 shows the elemental composition of rice straw. It was found that oxygen is the major component in rice straw followed by carbon and hydrogen. Table 4.2 shows the chemical composition of rice straw. And Cellulose was found to be the major component, followed by hemicellulose.

Table 4.1 Elemental composition of rice straw

Elemental composition	wt%, dry basis
Carbon	35.61
Hydrogen	5.57
Oxygen	58.03
Nitrogen	0.70
Sulfur	0.09

Table 4.2 Chemical composition of rice straw

Chemical composition of rice straw	wt%, dry basis
Cellulose	46.71
Hemicellulose	25.05
Lignin	4.69
Ash	16.64

4.2 Hydrolysis Capacity Value (HC Value)

The HC values are related to the activity of endoglucanase enzyme, which controls the cellulose hydrolysis rate in the primary hydrolysis step (Zhang *et al.*, 2006). Thus, in order to ensure the activity of the bacteria strian. The determination of hydrolysis capacity of strain A 002 and strain M 015 was perfomed. The HC value can be determind by calculating the ratio of the diameter of the appeared clear-zone and that of the bacterial colony. From Table 4.3, the HC values of strain A 002 and M 015 are 1.99 and 2.41, respectively.

Table 4.3 Hydrolysis capacity values (HC value) of bacteria strian A 002 and strian M 015

Bacteria strain	Hydrolysis capacity value (HC value)
A 002	1.99
M 015	2.41

4.3 Structure of Enzymatically Hydrolyzed Rice Straw Sample

The scanning electron micrographs at 1,000 magnifications clearly show the morphological changes of rice straw due to the hydrolysis process. The rice straw before it was undergo hydrolysis process shows smooth surfaces as shown in Figure 4.1 (a). However, the morphology of rice straw was significantly changed, after the rice straw was hydrolyzed as shown in Figure 4.1 (b) and (c). This indicates that there was a physical transformation of the rice straw after the hydrolysis.

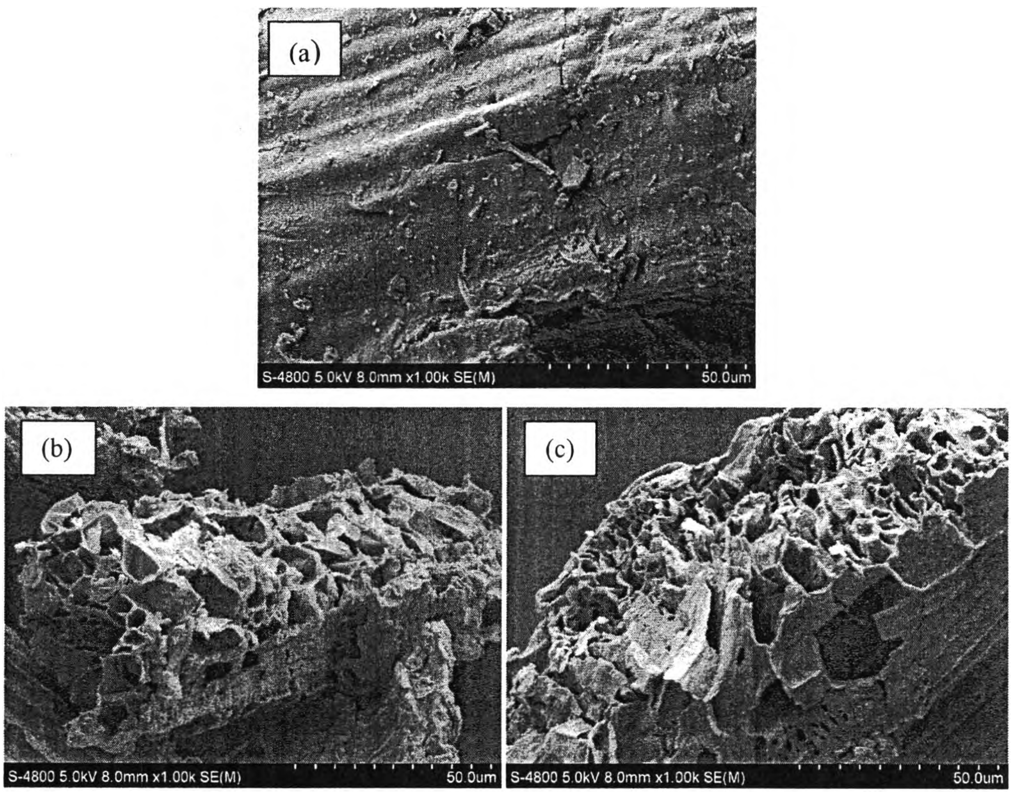

Figure 4.1 Scaning electron microraphs of < 80 mesh size of rice straw surface (a) befor hydrolysis (b) after hydrolysis at 37°C with the strain A 002 and (c) after hydrolysis at 37°C with the strain M 015.

4.4 Enzymatic Hydrolysis Results

4.4.1 Effects of Rice Straw Particle Size on the Glucose Production

Three different sizes (40 - 60 mesh, 60 - 80 mesh and < 80 mesh) of rice straw particle were investigated in order to obtain the optimum size of the rice starw for the microbial hydrolysis. The experiments were carried out under a fixed substrate concentration of 1.0 g/L-1.1 g/L. Figure 1 (a) shows the effect of the three rice straw sizes on the hydrolysis at 37 °C with strain A 002. The glucose concentration from the hydrolysis of the < 80 mesh size rice straw gradually incresed and it reached the maximum, about 0.97 g/L, at 9 h. After that, it drastically decreased. However, the glucose concentration from the hydrolysis of either the 60 - 80 mesh and 40 - 60 mesh sizes of rice straw under the same hydrolysis conditions increased at a slower rate and reached the maximum glucose concentration about 0.52 g/L and 0.51 g/L at 8 h and 9 h, respectively. The effect of rice straw size on the hydrolysis at 37°C with strain M 015 is shown in Figure 1 (b). The glucose concentration produced from the < 80 mesh size rice straw under the same conditions gradually increased and reached the maximum glucose production, about 0.55 g/L, at 9 hr. However, the hydrolysis of the 60 - 80 mesh and 60 - 40 mesh sizes of the rice straw at 37 °C with strain M 015 incresed at a faster rate and reached the maximum glucose production of 0.54 and 0.41 g/L at 9 and 8h, respectively. In a comparison, the glucose production from strain A 002 at 37 °C was higher than that from strain M015 under the same hydrolysis condition. These results indicate that the smaller the size of rice starw, the higher the surface area, the easier the emzyme to hydrolyze the rice straw which resulted in the higher glucose production.

As shown in Figure 4.3 (a), the glucose production from the hydrolysis of < 80 mesh, 60 - 80 mesh and 40 - 60 mesh sizes rice straw at 30 °C gradually increased until it reached maximun glucose production at 9 hr, about 0.38 g/L, 0.38 g/L and 0.36 g/L, respectively, before graudually decreasing. The same trends can be observed for the hydrolysis of the three rice straw sizes with strain M 015 at 30°C as shown in Figure 4.3 (b). The maximun glucose production were reached at 9 h, about 0.33 g/L, 0.25 g/L and 0.20 g/L for < 80 mesh, 60 - 40 mesh and 40 - 60 mesh rice straw.

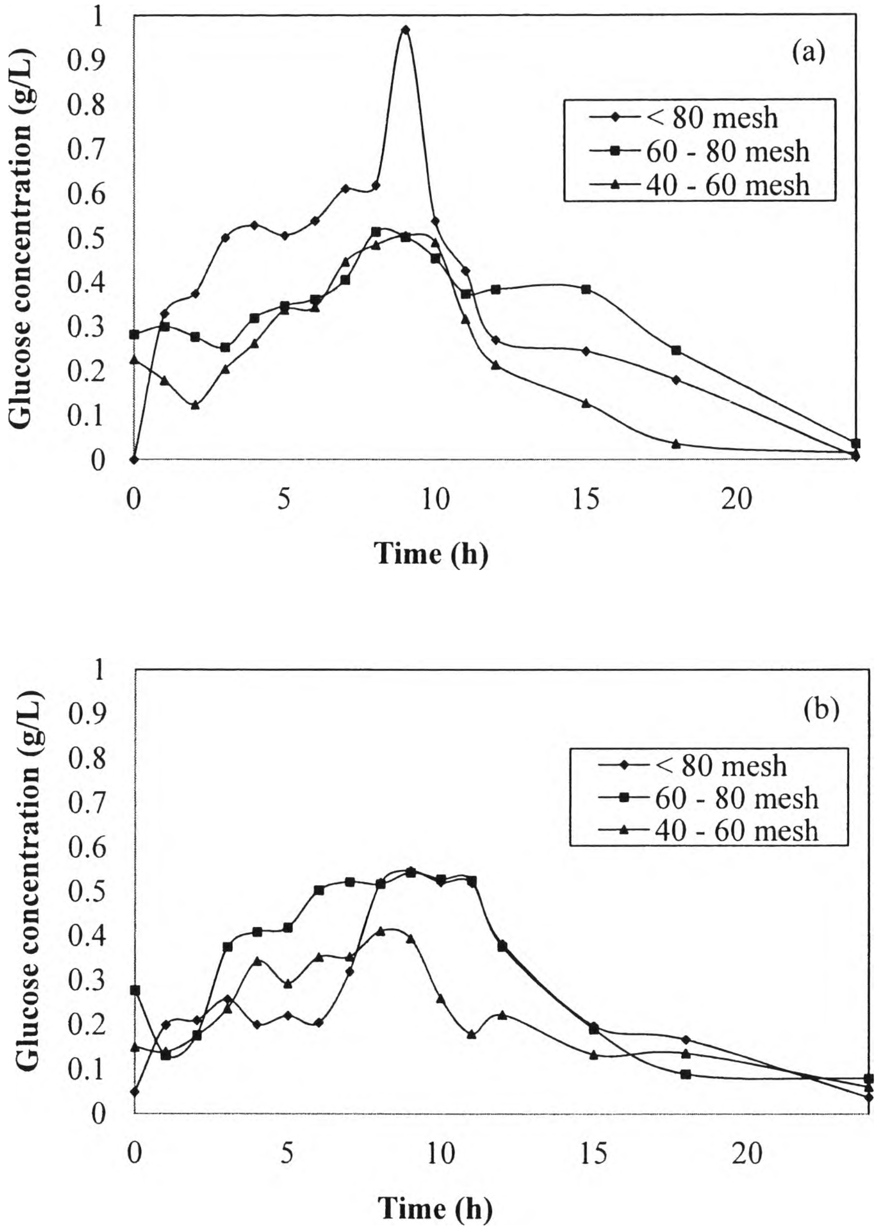

Figure 4.2 Effect of rice straw particle size on the glucose concentration profile at 37 °C using bacteria (a) strain A 002 and (b) strain M015.

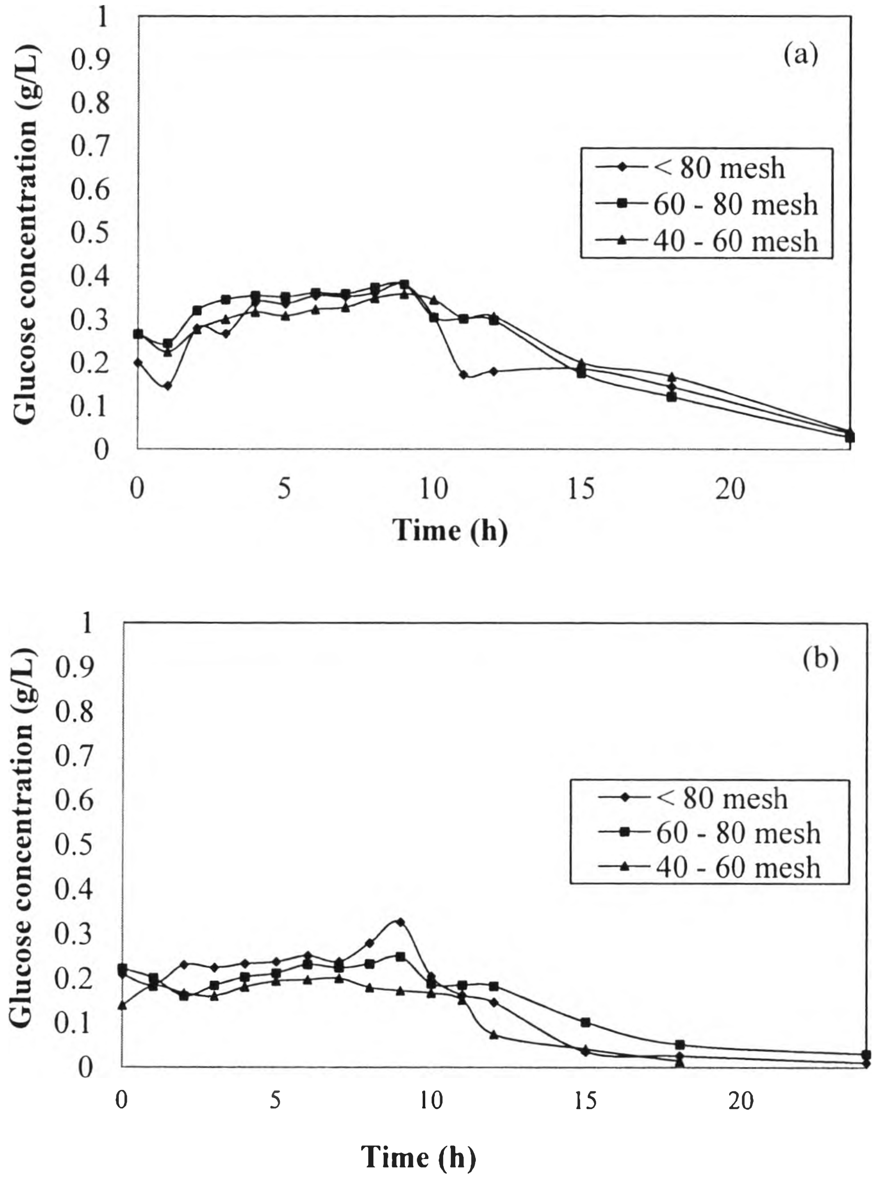

Figure 4.3 Effect of rice straw particle size on the glucose concentration profile at 30 °C using bacteria (a) strain A 002 and (b) strain M015.

4.4.2 Effects of Hydrolysis Temperature on the Glucose Production

In order to determine the optimum temperature for the microbial hydrolysis of rice straw, two different hydrolysis temperatures of 30 °C and 37 °C were investigated. Figure 4.4 (a) shows that the maximum glucose production for strain A 002 was obtained, about 9.67 g/L, at 9h for the hydrolysis temperature at 37 °C while the maximum amount of glucose, about 3.81 g/L, was produced at 9 h for the hydrolysis temperature at 30 °C. It indicates that the hydrolysis temperature at 37 °C resulted in higher glucose production than that at 30 °C. As shown in Figure 4.4 (b), the same effect of the hydrolysis temperature can be obserevd when using strain M 015 to hydrolyze the rice straw.The obtained results are in good agreement with the results from Taechapoempol *et al.* (2010), who reported that the incubation temperature of 37°C provided the highest cellulase activities for both strains.

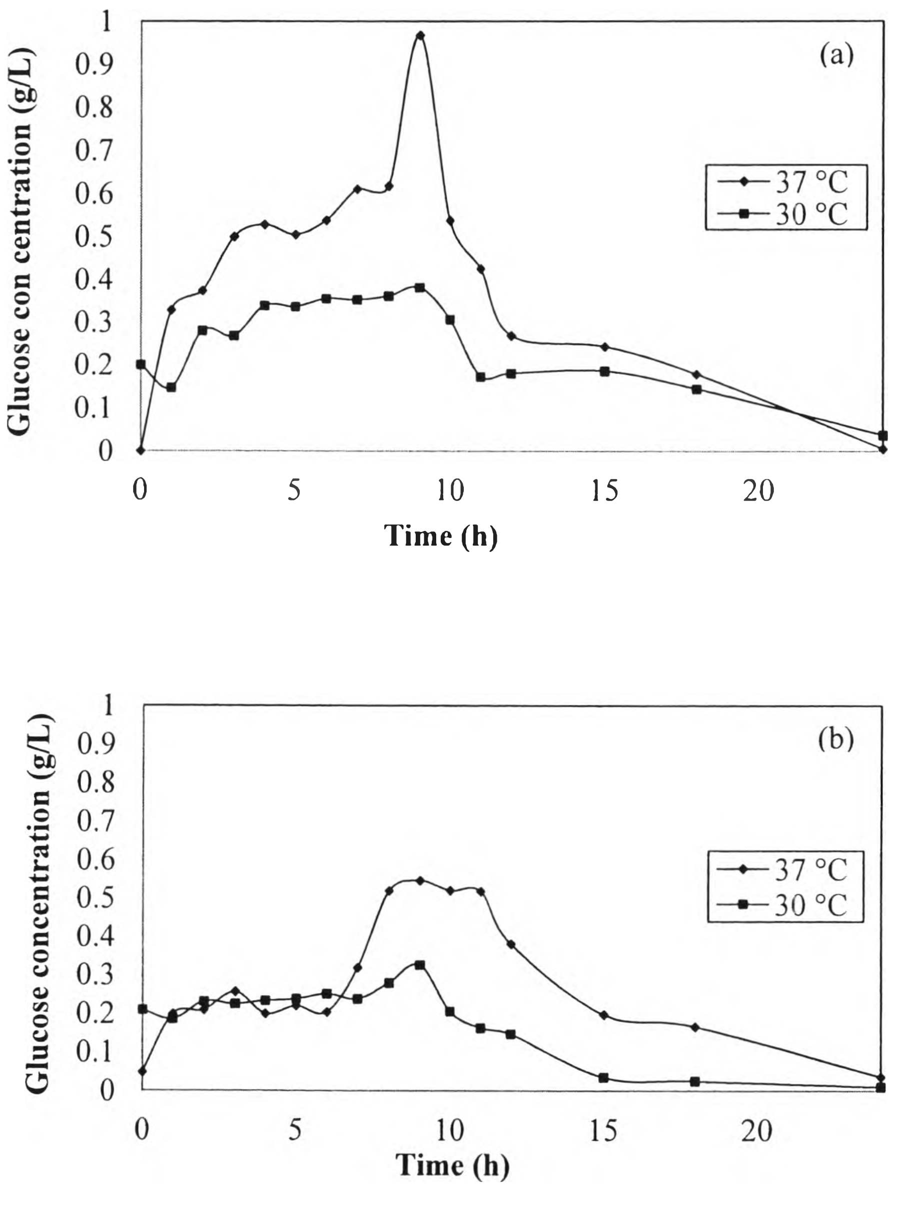

Figure 4.4 Effect of hydrolysis temperature on the glucose concentration profile from the hydrolysis of < 80 mesh particle size rice straw using bacteria (a) strain A 002 and (b) strain M 015.

4.4.3 Effects of Bacteria Strain on the Glucose Production

The performance of two bacteria strains, strain A002 and strain M015, on the microbial hydrolysis of rice straw were investigated. The glucose production by using strain A 002 slightly increased until it reached the maximum amount of glucose produced, about 9.67 g/L, at 9 h from the hydrolysis of 80 mesh size rice straw at 37 °C. as shown in Figure 4.5 (a). However, The glucose concentration profile by using strain M 015 slightly constant for 6 h until it increased to the maximum amount of glucose produced, about 5.47 g/L, at 9 h under the same hydrolysis condition. Figure 4.5 (b) also shows that the glucose production from strain A 002 was higher than that from strain M 015. This is due to the higher β-glucosidiase activity of strain A 002 over strain M 015 since the β-glucosidiase produced by strain A 002 can hydrolyze more cellulobiose to glucose.

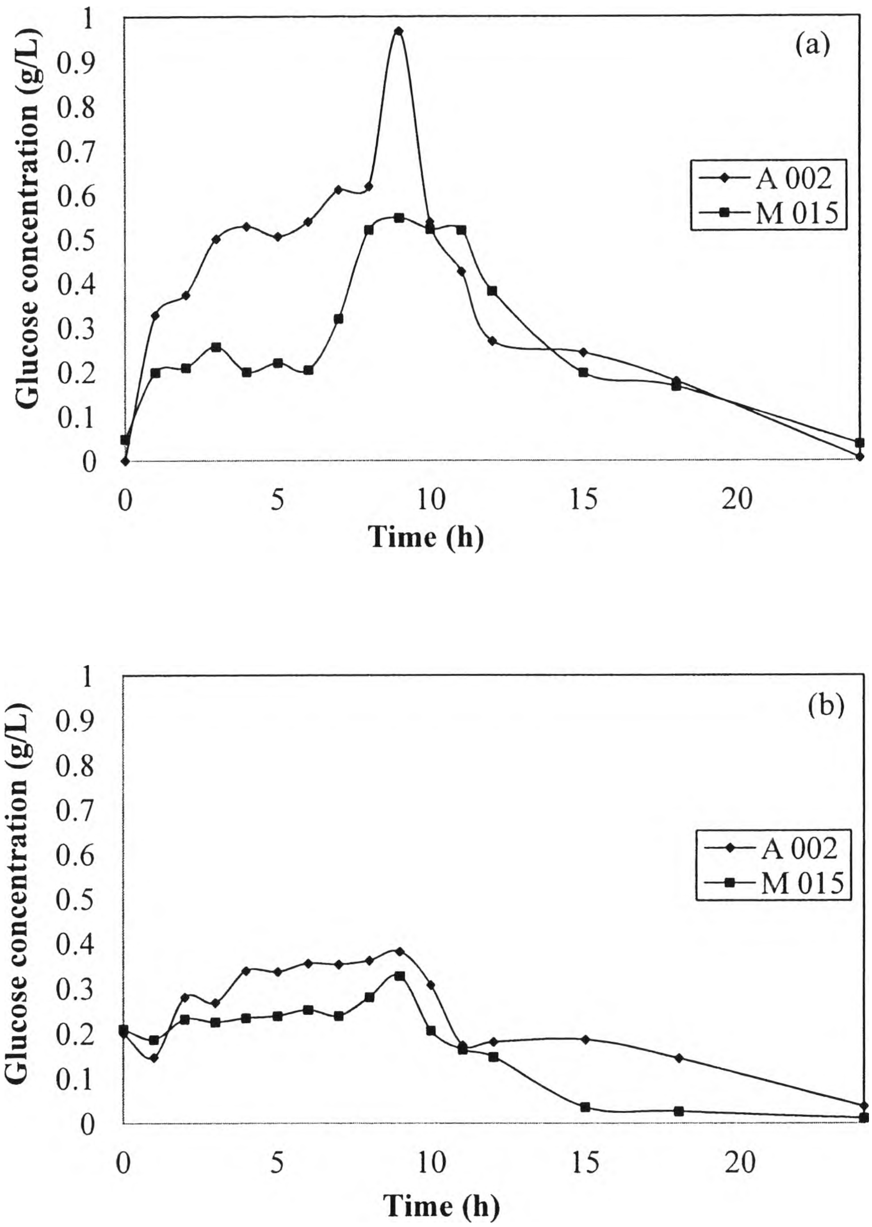

Figure 4.5 Effect of bacteria strains on the glucose concentration profile from the hydrolysis of < 80 mesh rice straw at 37 °C (a) and 30 °C (b).

4.4.4 Effects of Concentration of Secondary Carbon Source on the Glucose Production

To investigate the effect of concentration of secondary carbon source in production medium, 65 modified DSMZ broth medium 2, on the glucose production, the amount of malt extract in the production medium (10 g/L, 5g/L and 1 g/L) were varied. Figure 4.6 clearly shows that by adding 10 g/L of malt extract in the production medium, it resulted in the highest glucose production compared to the addition of 5 g/L and 1 g/L of malt extract in the production medium. These results conform to the report of Eourarekullart (2011), who reported that the nitrogen sources such as malt extract and yeast in the production medium are essential for bacteria to grow and hydrolyze the lignocellulosic materials to glucose.

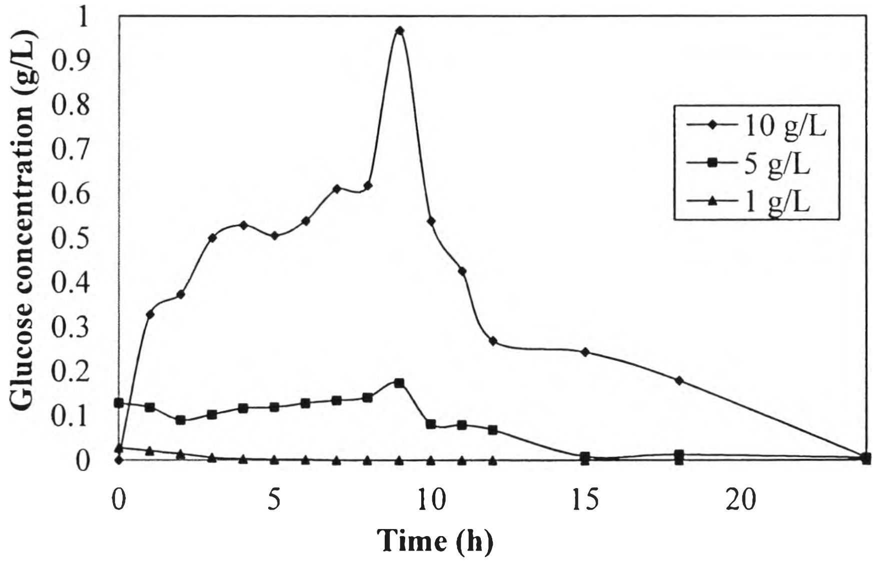

Figure 4.6 Effect of concentration of secondary carbon source on the glucose concentration profile from the hydrolysis of < 80 mesh particle size rice straw at 37 °C using bacteria strain A 002.

4.4.5 Bacteria Concentration and Glucose Production vs. Time

Figure 4.7 shows bacteria concentration and glucose concentration profile from the hydrolysis of 80 mesh rice starw with strain A 002 at 37 °C. The glucose production gradually increased until it reached the maximun, about 0.87 g/L, at 9 h, then started to decrease along the hydrolysis time. For the bacteria concentration, it continuously increased for 7 h and started to increase rapidly at 9 h. After that, the bacteria concentration continued to increase along the hydrolysis time. This indicates that the decreasing of glucose concentration after 9 h may resulted from the glucose consumption by the bacteria.

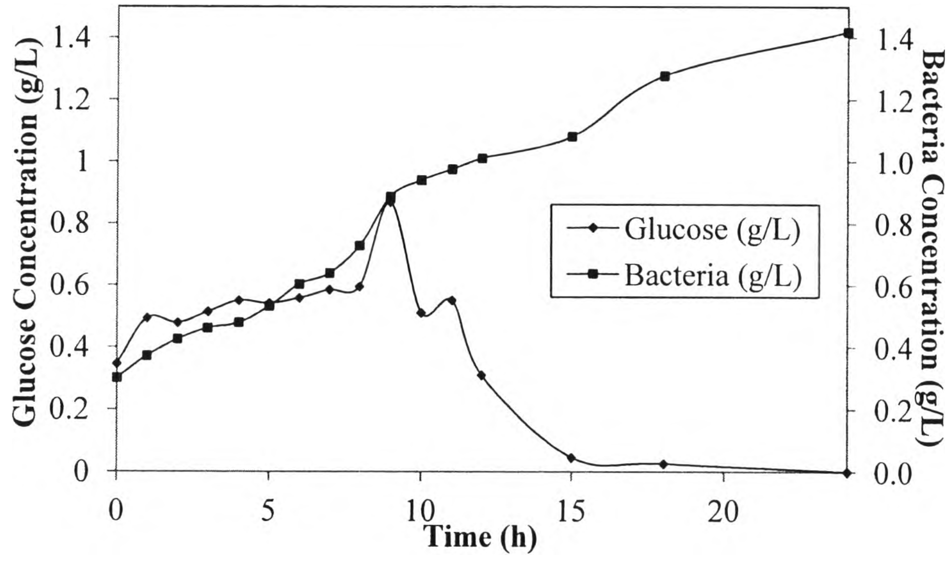

Figure 4.7 Glucose concentration and bacteria concentration profile from the hydrolysis of < 80 mesh rice straw with strain A 002 at 37 °C.

4.4.6 Cellulose Conversion and Glucose Production vs. Time

The glucose concentration and the percentage of cellulose conversion during the hydrolysis of < 80 mesh rice straw with strian A 002 at 37 °C were shown in Figure 4.8. The conversion of cellulose rapidly increased along the hydrolysis time which corresponding to the increasing in the glucose concentration until it reached the maximum glucose concentration at 9 h. After that the conversion of cellulose gradually increased at a slower rate and reached 50 % cellulose conversion at 24 h. The slow rate of cellulose conversion after reached the maximum glucose concentration indicates that after 9 h, the bacteria tends to consume the produced glucose instead of the rice straw which resulted in a slower rate of cellulose conversion.

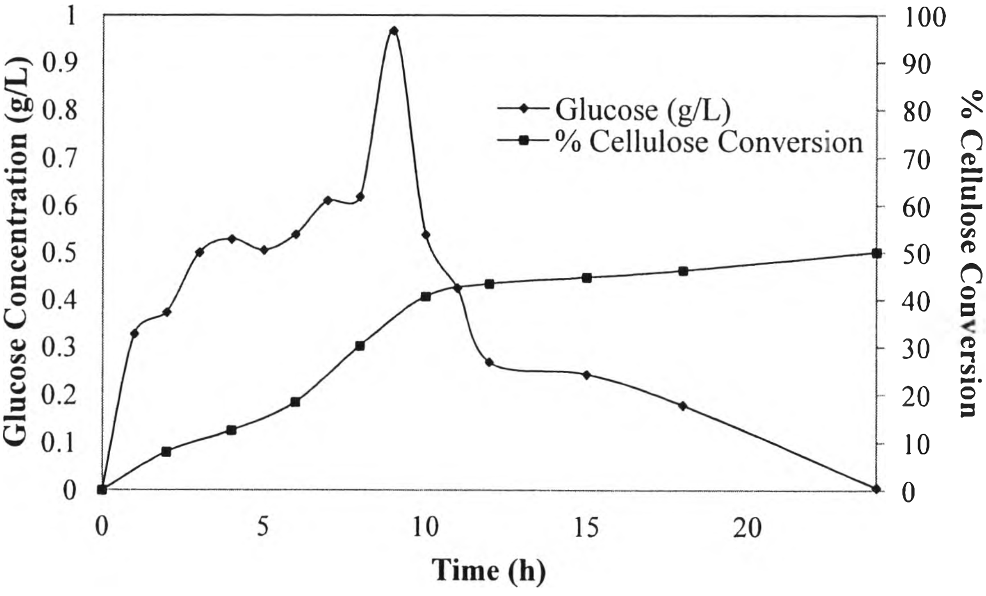

Figure 4.8 Glucose concentration profile and the percentage of cellulose conversion from the hydrolysis of < 80 mesh rice straw with strain A 002 at 37 °C.