CHAPTER IV
RESULTS AND DISCUSSION

4.1 Cassava Residue Composition

Results of elemental and chemical composition of cassava residue are shown in Tables 4.1 and 4.2, respectively. The major components of cassava residue are carbon, followed by oxygen, and hydrogen.

Table 4.1 Elemental composition of the cassava residue

Elemental composition	wt%, dry basis
Carbon	39.66
Hydrogen	5.38
Nitrogen	1.52
Oxygen	53.36
Sulfur	0.08

Cassava residue was analyzed for its composition as shown in Table 4.2. The hundred percent of composition consisted of protein, fat, fiber, ash, and starch. The major component, starch, was approximately 49.66%. The second highest component was fiber at 23.30%. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) were used to calculate the composition of cellulose, hemicelluloses, and lignin. The insoluble after dissolving the sample in a neutral detergent consisted of cellulose, hemicelluloses, and lignin or NDF. After dissolving the sample in an acid detergent, the hiatus was hemicelluloses whereas the leftovers, which were insoluble, were cellulose and lignin or ADF. The difference between NDF and ADF was equal to the hemicellulose composition. The results in Table 4.2 showed the maximum component of lignin followed by cellulose and hemicelluloses, respectively.

Table 4.2 Chemical composition of the cassava residue

Chemical composition	wt%, dry basis
Protein	13.31
Fat	1.18
Fiber	23.30
Ash	12.60
Starch	49.66
ADF	43.33
NDF	56.29
Cellulose	21.47
Hemicellulose	12.97
Lignin	21.86

4.2 Hydrolysis Capacity Value (HC)

Cellulase-producing bacteria obtained from Thai higher termites were preliminarily investigated for their potential to degrade cellulose by using the hydrolysis capacity (HC) value, shown in Table 4.3. Bacteria strain A 002 and M 015 showed clear zones on the culture plates, suggesting that cellulase producing bacteria can be used as the effective isolates for further investigation (Taechapoempol *et al.* 2010).

Table 4.3 Hydrolysis capacity value for strain A 002 and strain M 015 for their potential to degrade the cellulose

Strain	Average diameter of colony (cm)	Average diameter of clear zone (cm)	HC value
A 002	1.125	2.242	1.993
M 015	1.100	2.650	2.409

The bacteria isolated from Thai higher termites were also preliminarily investigated for their potential to degrade starch by using the hydrolysis capacity (HC) value. Starch was used instead of CMC in the culture plate of 65 modified DSMZ broth medium 2. Bacteria strain A 002 and M 015 showed clear zones on the culture plates, suggesting that bacteria can be used as the effective isolates for further investigation. The results were shown in Table 4.4.

Table 4.4 Hydrolysis capacity value for strain A 002 and strain M 015 for their potential to degrade starch

Strain	Average diameter of colony (cm)	Average diameter of clear zone (cm)	HC value
A 002	1.711	2.156	1.160
M 015	1.980	2.327	1.175

4.3 Enzymatic Hydrolysis

The cassava residue was hydrolyzed for 24 h in order to determine the concentration profile of glucose by two effective isolates (strain A 002 and M 015) obtained from Thai higher termites *Microcerotermes* sp,. Effects of cassava residue particle size (40, 60, and 80 mesh), hydrolysis temperature (30°C and 37°C), and quantity of malt extract (10, 5, 1 g/L) on glucose concentration were also determined.

4.3.1 Effects of Cassava Residue Particle Size

The optimum cassava residue particle size for the hydrolysis at 30°C and 37°C was determined with three different particle sizes – 40, 60, and 80 mesh, at the fixed concentration of raw material in the production medium of 1.0 – 1.1 g/L. As shown in Figure 4.1, the glucose evolution from the hydrolysis of 80 mesh cassava residue with bacteria strain A 002 gradually increased and reached the maximum point, about 1.44 g/L, at 7 h before sharply decreasing in the next hour and remained constant until 24 h. Although the glucose concentration evolution from the hydrolysis of 40 mesh and 60 mesh cassava residue were similar to that of the 80

mesh size, the concentration was much lower than that of 80 mesh, about 0.54 and 0.81 g/L, respectively at 10 h. About the same glucose evolution pattern was also obtained from the hydrolysis with strain M 015 (Figure 4.2). For example, for the glucose concentration from the hydrolysis of the 80 mesh cassava residue, it increased until 4 h and then decreased before reaching the maximum at 7 h. However, the increase and decrease were not as dramatic as shown in Figure 4.1. The 80 mesh particle size in this experiment still gave the highest glucose concentration whereas the lowest was obtained from the hydrolysis of 40 mesh. The maximum glucose concentrations from the hydrolysis of 40, 60, and 80 mesh with strain M 015 were 0.41 g/L at 9 h, 0.46 g/L at 8 h, and 0.98 g/L at 7 h, respectively, as shown in Figure 4.2. Comparison among the glucose concentration from the hydrolysis from these sample sizes with both strains A 002 and strain M 015 at 30°C showed that the smaller the particle size of cassava residue, the higher the glucose concentration.

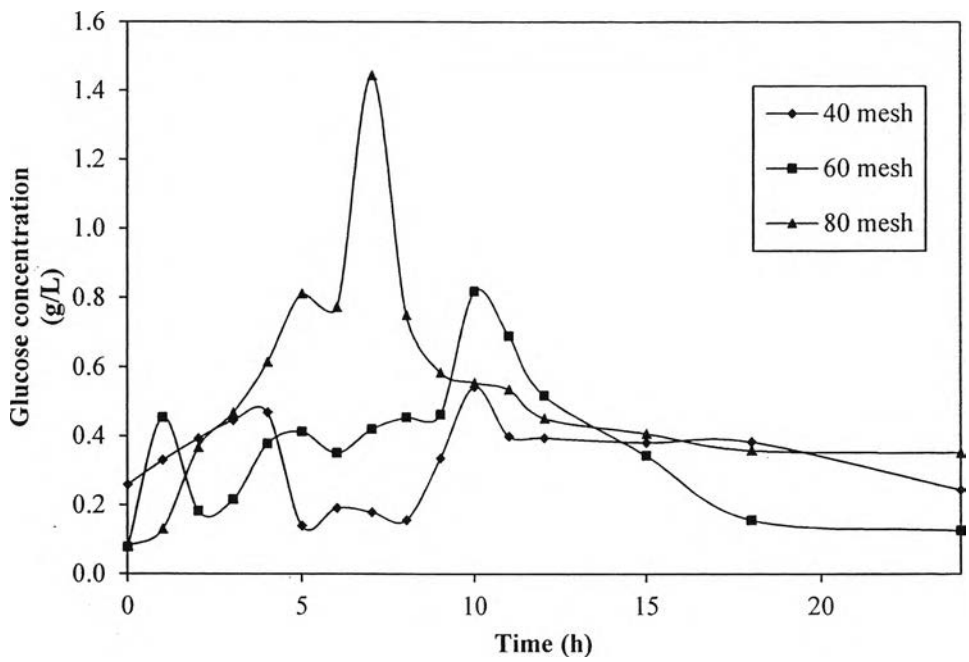

Figure 4.1 Effects of cassava residue particle size on the produced glucose concentration from the hydrolysis of cassava residue at 30°C using strain A 002.

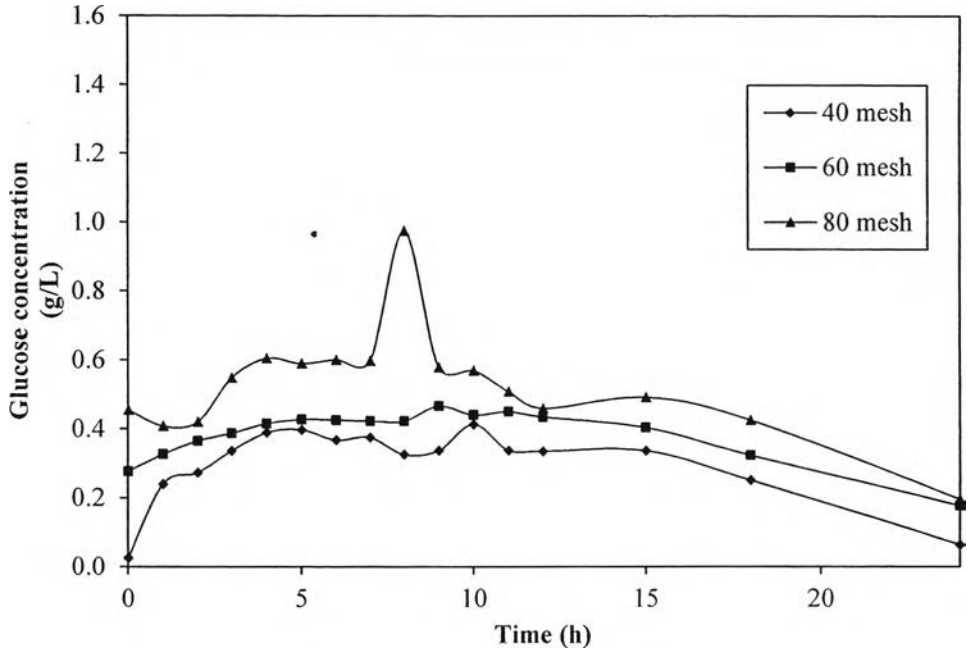

Figure 4.2 Effects of cassava residue particle size on the produced glucose concentration from the hydrolysis of cassava residue at 30°C using strain M 015.

Figures 4.3 and 4.4 showed the glucose evolution from the hydrolysis of the three different particle sizes at 37 °C with strain A 002 and M 015, respectively. The results in Figure 4.3 indicated that both strains were not effective to hydrolyze the residue to produce glucose.

In summary, it clearly showed that cassava residue particle sizes affected the glucose evolution from the enzymatic hydrolysis with Thai higher termites for both strain A 002 and strain M 015. The effects were clearly observed at 30 °C. The smaller the particle size, the higher the specific surface area. Hence, enzymatic hydrolysis of small particle size can proceed faster than the larger particle size. The results were in agreement with that from Gabriela *et al.* (2012), who reported that particle size reduction affects internal changes in the lignocellulosic structure, where the degradability of substance was enhanced by decreasing of particle size until a limit. Yeh *et al.* (2010) reported that the yield of glucose was significantly increased when the average particle size was in the submicron scale. In addition, the results of hydrolysis time at the highest concentration of glucose on each condition (Figures 4.1 to 4.4) showed that the 80 mesh size can be hydrolyzed faster at around 7 h followed by the 60 and 40 mesh, respectively. The results were

consistent with that from Abasaeed and Mansour (1992), who reported that the increase in the particle size resulted in longer reaction times.

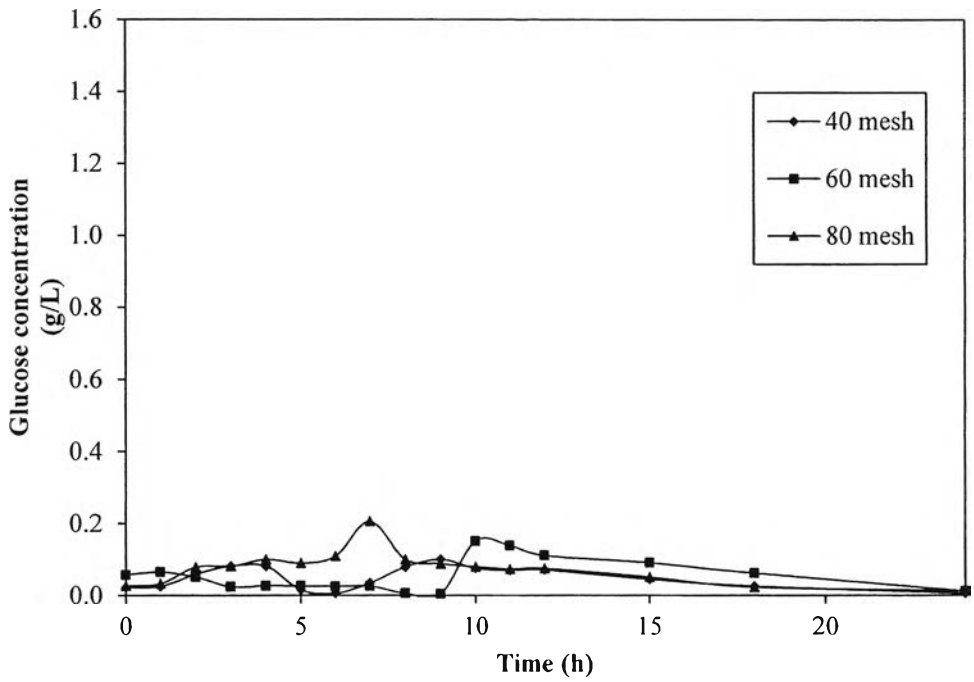

Figure 4.3 Effects of cassava residue particle size on the produced glucose concentration from the hydrolysis of cassava residue at 37°C using strain A 002.

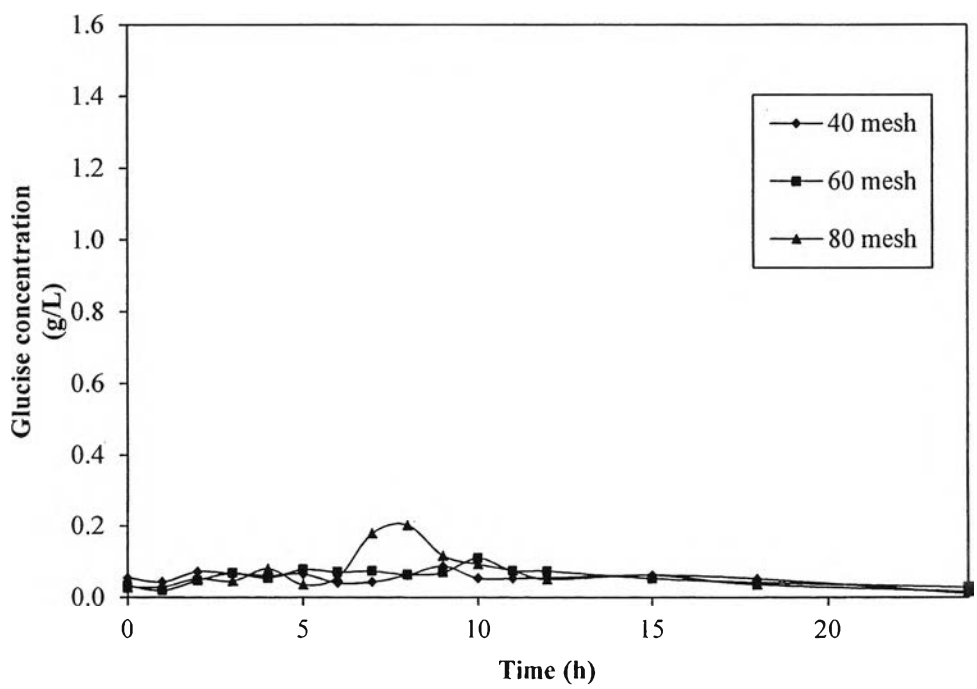

Figure 4.4 Effects of cassava residue particle size on the produced glucose concentration from the hydrolysis of cassava residue at 37°C using strain M 015.

4.3.2 Effects of Bacteria Strains

The performance of both strain A 002 and strain M 015 on the enzymatic hydrolysis of cassava residue was investigated. Comparison between results from both strains at the 80 mesh size cassava residue, Figures 4.5 and 4.6, showed that using strain A 002 resulted in a higher amount of glucose at 30 °C. It also seems that the hydrolysis activity of strain M 015 was rather constant at 30 °C. Although strain M 015 had higher specific endoglucanase activity to attack and create more free chain-ends of cellulose than strain A 002, it had less specific exoglucanase activity to change the free chain-ends, which were the product of endoglucanase enzyme to cellobiose and also less β-glucosidase activity to convert cellobiose to glucose than strain A 002 (Taechapoempol *et al.* 2010). So stain M 015 had higher stability but produced lower glucose concentration than from using strain A 002. According to Öhgren *et al.* (2007), β-glucosidases are strongly inhibited by glucose, while endoglucanase and exoglucanase are inhibited by cellobiose, shown in Figure 4.7. That explains why glucose concentration decreased after reaching the highest value. At 37 °C, both strains showed minimal activity in the hydrolysis.

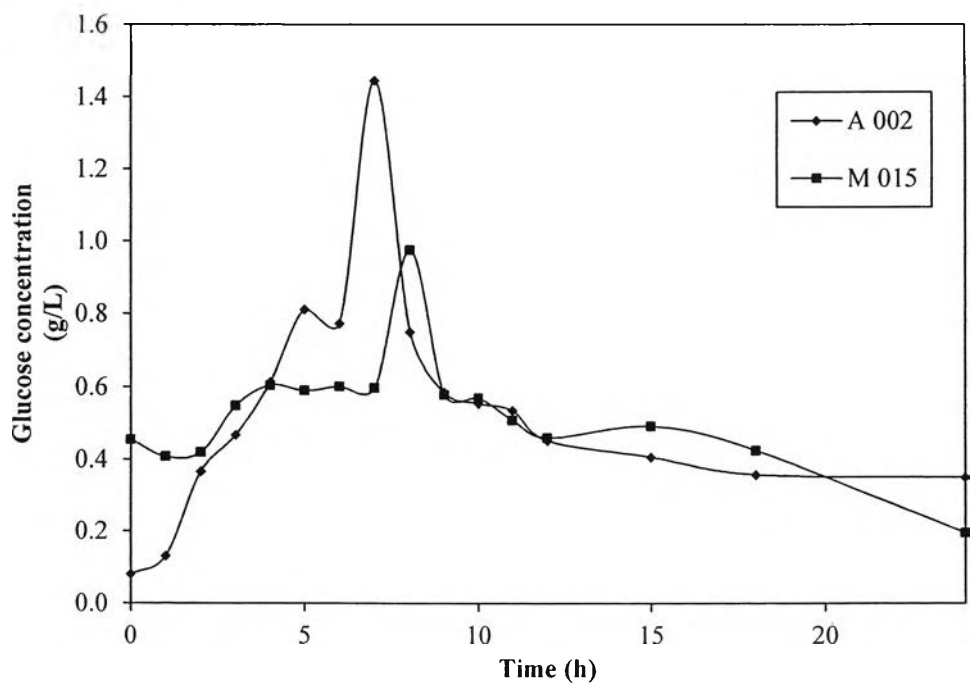

Figure 4.5 Effects of enzymatic hydrolysis temperature on the produced glucose concentration from the hydrolysis of 80 mesh cassava residue using strain A 002.

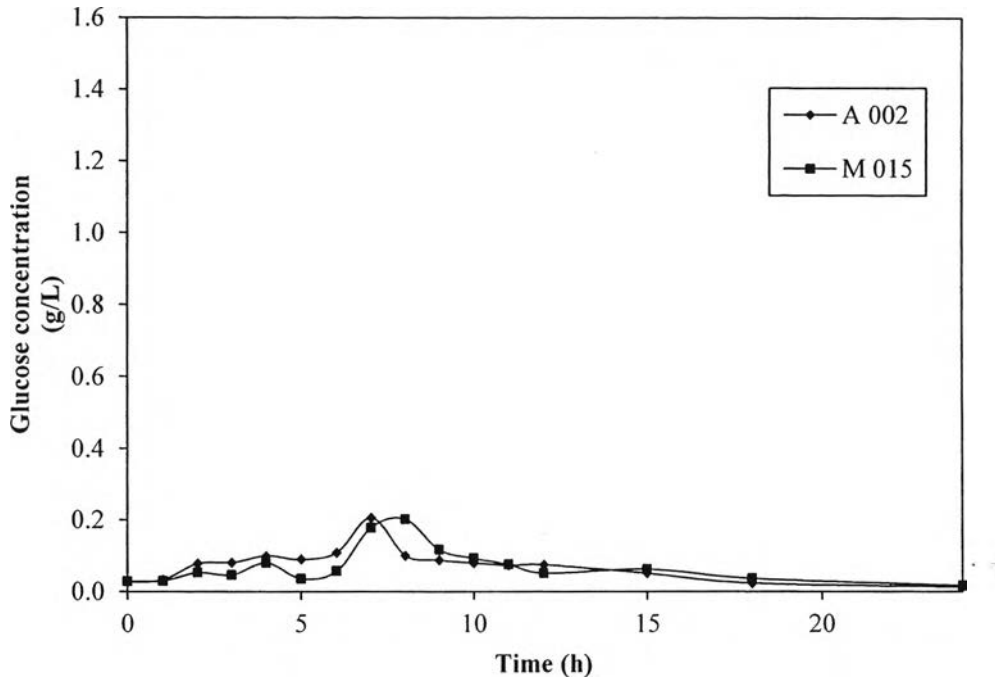

Figure 4.6 Effects of enzymatic hydrolysis temperature on the produced glucose concentration from the hydrolysis of 80 mesh cassava residue using strain M 015.

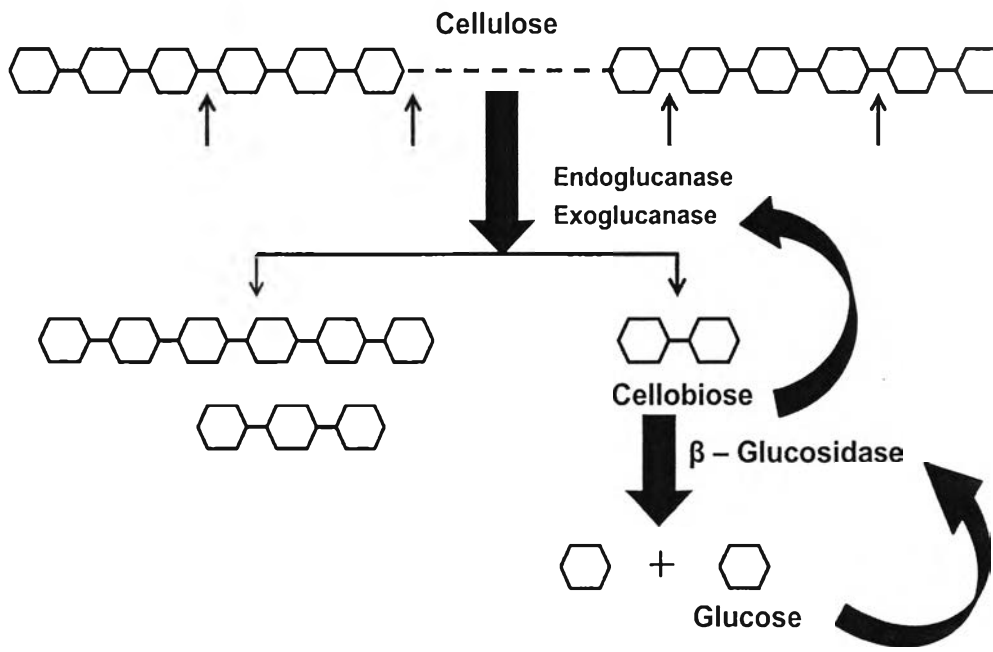

Figure 4.7 Schematic for the product inhibition by cellobiose and glucose (Lee *et al.* 2010).

The cellobiose molecule is utilized simultaneously to glucose by β-glucosidases. So the decrease in cellobiose means the increase in glucose molecule. The theoretical was consistence with the results in Figures 4.8 - 4.19 that showed the comparison between the cellobiose and glucose evolutions from each experimental condition. The results can be divided into two parts. The first part belonged to the results from the hydrolysis at 30 °C, Figures 4.8 - 4.13. The results from the hydrolysis with strain A 002 in Figures 4.8, 4.10, and 4.12 showed that the glucose concentration fluctuation was more pronounced than that from stain M 015, Figures 4.9, 4.11, and 4.13. A possible reason may be because of the lower endoglucanase activity of strain A 002. Consequently, the free chain ends were needed to be converted to cellobiose and glucose depended on the effectiveness of exoglucanase and β-glucosidase activity, which converted free chain ends. Much or few of free chain ends in the solution, almost all of them can be converted due to its high activity of the two enzymes (exoglucanase and β-glucosidase). On the other hand, the free chain ends which were converted using strain M 015 were gradually converted to cellobiose and glucose due to its less activity of exoglucanase and β-glucosidase so the glucose concentration stayed relatively constant. As the result of higher specific surface area of 80 mesh size, the glucose concentration from Figure 4.13 showed more fluctuation than that in Figures 4.9 and 4.11 because the residue was easier for enzyme to hydrolyze. After the highest value from each batch of both strains at 30 °C, similar trends were observed for both sugars. The decrease in the glucose and cellobiose concentrations was observed. This may be resulted from the high the glucose concentration, the lower the activity of β-glucosidases. This resulted in the high cellobiose concentration, which inevitably acted as an inhibitor for the activity for the other two enzymes (endoglucanase and exoglcanase) as shown in Figure 4.7. So glucose and cellobiose evolutions were similar. The results of glucose and cellobiose concentrations from the hydrolysis at 37 °C, shown in Figures 4.14 to 4.19, were not as high and fluctuated as that at 30 °C because of the lower activity of both strains at 37 °C.

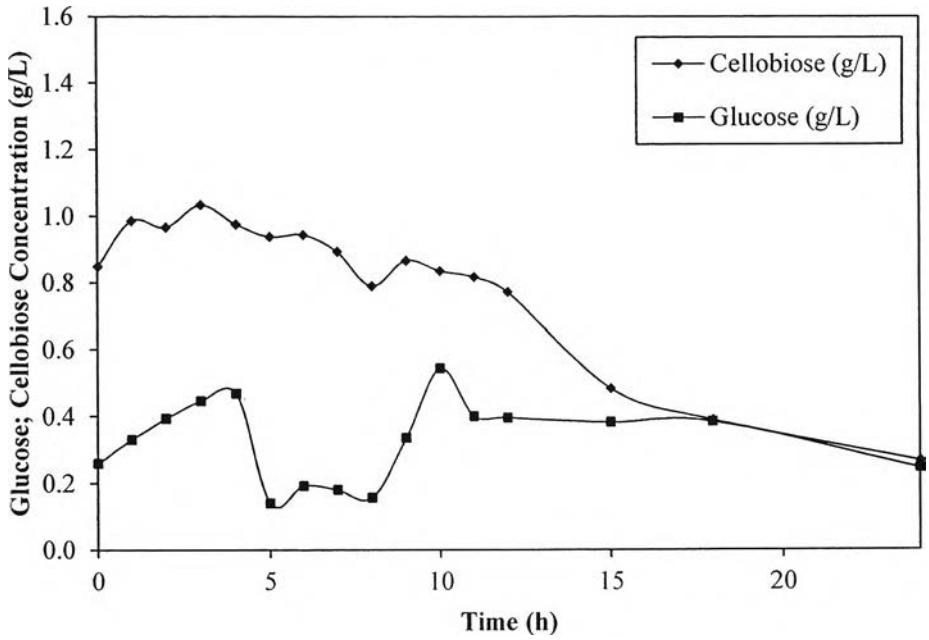

Figure 4.8 Comparison of cellobiose and glucose concentration from the hydrolysis of 40 mesh cassava residue with strain A 002 at 30 °C.

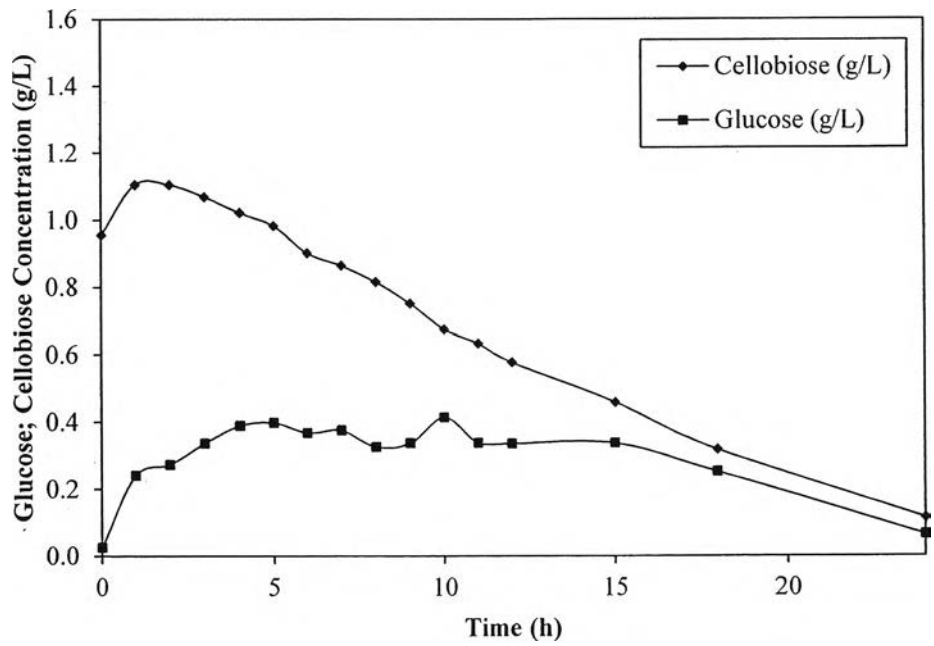

Figure 4.9 Comparison of cellobiose and glucose concentration from the hydrolysis of 40 mesh cassava residue with strain M 015 at 30 °C.

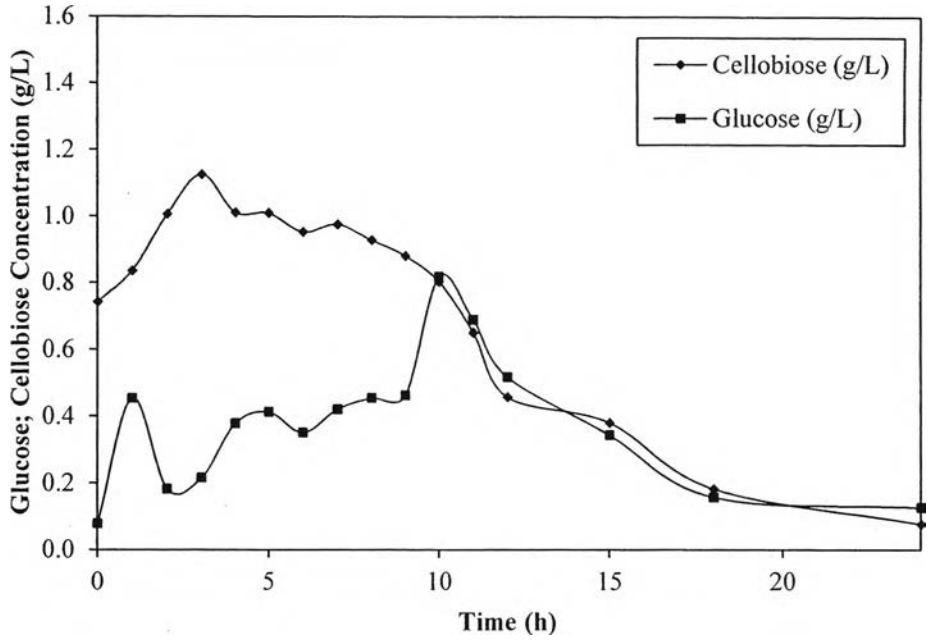

Figure 4.10 Comparison of cellobiose and glucose concentration from the hydrolysis of 60 mesh cassava residue with strain A 002 at 30 °C.

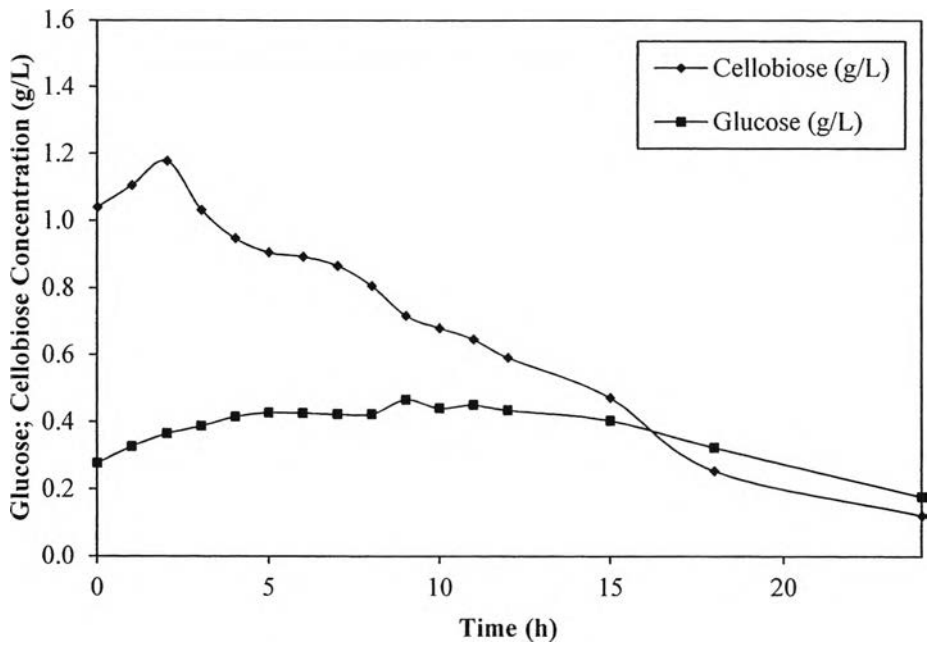

Figure 4.11 Comparison of cellobiose and glucose concentration from the hydrolysis of 60 mesh cassava residue with strain M 015 at 30 °C.

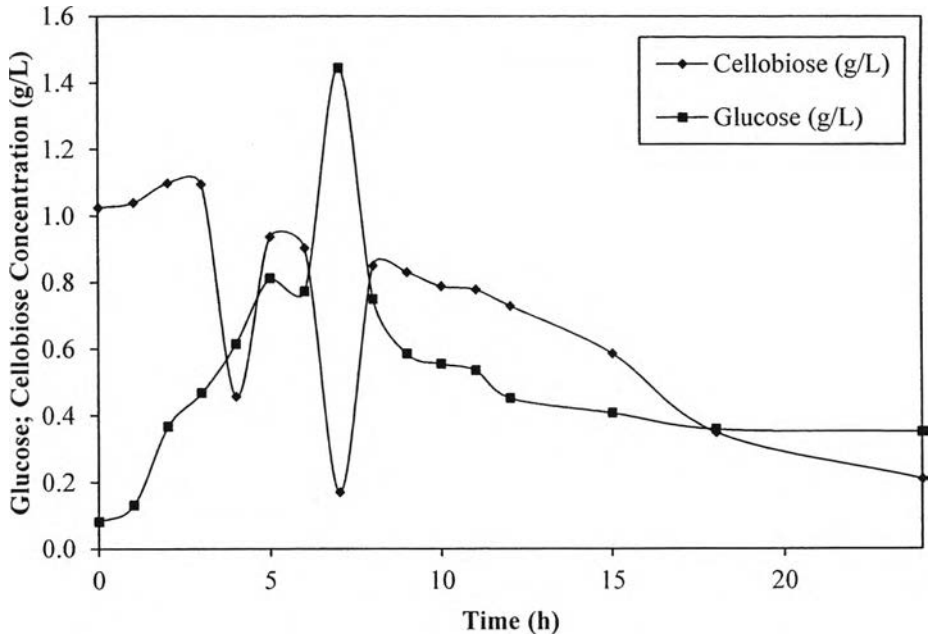

Figure 4.12 Comparison of cellobiose and glucose concentration from the hydrolysis of 80 mesh cassava residue with strain A 002 at 30 °C.

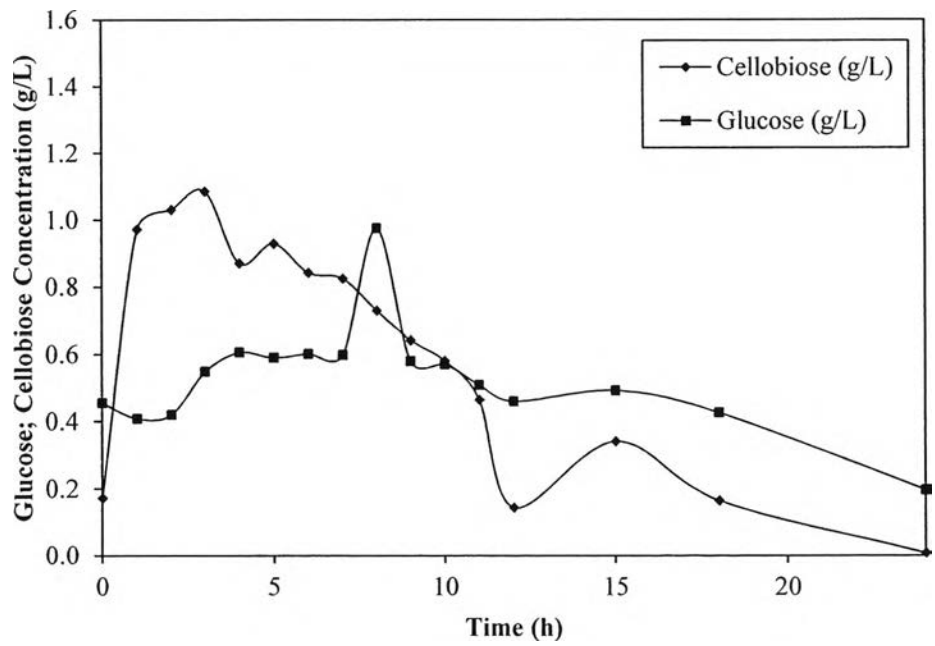

Figure 4.13 Comparison of cellobiose and glucose concentration from the hydrolysis of 80 mesh cassava residue with strain M 015 at 30 °C.

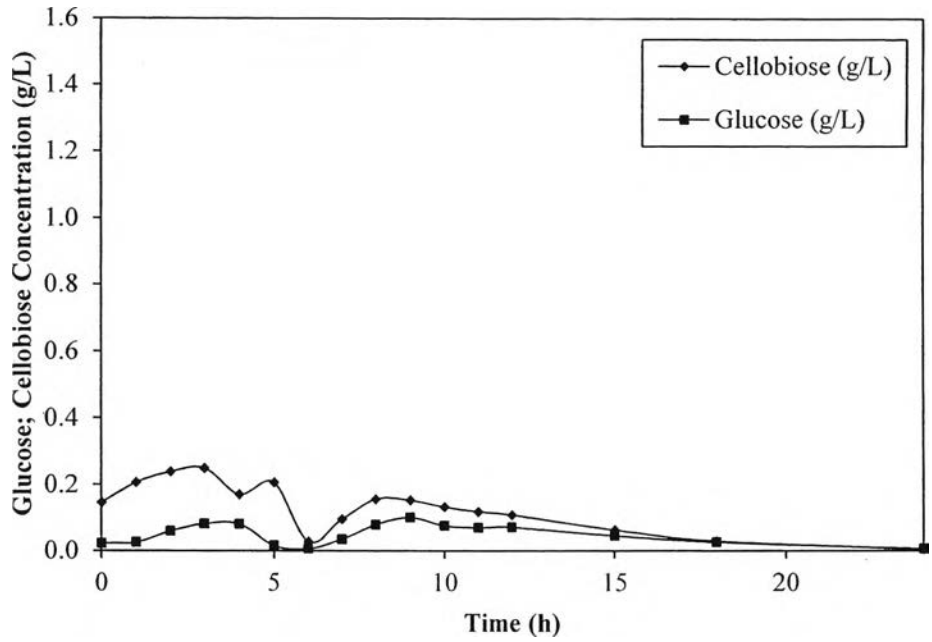

Figure 4.14 Comparison of cellobiose and glucose concentration from the hydrolysis of 40 mesh cassava residue with strain A 002 at 37 °C.

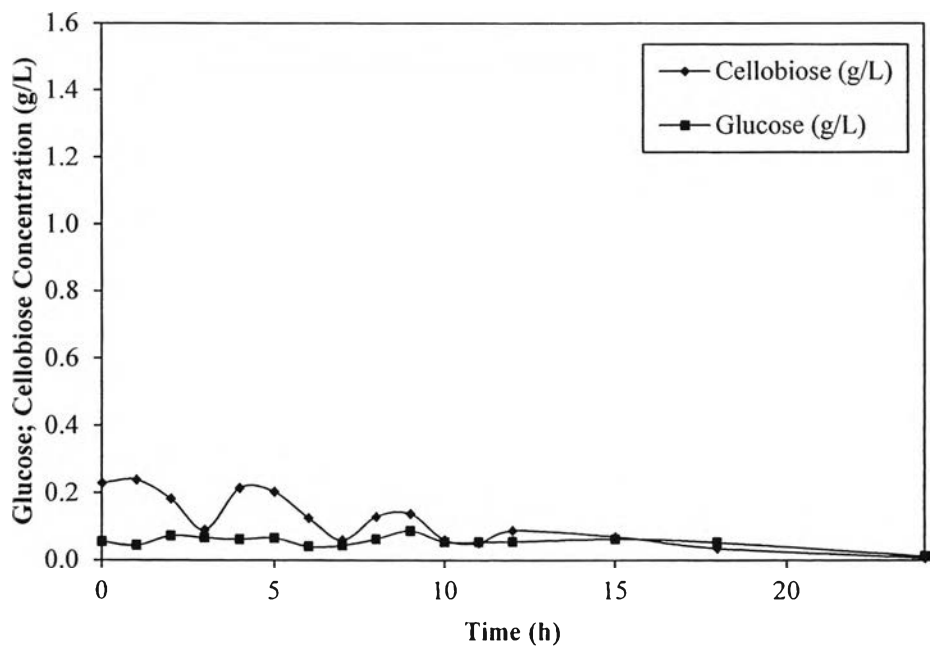

Figure 4.15 Comparison of cellobiose and glucose concentration from the hydrolysis of 40 mesh cassava residue with strain M 015 at 37 °C.

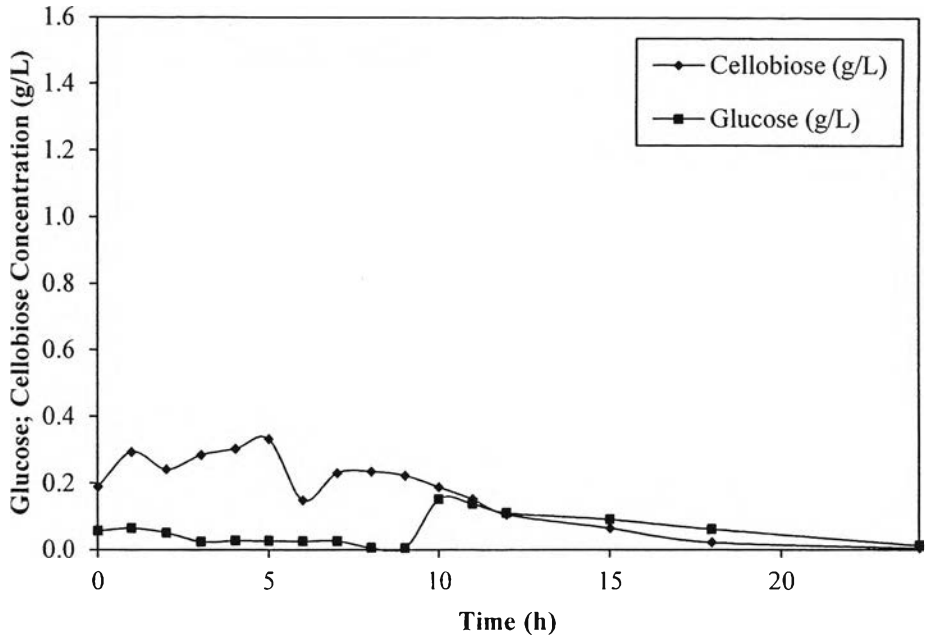

Figure 4.16 Comparison of cellobiose and glucose concentration from the hydrolysis of 60 mesh cassava residue with strain A 002 at 37 °C.

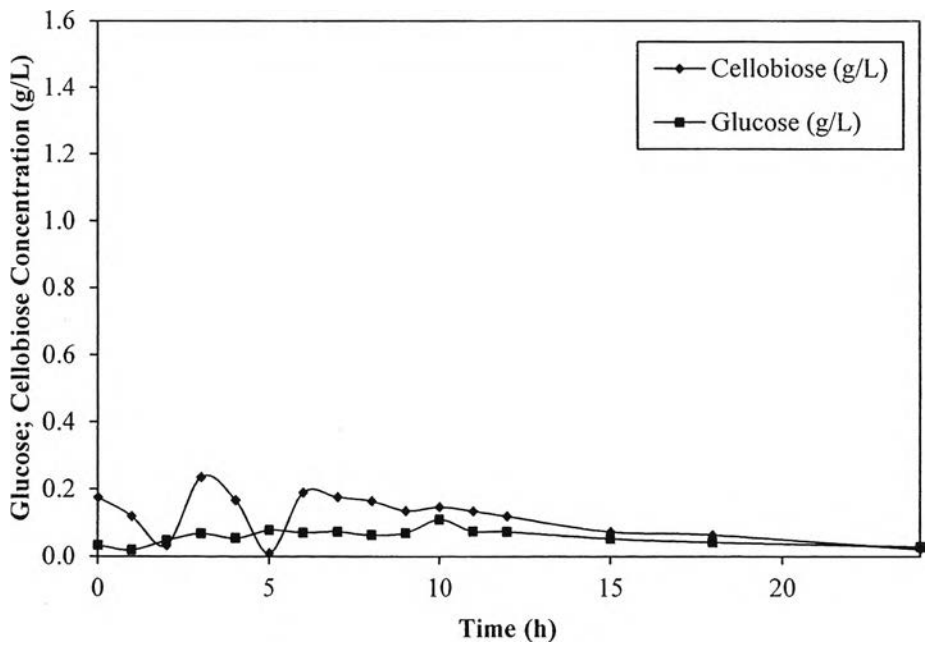

Figure 4.17 Comparison of cellobiose and glucose concentration from the hydrolysis of 60 mesh cassava residue with strain M 015 at 37 °C.

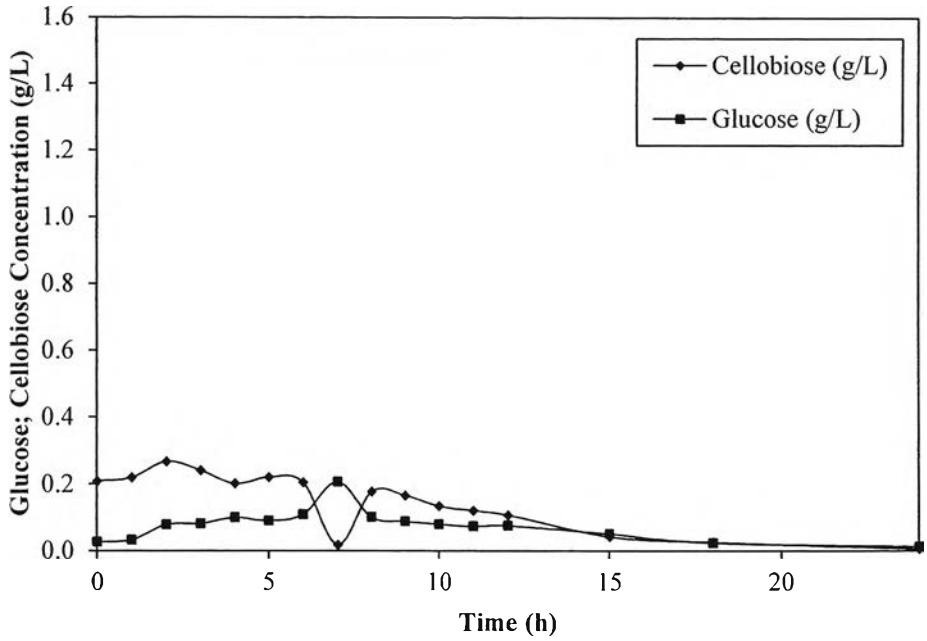

Figure 4.18 Comparison of cellobiose and glucose concentration from the hydrolysis of 80 mesh cassava residue with strain A 002 at 37 °C.

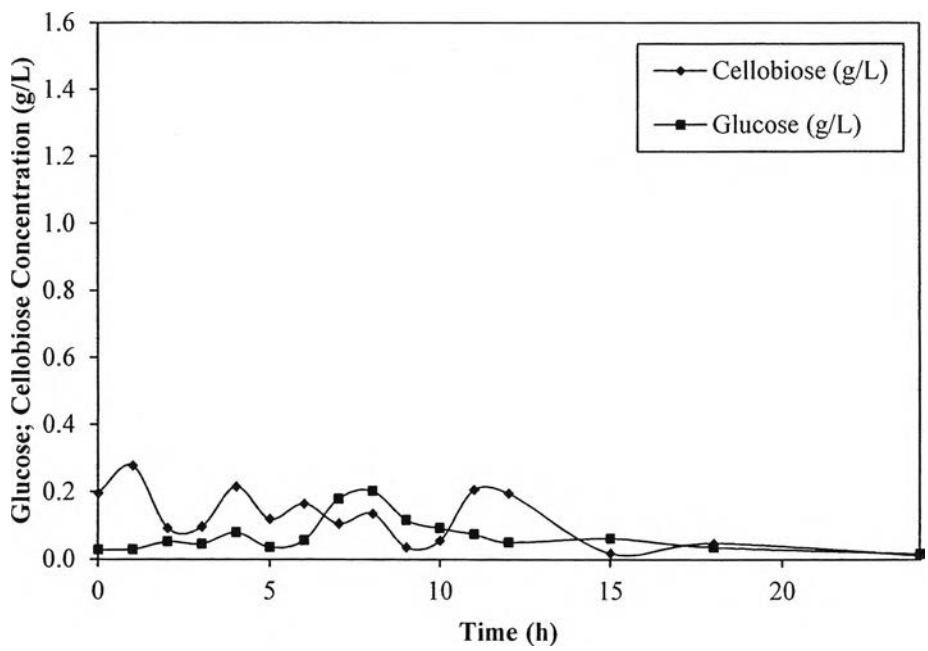

Figure 4.19 Comparison of cellobiose and glucose concentration from the hydrolysis of 80 mesh cassava residue with strain M 015 at 37 °C.

4.3.3 Effects of Operating Temperature

Effects of temperature on the enzymatic hydrolysis of cassava residue were investigated at 30 °C and 37 °C. Figure 4.20 showed that much higher glucose concentration was obtained from the hydrolysis of 80 mesh cassava residue with strain A 002 at 30 °C than that at 37 °C. The same phenomenon was observed with bacteria strain M 015, Figure 4.21. It is interesting to note that, although both strains A 002 and M 015 were cultured at 37 °C, the hydrolysis results showed that both strains were more effective at 30 °C. The results may be due to the presence of starch in a large amount. So what was observed was the results from amylase acyivity rather than cellulase, which is effective at 30 °C. The results were consistent with that from Dutta *et. al* (2005) and Khunt *et al.* (2011), who reported that the optimum temperature for starch hydrolysis using amylase was 30 °C.

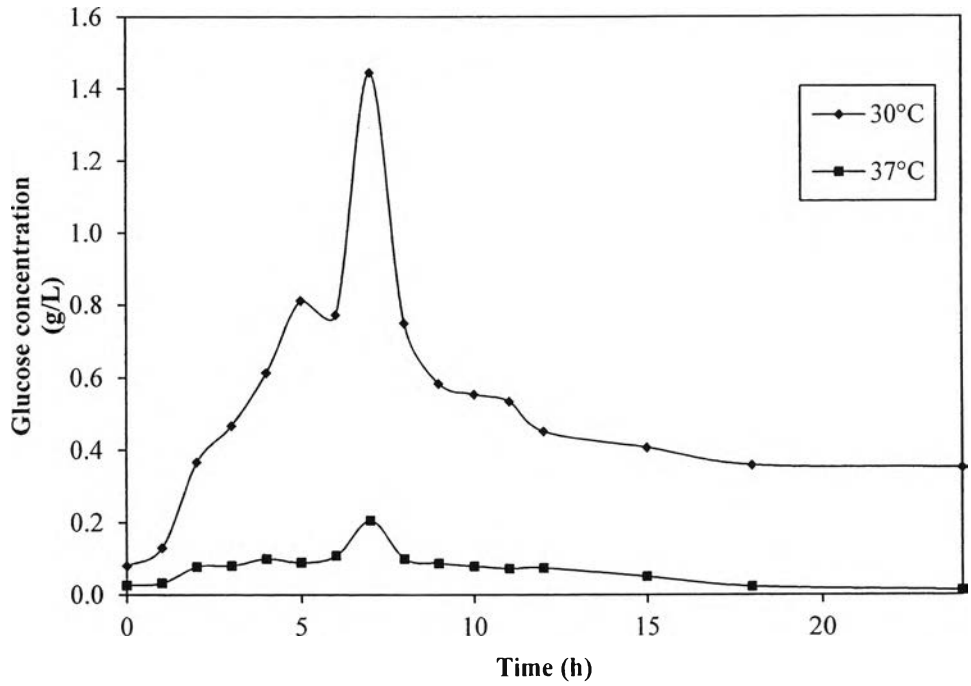

Figure 4.20 Effects of temperature on the produced glucose concentration from the hydrolysis of 80 mesh particle size cassava residue with strain A002.

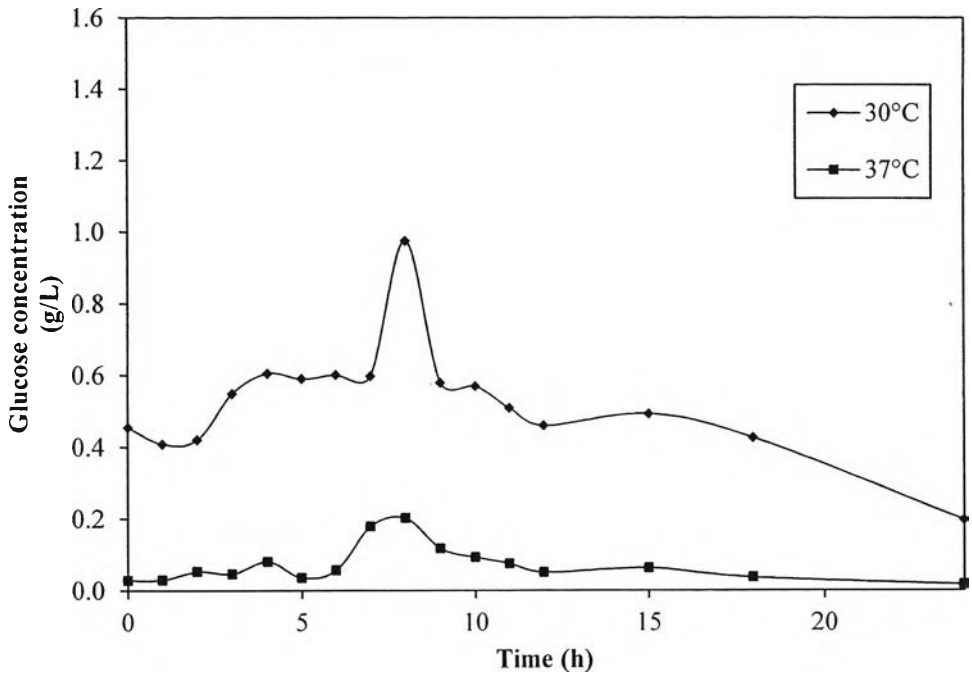

Figure 4.21 Effects of temperature on the produced glucose concentration from the hydrolysis of 80 mesh particle size cassava residue with strain M 015.

4.3.4 Effects of Concentration of Secondary Carbon Source

In general, growth medium, also known as a culture or production medium, consists of malt and yeast extract. In this work 65, modified DSMZ broth medium 2 was used as a production medium for the hydrolysis. It consisted of yeast extract 4.0 g/L, and malt extracts 10 g/L. Here, the amount of malt extract (10 g/L, 5 g/L, and 1 g/L) was varied to check if it had any effects on the glucose production. Results in Figure 4.22 showed that the malt extract at 10 g/L was suitable for bacteria from Thai higher termites to grow. As malt extract provides the carbon, protein and nutrient sources required for the growth of microorganisms, a sufficient carbon source is needed for bacteria to grow and degrade the cassava residue (Altaf *et al.* 2007).

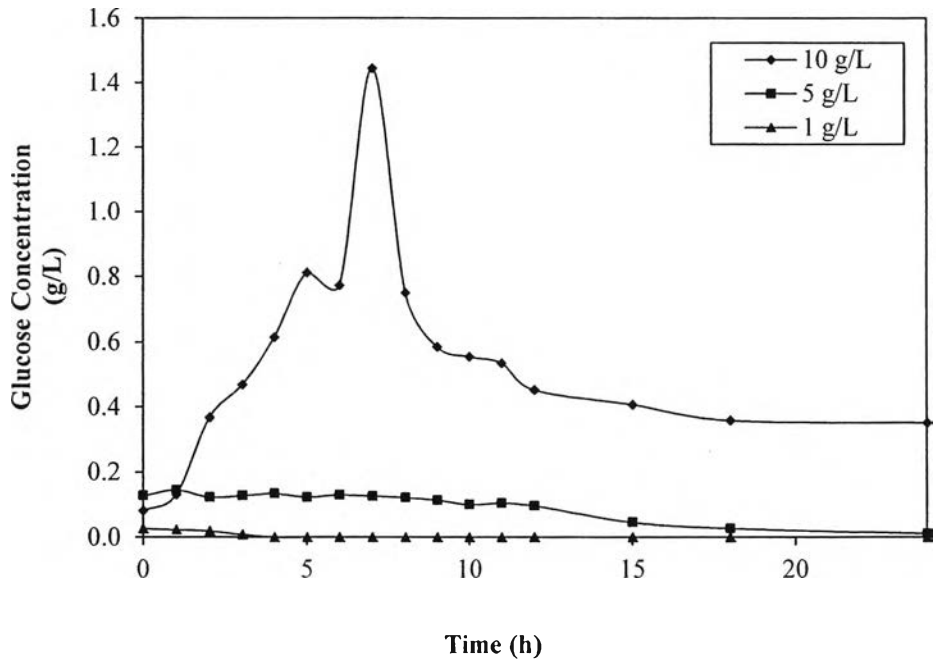

Figure 4.22 Effects of malt extract quantity on the produced glucose concentration from the hydrolysis of 80 mesh cassava residue using strain A 002 at 30 °C.

4.3.5 Glucose and Bacteria Evolutions

The produced glucose profiles obtained from the hydrolysis of 80 mesh cassava residue with strain A 002 at 30 °C were compared with the bacteria concentration, shown in Figure 4.23. Glucose significantly increased after 1 h until reaching the highest value and continued to decrease until 24 h. On the other hand, the bacteria concentration continued to increase. It implied that the glucose was consumed by the bacteria after 7 h.

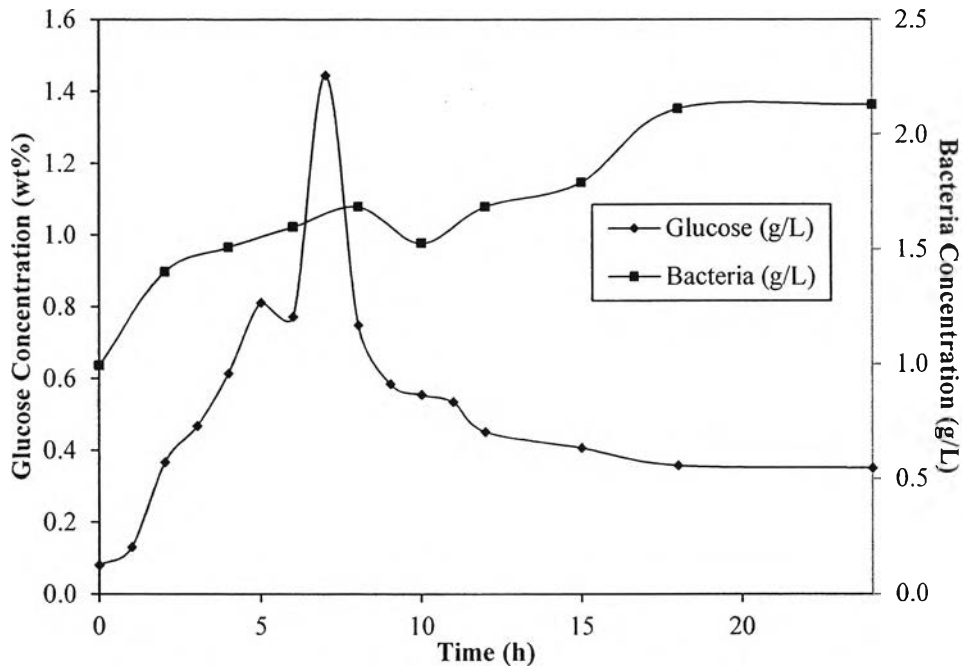

Figure 4.23 Effects of bacteria strain on the glucose concentration produced from the hydrolysis of the 80 mesh size cassava residue at 30 °C.

4.3.6 Glucose, Starch, and Cellulose Concentration vs. Time

During the enzymatic hydrolysis, cellulose, and starch concentration was also determined to confirm that both components were hydrolyzed. The cellulose and starch concentrations after the hydrolysis of 80 mesh cassava residue with strain A 002 at 30 °C were shown in Figure 4.24 compared with the produced glucose along the time. The starch and cellulose concentrations continued to decrease until the glucose concentration reached the highest value and then remained almost constant. This result implied that the bacteria used glucose as a carbon source instead of using starch and cellulose to grow.

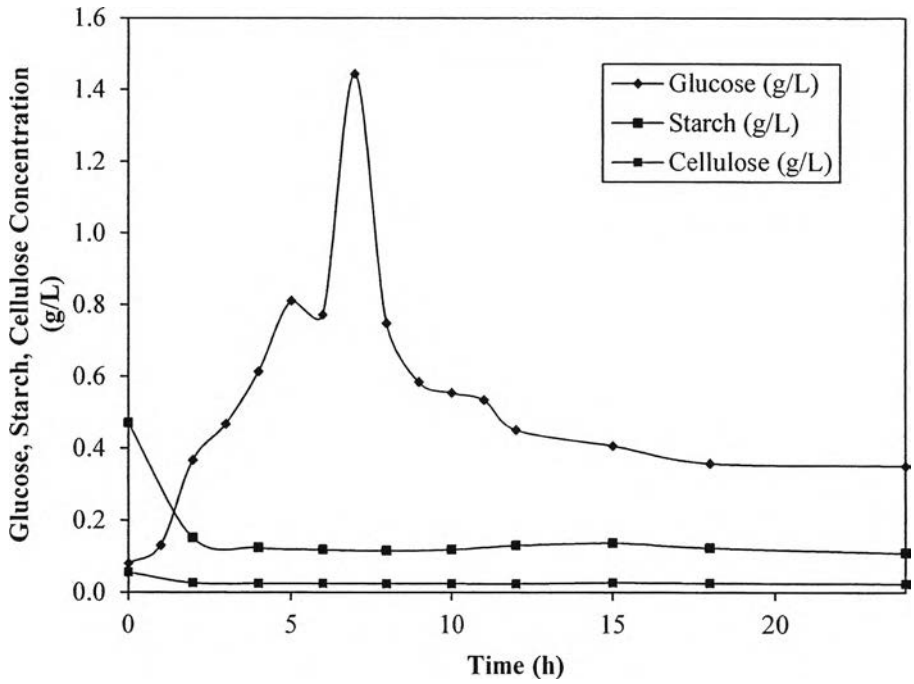

Figure 4.24 Concentration of glucose, starch, and cellulose after hydrolysis of 80 mesh cassava residue with strain A 002 at 30 °C.

4.4 Structure of Enzymatically Hydrolyzed Cassava Residue Samples

The morphological changes of cassava residue characteristics due to the enzymatic hydrolysis can be seen from the scanning electron micrographs at 1,000 magnifications. Figure 4.25 shows the smooth surface of cassava residue with the starch granules before hydrolysis. The morphology of cassava residue after the hydrolysis with strain A 002 and strain M 015 at 30°C was shown in Figure 4.26. The morphology was changed after the hydrolysis and was more pronounced with strain A 002 (Figure 4.26(a))

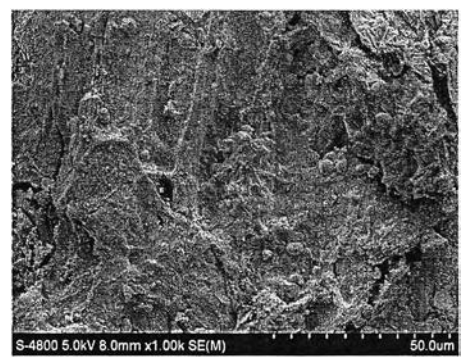

Figure 4.25 Scanning electron micrographs of the 80 mesh cassava residue surface before hydrolysis.

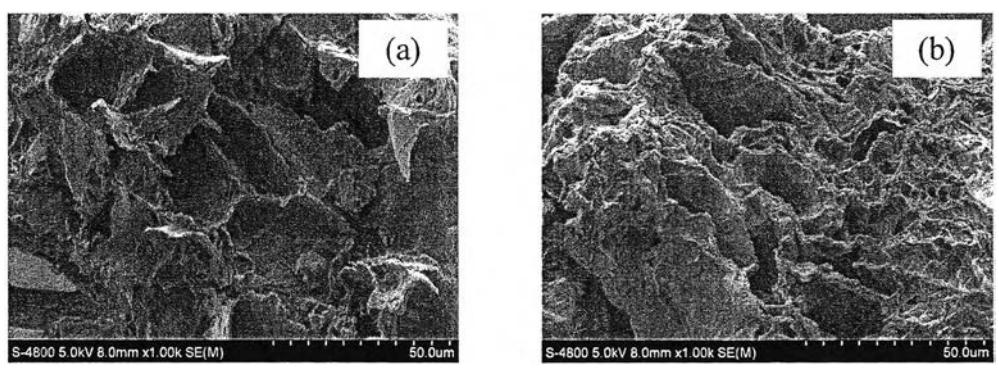

Figure 4.26 Scanning electron micrographs of the 80 mesh cassava residue surface at 30 °C (a) after hydrolysis with the strain A 002 and (c) after hydrolysis with the strain M 015.