CHAPTER IV
RESULTS AND DISCUSSION

4.1 Raw Material Characterization

4.1.1 Content Analysis

The component analysis and ultimate analysis of sugarcane bagasse were determined and are shown in Table 4.1. The results of the ultimate analysis from the CHNO/S analysis are 49.88, 6.34, 43.48, 0.28, and 0.02 for carbon, hydrogen, oxygen, nitrogen, and sulfur, respectively. Cellulose, hemicellulose, lignin, and extractives of sugarcane bagasse were analyzed by previously mentioned methods before the microbial hydrolysis experiments. The sugarcane bagasse sample consisted of 21.02 wt% cellulose, 45.63 wt% hemicellulose, 29.50 wt% lignin, and 3.85 wt% extractives. These values were found in the range with the same kind of material.

Table 4.1 Ultimate analysis and component analysis of ground bagasse

Parameter	Sugarcane bagasse sample
Ultimate analysis (wt% dry basis)	
Carbon	49.88
Hydrogen	6.34
Oxygen (by difference)	43.48
Nitrogen	0.28
Sulfur	0.02
Component analysis (wt% dry basis)	
Cellulose	45.63
Hemicellulose	21.02
Lignin	29.50
Extractives	3.85

4.1.2 Particle Size Analysis

Because we also studied the effects of biomass particle size on the microbial hydrolysis, we separated the ground bagasse by sieving through 40-mesh,

60-mesh, and 100-mesh screens. The samples are denoted as SCB (sugarcane bagasse) 40 (40-60 size) and SCB60 (60-100 size). Samples were analyzed by the particle size analyzer, and the results are shown in Table 4.2.

Table 4.2 Particle sizes of SCB40 and SCB60 samples

Biomass samples	D[4, 3], μm
SCB40	398.82
SCB60	261.63

4.1.3 Surface Area Analysis

Surface area of bagasse samples was measured because it is one of the structural features that can affect enzymatic activity and digestibility of bacteria. Table 4.3 shows the surface area results for SCB40 and SCB60 samples. The surface areas of the samples are low and relatively about the same.

Table 4.3 Results from surface area analyzer of SCB40 and SCB60 samples

Biomass samples	Surface area (m^2/g)
SCB40	7.92
SCB60	9.65

4.2 Sugar Production

Without the pretreatment, the bagasse samples (SCB40 and SCB60) were hydrolyzed for 24 h with M 015 and A 002 bacteria strains in DSMZ broth medium 2 without CMC, tap water, and mineral nutrient broth media at 30 and 37 °C. The liquid fraction of the hydrolysate from each batch was analyzed by the HPLC to determine monosaccharide concentration. The calibration equation was obtained

from the calibration graph by using various known concentrations of standard sugars. We found that glucose was the only monosaccharide in a significant amount in all experimental conditions.

4.2.1 Effects of Particle Size of Ground Bagasse

Results from using untreated 40 mesh and 60 mesh ground bagasse for the hydrolysis are shown in Figures 4.1 and 4.2. The glucose concentration from the hydrolysis of the 60 mesh sample with the M 015 strain increase sharply until reaching the peak at the fourth hour. Then, it significantly falls in the next four hours and starts to bottom off after that. For the 40 mesh sample with the M 015 strain, the rise in the glucose concentration starts faster but it takes longer to reach the maximum value, before dropping significantly and levelling off. The same pattern can also be observed from the hydrolysis with the A 002 bacteria strain. The glucose concentration is slightly higher in the batch that uses the smaller size ground bagasse. These results can be explained by the smaller particle size of the 60 mesh ground bagasse, which gives more contact surface for the bacteria to release enzyme to hydrolyse the material. As the direct physical adsorption onto the target molecules surface is required for cellulase activity, available accessible surface area is crucial for the hydrolysis (Leitão de Carvalho, 2009).

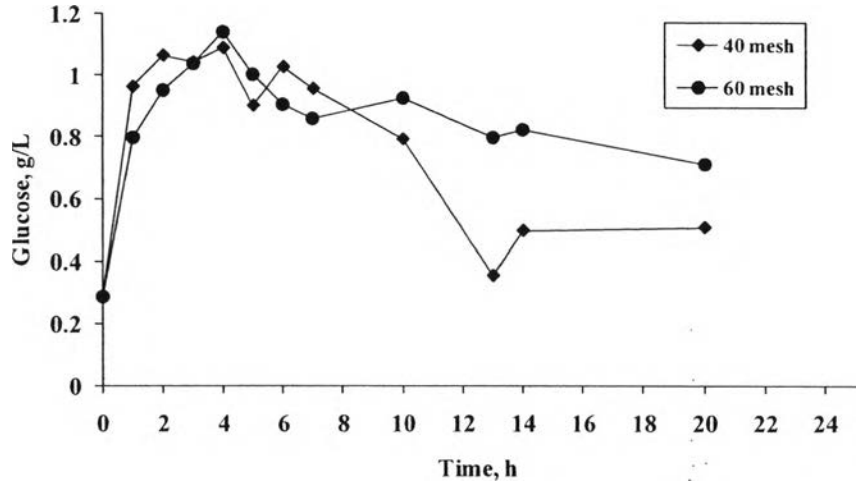

Figure 4.1 Glucose concentration from the hydrolysis of ground bagasse with the M 015 bacteria strain at 37°C.

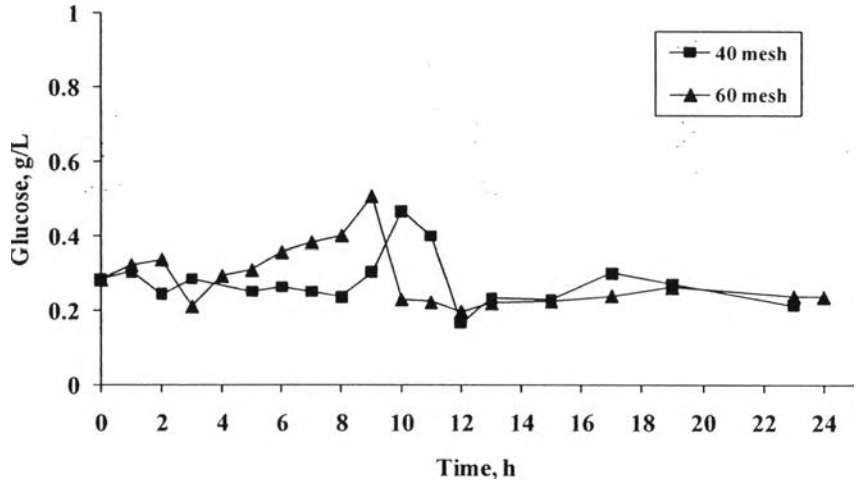

Figure 4.2 Glucose concentration from the hydrolysis of ground bagasse with the A 002 bacteria strain at 37°C.

4.2.2 Effects of Operating Temperature

Figures 4.3 to 4.6 show the comparison between the glucose produced from the hydrolysis of the 40 mesh and 60 mesh samples with the M 015 and A 002 bacteria strains at 30 and 37 °C operating temperature in the hydrolysis. At 37 °C,

the glucose content is clearly higher in the batch with the M 015 bacteria strain but only slightly higher in the batch with the A 002 bacteria strain. The glucose production kinetics seems to be slower with 30 °C operating temperature. The glucose concentration from the hydrolysis at 37 °C increases more sharply to reach its maximum than at 30 °C. The same phenomena can be observed from the hydrolysis of both 40 and 60 mesh sizes. After glucose concentration reaches the maximum value, the concentration decreases at a slower rate in the hydrolysis at 30 °C. This indicates that the bacteria may be less active at the low temperature.

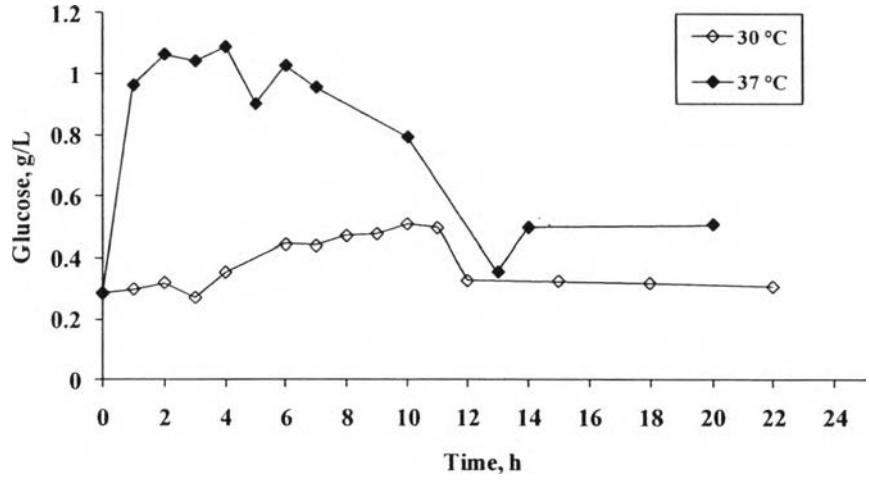

Figure 4.3 Glucose concentration from the hydrolysis of the 40 mesh ground bagasse with the M 015 bacteria strain at 30 and 37 °C.

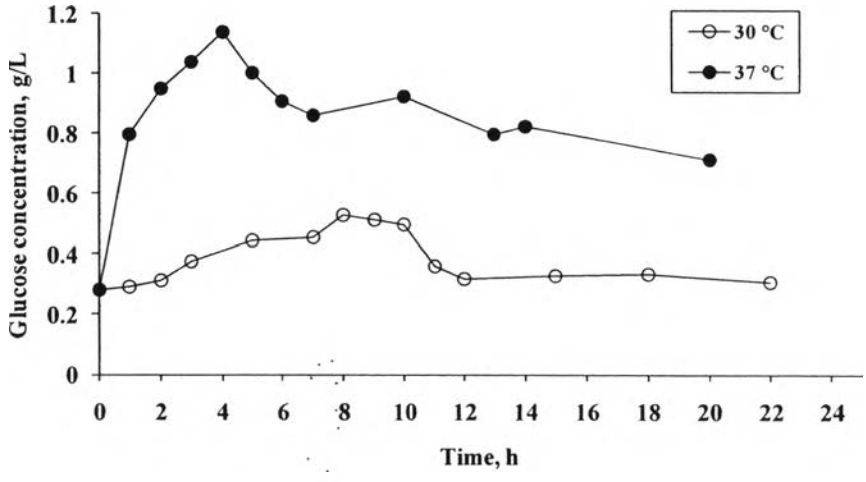

Figure 4.4 Glucose concentration from the hydrolysis of the 60 mesh ground bagasse with the M 015 bacteria strain at 30 and 37 °C.

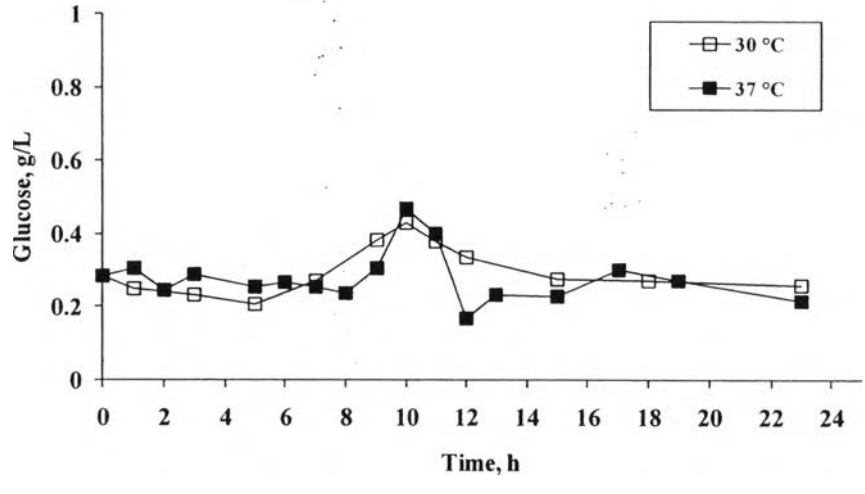

Figure 4.5 Glucose concentration from the hydrolysis of the 40 mesh ground bagasse with the A 002 bacteria strain at 30 and 37 °C.

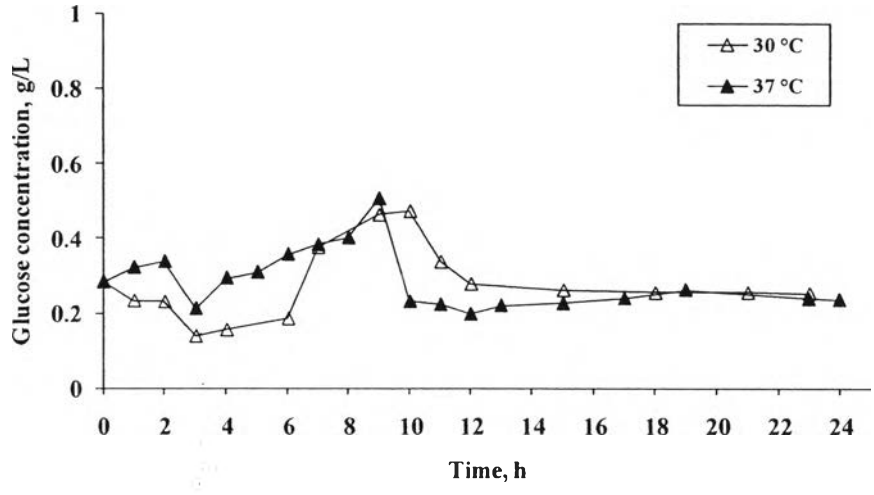

Figure 4.6 Glucose concentration from the hydrolysis of the 60 mesh ground bagasse with the A 002 bacteria strain at 30 and 37 °C.

4.2.3 Effects of Bacteria Strain

The M 015 strain gives much higher glucose concentration than the A 002 strain as shown in Figures 4.7 and 4.8. The glucose concentration from the hydrolysis of the untreated 60 mesh bagasse with the M 015 strain increases significantly in the first few hours and reaches its maximum value at 1.03 g/L. Another obvious difference is the time required to reach the maximum. The M 015 strain gives the highest glucose concentration at the fourth hour, while the A 002 strain needs nine hours. While the hydrolysis of the untreated 40 mesh bagasse gives slightly lower maximum value at the same time, the differences between the two strains are the same with the 60 mesh sample. These can be the result from the different selectivity of each bacteria strain to cellulose in sugarcane bagasse sample.

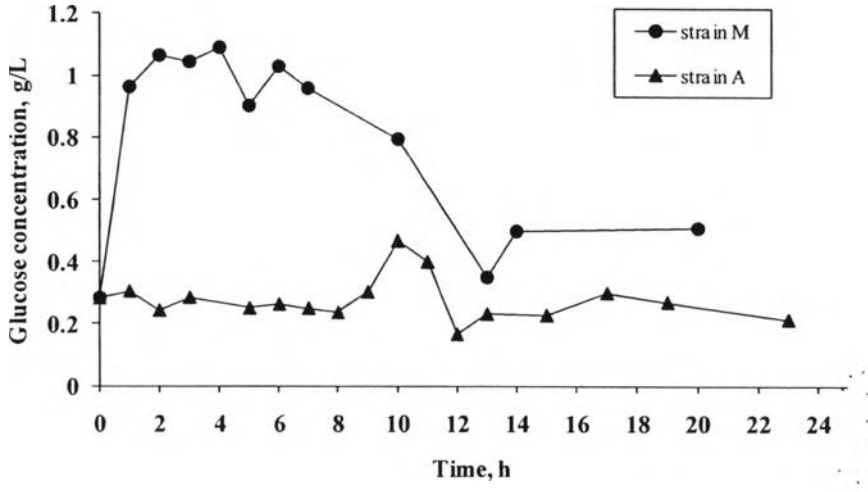

Figure 4.7 Glucose concentration from the hydrolysis of the 40 mesh ground bagasse with the M 015 and A 002 bacteria strain at 37 °C.

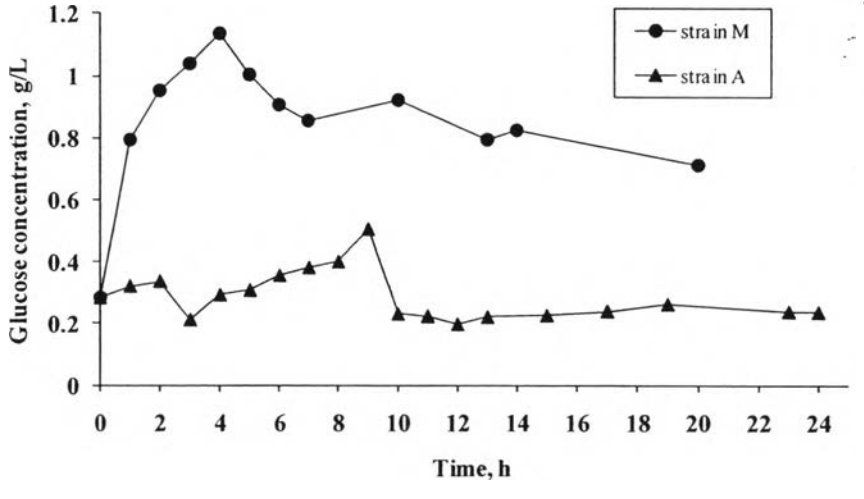

Figure 4.8 Glucose concentration from the hydrolysis of the 60 mesh ground bagasse with the M 015 and A 002 bacteria strain at 37 °C.

4.2.4 Effects of Fermentation Media

Types of fermentation media used in the hydrolysis are 65 modified DSMZ broth medium 2 without CMC, tap water, and mineral nutrient broth. The results from the HPLC analysis show that there is no sugar content in any samples

collected from the tap water and mineral nutrient broth media. Only the 65 modified DSMZ broth medium 2 without CMC has the appropriate components for the bacteria to grow, which are yeast extract and malt extract, the common component in microorganisms and cell cultures media.

4.2.5 Bacteria Growth

Although the bacteria loaded into the bioreactor was weighed on the wet basis, bacteria in the solid fraction of the hydrolysate periodically collected from hydrolysis of the untreated sugarcane bagasse were measured on the dry basis. The initial bacteria weight for each batch was 0.69 to 0.72 g. Figures 4.9 to 4.12 show the growth of bacteria and glucose concentration versus time for each experiment at 30 °C. As shown in Figures 4.9 and 4.10, the glucose concentration of the hydrolysis with the M 015 bacteria strain at 37 °C significantly increases until reaching its maximum. In the meantime, the growth of bacteria increases gradually. After that, the glucose concentration decreases, while the bacteria growth slightly increases. The results can be explained that during the first period, the bacteria grow by consuming the raw material and producing glucose. Then, the bacteria start to consume the produced glucose instead of the raw material; hence, the decrease in the glucose concentration and the increase in the bacteria. Compared to the M 015 bacteria, the A 002 bacteria grow at a slower rate in the first six hours before dramatically increasing at a higher rate than the M 015 bacteria strain. When the glucose concentration drops, the bacteria growth decreases to at a slow rate. The results are shown in Figures 4.11 and 4.12.

Considering the effect of difference ground bagasse sizes, the trend of the 40 mesh ground bagasse with the M 015 and A 002 bacteria strains give slightly slower growth rate than that of the 60 mesh size with the same strain. These are associated with the difference in the increased rate and time of glucose production, which are slightly slower in the hydrolysis of the larger size ground bagasse. However, the concentration of the bacteria at the end is about the same for both sizes with each bacteria strain.

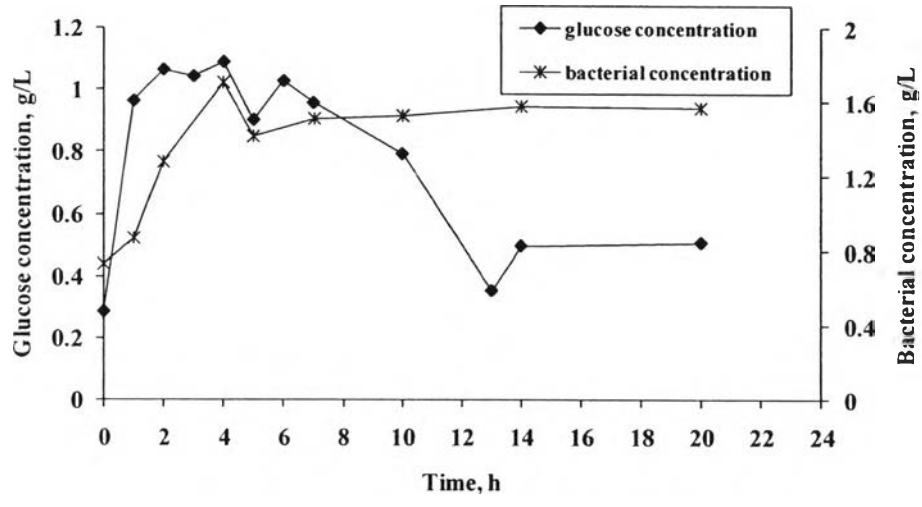

Figure 4.9 Bacteria growth and glucose concentration from the hydrolysis of the 40 mesh ground bagasse with the M 015 bacteria strain at 37 °C.

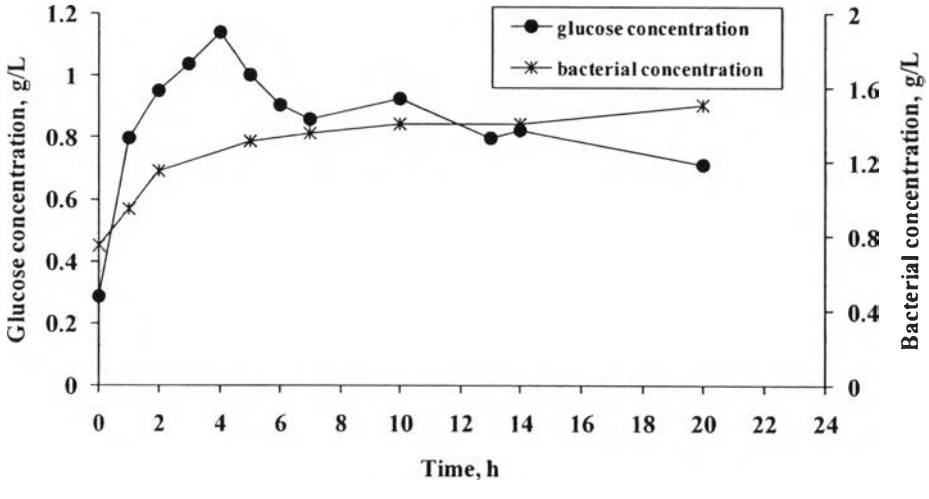

Figure 4.10 Bacteria growth and glucose concentration from the hydrolysis of the 60 mesh ground bagasse with the M 015 bacteria strain at 37 °C.

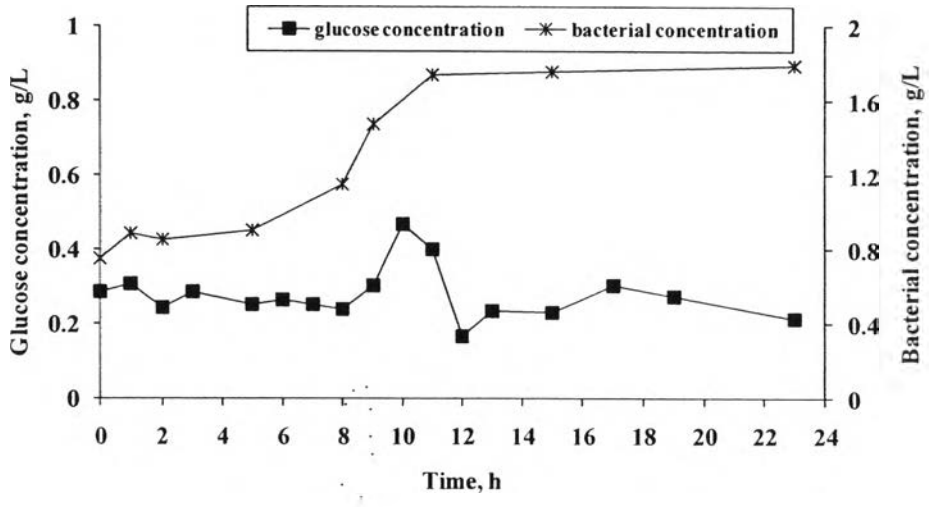

Figure 4.11 Bacteria growth and glucose concentration from the hydrolysis of the 40 mesh ground bagasse with the A 002 bacteria strain at 37 °C.

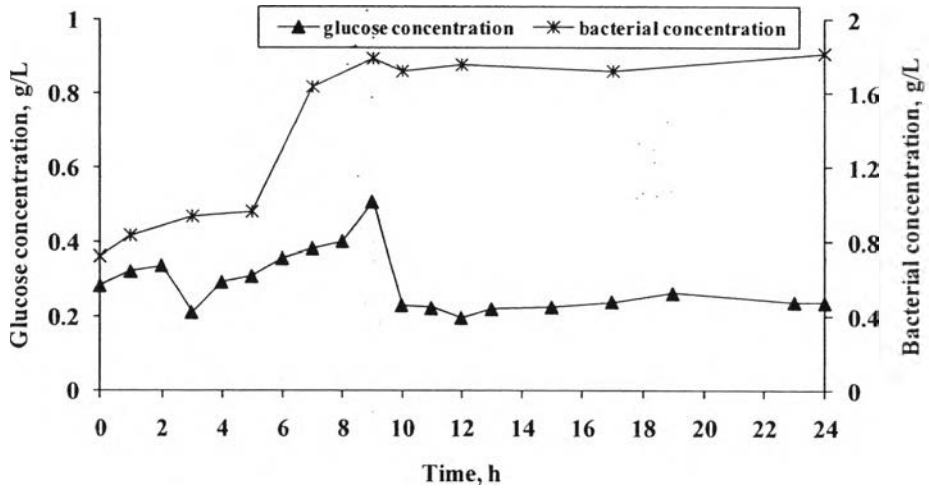

Figure 4.12 Bacteria growth and glucose concentration from the hydrolysis of the 60 mesh ground bagasse with the A 002 bacteria strain at 37 °C.

The same relation between the glucose concentration and the bacteria growth also occurred in every batch at 30 °C with the M 015 and A 002 bacteria strains as shown in Figures 4.13 to 4.16. However, there is a difference in the initial glucose concentration and bacteria growth, which is possibly due to the lower

hydrolysis rate at 30 °C. Overall, the hydrolysis of the ground bagasse samples at 30 °C gives moderately slower bacteria growth rate than at 37 °C with the same strain and same size of ground bagasse. Furthermore, at the end of the hydrolysis, the bacteria at 30 °C are lower than at 37 °C.

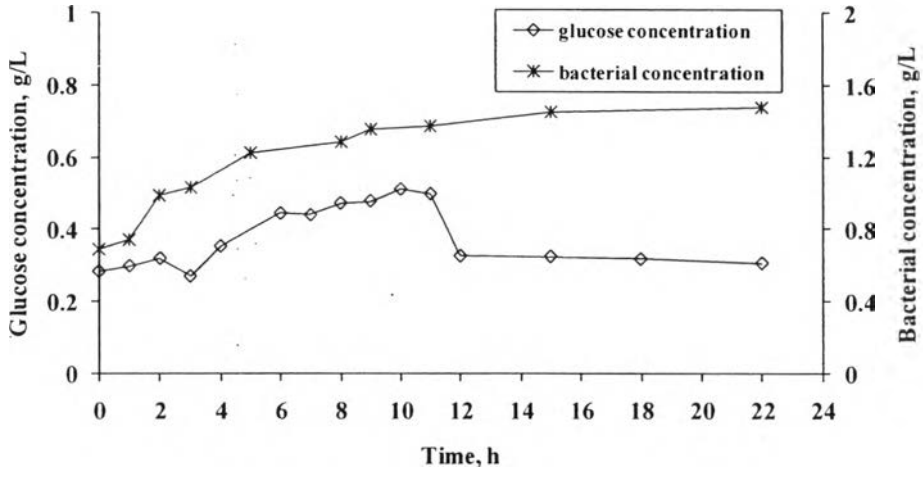

Figure 4.13 Bacteria growth and glucose concentration from the hydrolysis of the 40 mesh ground bagasse with the M 015 bacteria strain at 30 °C.

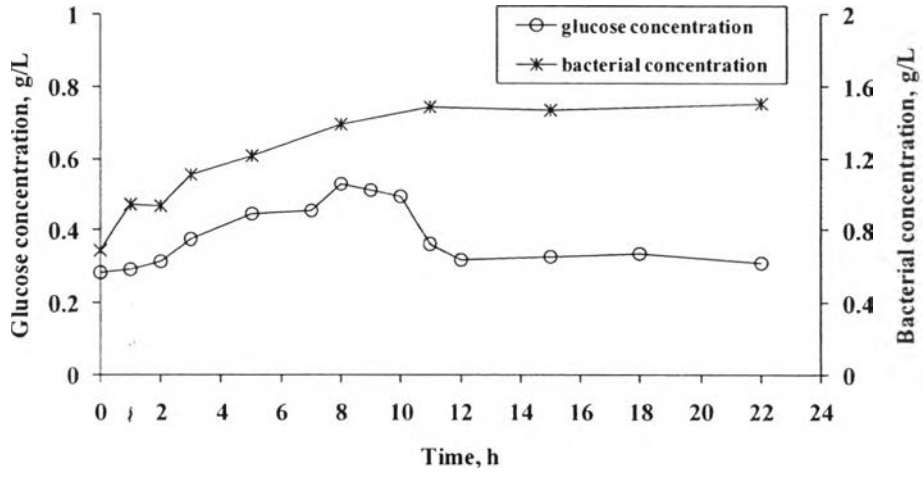

Figure 4.14 Bacteria growth and glucose concentration from the hydrolysis of the 60 mesh ground bagasse with the M 015 bacteria strain at 30 °C.

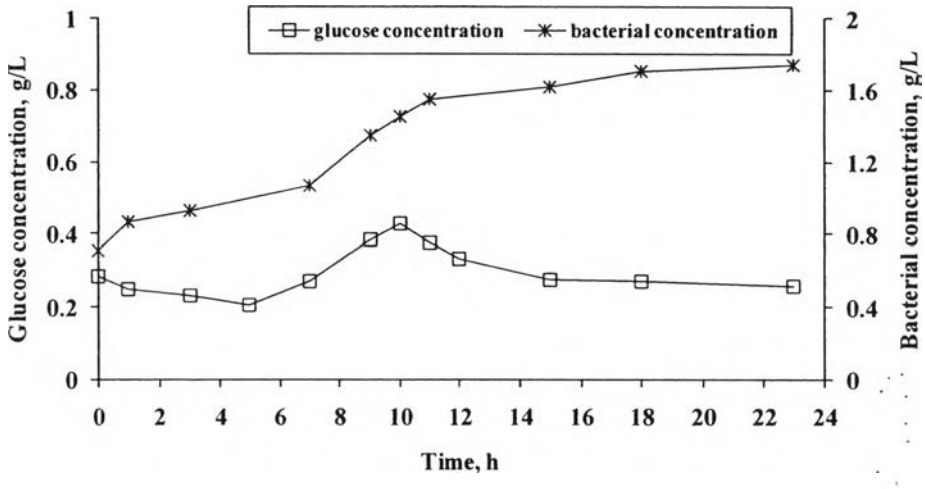

Figure 4.15 Bacteria growth and glucose concentration from the hydrolysis of the 40 mesh ground bagasse with the A 002 bacteria strain at 30 °C.

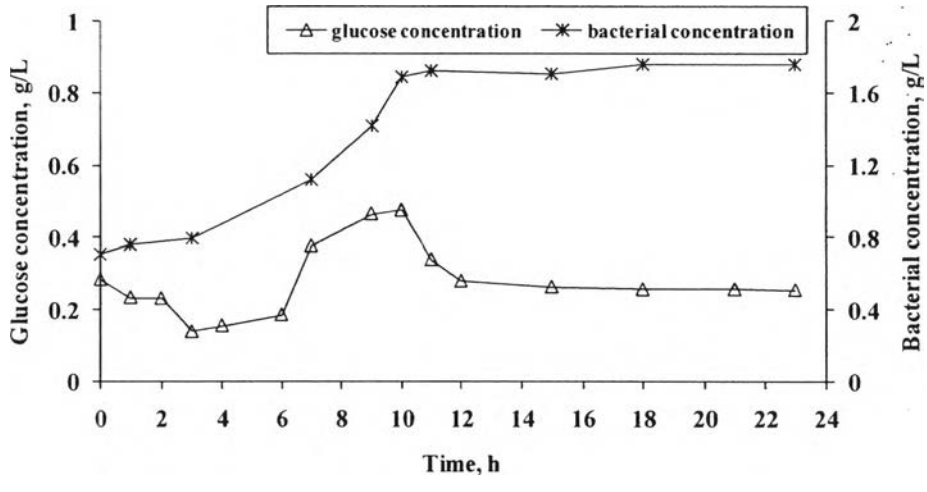

Figure 4.16 Bacteria growth and glucose concentration from the hydrolysis of the 60 mesh ground bagasse with the A 002 bacteria strain at 30 °C.

Table 4.4 shows the composition of sugarcane bagasse samples after the hydrolysis experiments. The cellulose composition in each sample is lower than that of the material before the hydrolysis (Table 4.1). Hence, lignin and hemicellulose are left as the main compositions, especially for the hydrolysis with

the M 015 strain. These can be explained in that the cellulose portion in the raw material was consumed by the bacteria and hydrolyzed to glucose, which was mainly found in the liquid products. For this reason, the production of glucose from sugarcane bagasse by the bacteria was verified.

Table 4.4 Component analysis of ground bagasse after the hydrolysis experiment at 37 °C

Component (wt% dry basis)	The 40 mesh with the M 015	The 60 mesh with the M 015	The 40 mesh with the A 002	The 60 mesh with the A 002
Cellulose	11.55	9.47	34.87	34.23
Hemicellulose	34.22	34.96	25.76	25.93
Lignin	47.96	49.02	35.26	35.13
Extractives	6.27	6.55	4.11	4.71