CHAPTER IV
RESULTS AND DISCUSSION

4.1 Bagasse Composition

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

Table 4.1 Elemental composition of bagasse

Elemental composition	**wt%, dry basis**
Carbon	39.42
Hydrogen	6.08
Oxygen	54.14
Nitrogen	0.34
Sulfur	0.02

Table 4.2 Chemical composition of bagasse

Chemical composition of bagasse	**wt%, dry basis**
Cellulose	46.7
Hemicellulose	28.89
Lignin	13.83
Ash	10.58

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 strain, the determination of hydrolysis capacity of strain A 002 and strain M 015 was perfomed. The HC value can be determined 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 1.79 and 1.52, respectively. This concludes that strain A 002 tends to have higher ability to degrade cellulose than strain M 015.

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

Bacteria strain	Hydrolysis capacity value (HC value)
A 002	1.79
M 015	1.52

4.3 Microbial Hydrolysis Results

The microbial hydrolysis of bagasse was performed in batvh reactor, containing 1000 ml of the 65 modified DSMZ broth medium 2 without CMC and 1 g of bagasse powder with pH adjusted to 7.2. In this research, two effective strains (strain A 002 and M 015) obained from Thai higher termite Microceroterm sp., were used to hydrolyze bagasse with different bagasse particle sizes and differnet concentration of secondary carbon source on the glucose production.

4.3.1 Effects of Bagasse Particle Size on the Glucose Production

Three different sizes (40−60 mesh, 60−80 mesh and > 80 mesh) of bagasse were investigated in order to obtain an optimum size for the microbial hydrolysis. The experiments were operated under a fixed substrate concentration of 1.0 g/L.

Figure 4.1 (a) shows the effect of the three bagasse sizes on microbial hydrolysis at 37 °C with strain A 002. The glucose concentration from the hydrolysis of the > 80 mesh size bagasse gradually increased and reached the maximum, about 0.46 g/L, at 8 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 bagasse under the same hydrolysis conditions increased at a slower rate and reached the maximum glucose concentration about 0.41 g/L and 0.38 g/L at 8 h, respectively. The effect of bagasse size on the hydrolysis at 37°C with strain M 015 is shown in Figure 4.1 (b). The glucose concentration produced from the > 80 mesh size bagasse under the same conditions gradually increased and reached the maximum glucose production, about 0.44 g/L, at 12 hr. However, the hydrolysis of the 60—80 mesh and 40—60 mesh sizes of the bagasse at 37 °C with strain M 015 slightly increased and reached the maximum glucose production of 0.41 and 0.40 g/L at 14 and 16 h, respectively. In a comparison, the glucose production from strain A 002 was higher than that from strain M 015 under the same hydrolysis condition. These results indicate that the smaller the size of bagasse, the higher the surface area, the easier the enzyme to hydrolyze the bagasse which resulted in the higher glucose production. In addition, BET experiment was done to support the results why the smaller size of bagasse provided the higher glucose production. As shown in Table 4.4, the smaller particle size provided the higher specific surface area. Thus, this can be confirmed that the higher specific area, the higher in accessibility between cellulose and enzyme, which increased enzymatic digestibility and glucose production. The obtained results were consistent with several researchers (Caulfield & Moore, 1974; Fierobe et al., 2002; Howell & Stuck, 1975) who have pointed out that the reduction of particle size significantly improved the hydrolysis reaction rate.

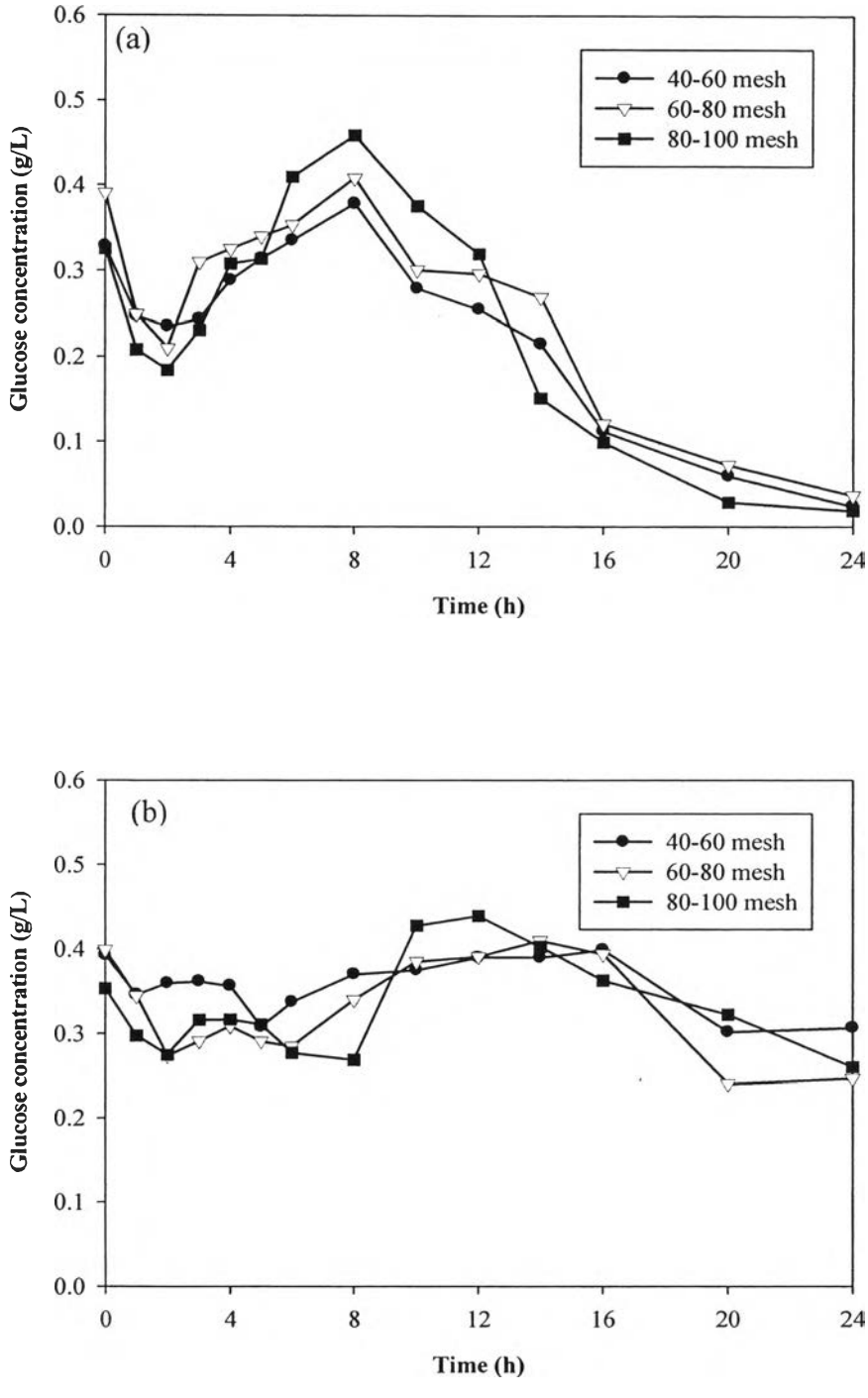

Figure 4.1 Effect of bagasse particle size on the glucose concentration profile using bacterial (a) strain A 002 and (b) strain M015.

Table 4.4 Specific surface area of bagasse particle

Bacteria size	**Surface area (m^2/g)**
40–60 mesh	7.44
60–80 mesh	8.97
> 80 mesh	13.59

4.3.2 Effects of Bacteria Strain on the Glucose Production

The performance of two bacteria strains (strain A002, and strain M 015) on the microbial hydrolysis of bagasse were studied. Figure 4.2 shows that the glucose production from strain A 002 was higher than that from strain M 015. Moreover, using strain A 002 requires less hydrolysis time to reach maximum glucose concentration than using strain M 015. According to Figure 4.2, the glucose concentration slightly increased until it reached the maximum amount of glucose produced, about 0.46 g/L, at 8 h from the hydrolysis of > 80 mesh size bagasse with strain A 002. However, The glucose concentration profile by using strain M 015 slightly increased until it reached the maximum glucose concentration, about 0.44 g/L, at 12 h under the same hydrolysis condition. The present results are supported by the results from Taechapoempol *et al.* (2010), who reported that strain A 002 had higher β-glucosidases activity than strain M 015 to cleave cellulobiose into glucose resulting in a higher glucose production.

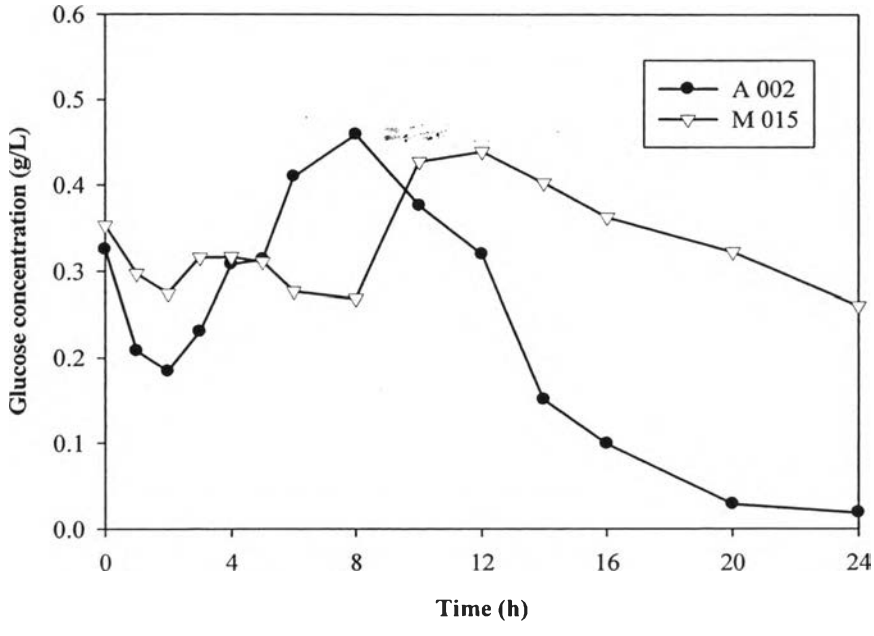

Figure 4.2 Effect of bacteria strains on the glucose concentration profile from the hydrolysis of < 80 mesh bagasse.

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

Production medium generally consists of malt and yeast extract. In this research, modified DSMZ broth medium 2 was used as a production medium. It consists of 10 g/L malt extracts, and 4.0 g/L yeast extract. To investigate the effect of concentration of secondary carbon source in production medium, on the glucose production, the amount of malt extract in the production medium was varied (12, 10, 8, and 6 g/L). Figure 4.3 clearly shows that the addition of 12 g/L or 10 g/L of the malt extract in the production medium resultes in a higher glucose production rate as compared to the addition of 8 g/L or 6 g/L of malt extract in the production medium. These results are also supported by 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. The maximum glucose concentrations obtained from adding 12 g/L and 10 g/L malt

extract were 0.46 and 0.47 g/L, respectively, which were insignificantly different. This indicated that the optimum concentration of malt extract in production medium was 10 g/L.

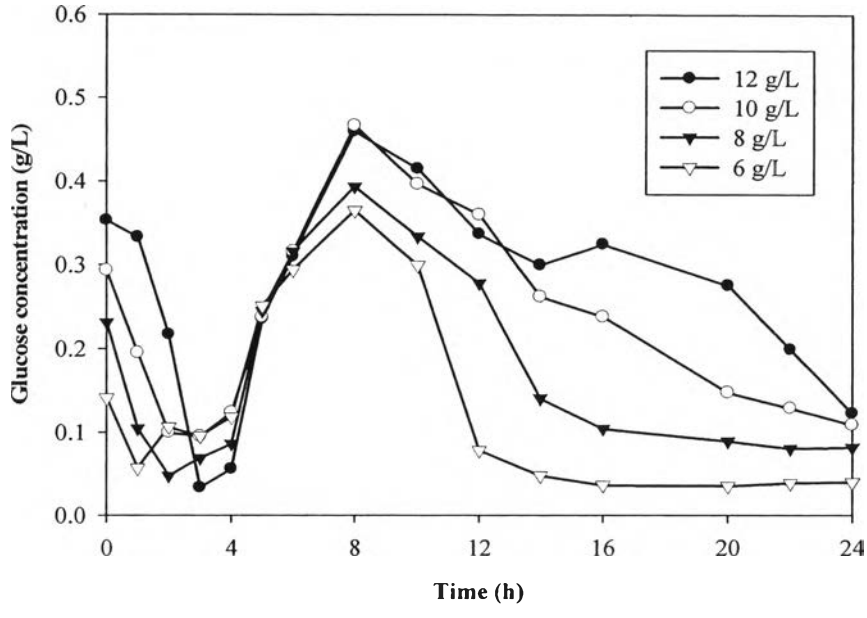

Figure 4.3 Effect of concentration of secondary carbon source on the glucose concentration profile from the hydrolysis of > 80 mesh particle size bagasse using bacteria strain A 002.

4.3.4 Bacteria Concentration and Glucose Production vs. Time

Figure 4.4 (a) shows bacteria concentration and glucose concentration profile from the hydrolysis of > 80 mesh bagasse with strain A 002 at 37 °C. The glucose significantly increased after 2 h until it reached the highest value at 8 h and continued to decrease until 24 h. On the other hand, the bacteria concentration continued to increase. It can be implied that the glucose was consumed by the bacteria after 8 h. Moreover, the produced glucose profiles obtained from the hydrolysis of > 80 mesh bagasse with strain M 015 at 37 °C were also compared with the bacteria concentration, as shown in Figure 4.4 (b). Glucose production slightly increased and reached the highest value at 12 h and then decrease until 24 h. However, the bacteria concentration continued to increase along the hydrolysis time. This also indicates that the decreasing of glucose concentration after 12 h may resulted from the glucose consumption by the bacteria.

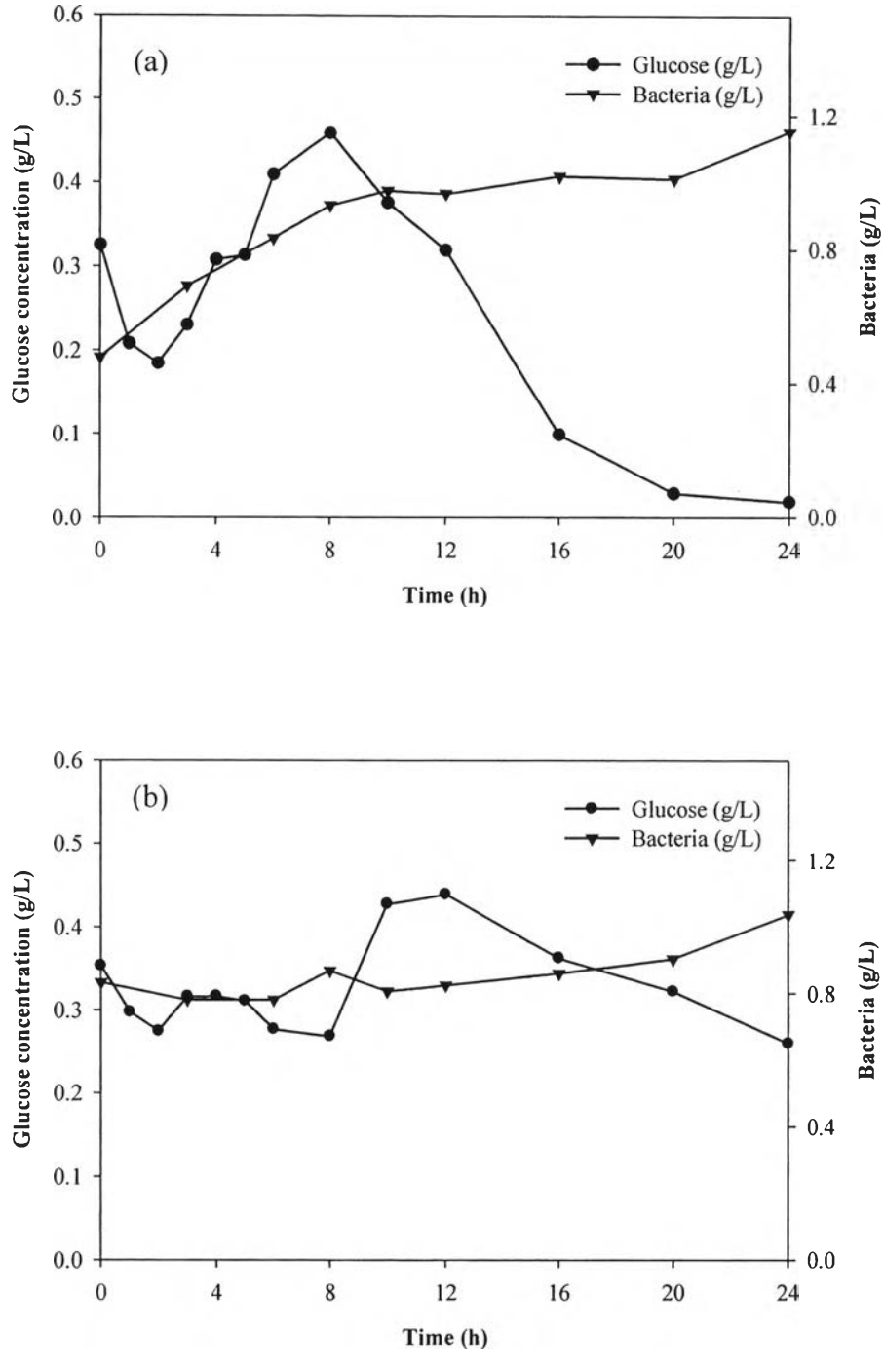

Figure 4.4 Glucose concentration and bacteria concentration profile from the hydrolysis of > 80 mesh bagasse with (a) strain A 002 and (b) strain M 015.

4.4 Enzymatic Hydrolysis Results

4.4.1 Effects of Enzyme Loading on the Glucose Production

In order to compare glucose production, the > 80 mesh bagasse was hydrolyzed enzymatically using a commercial enzyme, which was cellulase produced from *Aspergillus niger*. Various concentrations (100, 200, 300, 400, and 500 U) of enzyme loading were used for hydrolysis of bagasse at pH 5.5 and 37 °C. The profiles of glucose production were examined over 72 h period as shown in Figure 4.5. From the results, 500 U cellulase provided the highest glucose concentration of 0.51 g/L followed by 400, 300 ,200, and 100 U at 72 h, respectively. The maximum glucose concentration from the microbial hydrolysis was 0.46 g/L which is equivalent to the glucose concentration obtained from the enzymatic hydrolysis using 415 U cellulase enzyme. The results indicate that the hydrolytic activities of both strains (strain A 002 and M 015) were found to be as high as that of commercial enzyme. Due to the high cost of commecial cellulase enzyme, microbial hydrolysis using bacterial strains from Thai higher termites is more economical than the enzymatic hydrolysis.

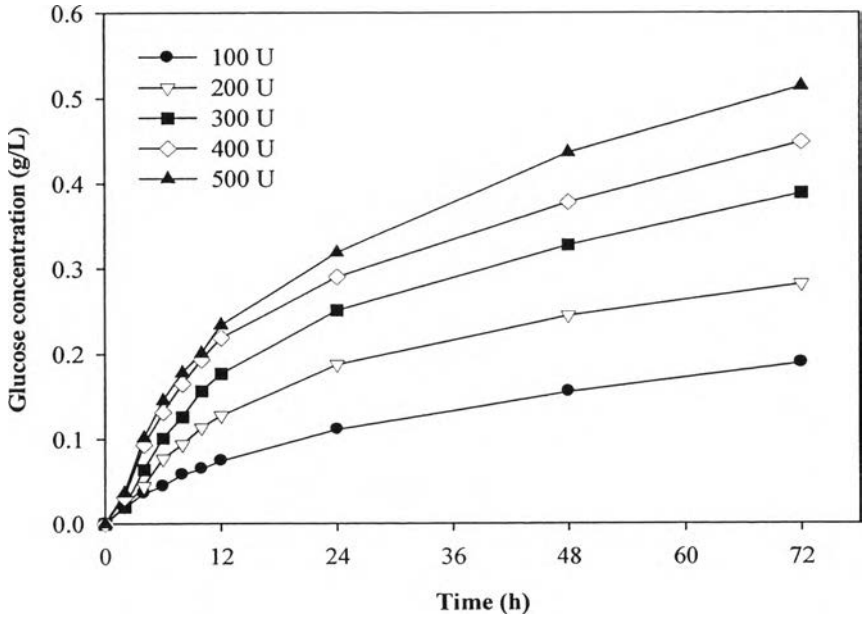

Figure 4.5 Effects of cellulase enzyme loading on the glucose concentration produced from the hydrolysis of the > 80 mesh bagasse at 37 °C.

4.6. Structure of Enzymatically Hydrolyzed Bagasse Sample

The scanning electron micrographs at 800 magnifications clearly show the morphological changes of bagasse due to the hydrolysis process using bacteria isolated from Thai higher termites (strain A 002, and M 015), and cellulase enzyme, produced from *Aspergillus niger*. Before undergoing hydrolysis process, bagasse showed smooth surfaces as shown in Figure 4.6 (a). However, the morphology of bagasse was significantly changed, after the bagasse was hydrolyzed as shown in Figure 4.6 (b), (c) and (d). This indicates that there was a physical transformation of the bagasse after the hydrolysis.

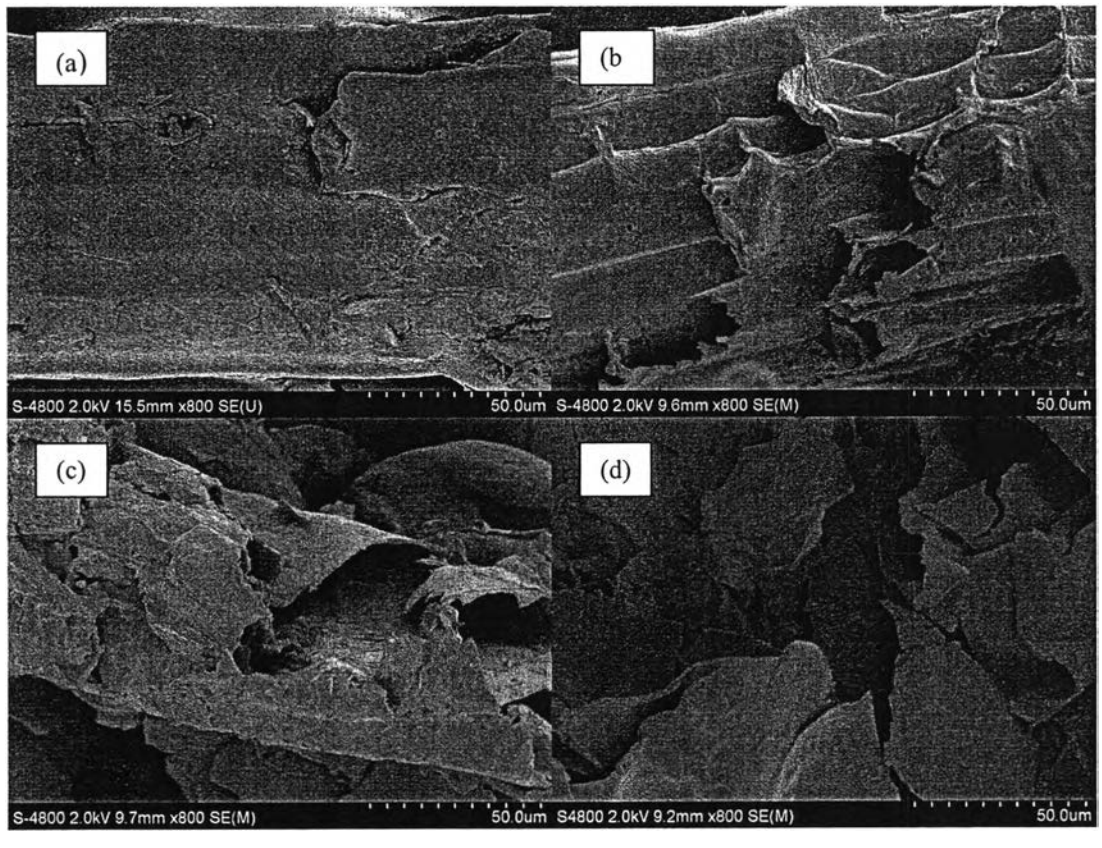

Figure 4.6 Scaning electron microraphs of > 80 mesh size of bagasse surface (a) before hydrolysis (b) after hydrolysis at 37 °C with the strain A 002, (c) after hydrolysis at 37 °C with the strain M 015, and (d) after hydrolysis at 37 °C with the cellulase enzyme.