CHAPTER IV
RESULTS AND DISCUSSION

4.1 Corncob and Bagasse Composition

For the elemental analysis of corncob and sugarcane bagasse residue, CHNS elemental analysis was used. The results are shown in Tables 4.1. The main elemental composition of untreated corncob contains about 50.55% oxygen, followed by 43.02% carbon and 6.28% hydrogen. For sugarcane bagasse, the main elemental composition is also oxygen (58.86 %), followed by carbon (35.34%) and hydrogen (5.69%). It can also be seen that the carbon composition in the corncob is higher than that in the sugarcane bagasse, while the oxygen content in the corncob is lower.

Chemical compositions of corncob and sugarcane bagasse before and after steam-explosion pretreatment are shown in Tables 4.2 - 4.3 and Figures 4.1 – 4.4. The major component of corncob and sugarcane bagasse is cellulose, followed by hemicellulose and lignin. The corncob has higher cellulose, hemicellulose, and lignin contents than the sugarcane bagasse.

Table 4.1 Elemental composition of corncob and sugarcane bagasse

Elemental Composition	**Corncob (% wt, dry basis)**	**Sugarcane bagasse (% wt, dry basis)**
Carbon (C)	43.02	35.34
Hydrogen (H)	6.28	5.69
Oxygen (O)	50.55	58.86
Sulfur (S)	0.16	0.11

Table 4.2 Chemical compositions of untreated corncob and steam-explosion pretreated corncob with H_2O and H_2SO_4 as preimpregnation agents and 1, 2, and 3 h pretreated time

Pretreatment conditions		Corncob compositions (%)			
		Cellulose	Hemicellulose	Lignin	Extractives
Untreated		43.35	31.86	18.25	6.54
H_2O	1 h	45.64	28.63	14.29	5.77
	2 h	44.32	32.16	14.58	5.87
	3 h	40.93	31.05	15.29	6.51
H_2SO_4	1 h	43.66	29.24	19.55	7.56
	2 h	39.46	29.36	22.50	7.71
	3 h	37.63	32.46	23.23	6.95

Table 4.2 shows the chemical composition changes of corncob after steam-explosion pretreatment by using H_2O and H_2SO_4 as a preimpregnation agent at difference pretreatment times. The results show that the cellulose fraction increases after the pretreatment with H_2O for 1 and 2 h, but the longer the steam-explosion pretreatment time leads to lower in the cellulose content. However, using H_2SO_4 as the preimpregnation agent and 1 h for pretreatment results in almost the same cellulose contents. Increasing the pretreatment time to 2 and 3 h decreases the cellulose content. The hemicellulose fraction slightly increases at 2 h for the H_2O

pretreated corncob and at 3 h for the H_2SO_4 pretreated corncob. On the contrary, the hemicellulose fraction for 1 and 3 h H_2O steam-explosion pretreated decreases and for the H_2SO_4 steam-explosion pretreated corncob, the hemicellulose fraction decreases at 1 and 2 h pretreatment time. This is because the hemicelluloses fractions are known to be readily hydrolyzed to oligosaccharides by steaming and some volatile organic compounds vaporize as volatile components (Joeh, 1998). The lignin contents of the H_2SO_4 steam-explosion pretreated corncob increase, while the lignin contents for the H_2O pretreated corncob decrease. For the increase in the lignin contents in the H_2SO_4 steam-explosion pretreated corncob, it can be explained that H_2SO_4 softens the lignin structure under the heat. Lignin is then released from the cell wall and distributed evenly onto the raw material. This indicates that the addition of the preimpregnation agent especially the chemical agents (i.e. alkaline, diluted acid or ammonia) seem to be an effective method to increase cellulose digestibility of 1,2, and 3 h H_2SO_4 pretreated corncob and solubilize a significant portion of the hemicellulosic component (Ballesteros *et al.*, 2006). This is due to the chemical preimpregnation agent helps to reduce the barriers posed by plant cell walls that limit hydrolytic enzymes access to the cellulose. The effects of using H_2O and H_2SO_4 for the pretreatment on the chemical composition of corncob are also summarized in Figures 4.1 and 4.2.

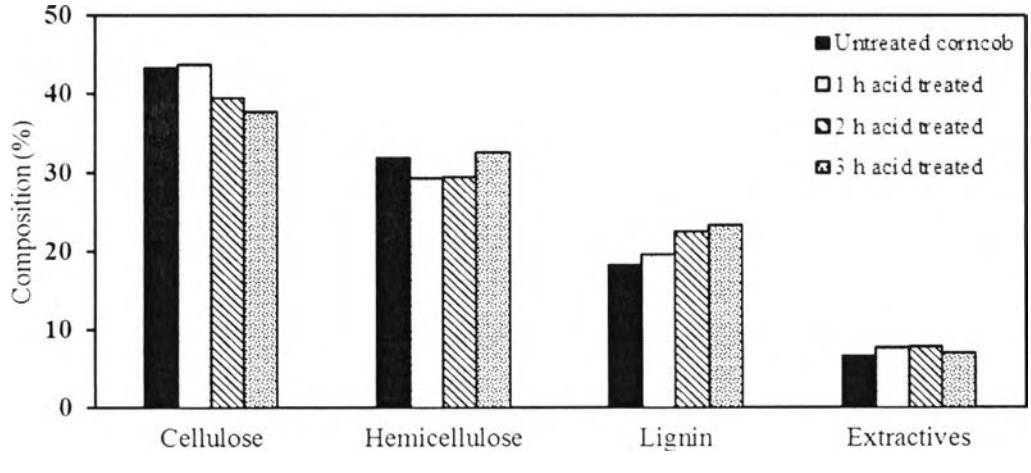

Figure 4.1 Chemical compositions of untreated corncob and steam-explosion pretreated corncob by using H_2SO_4 as a preimpregnation agent with difference times.

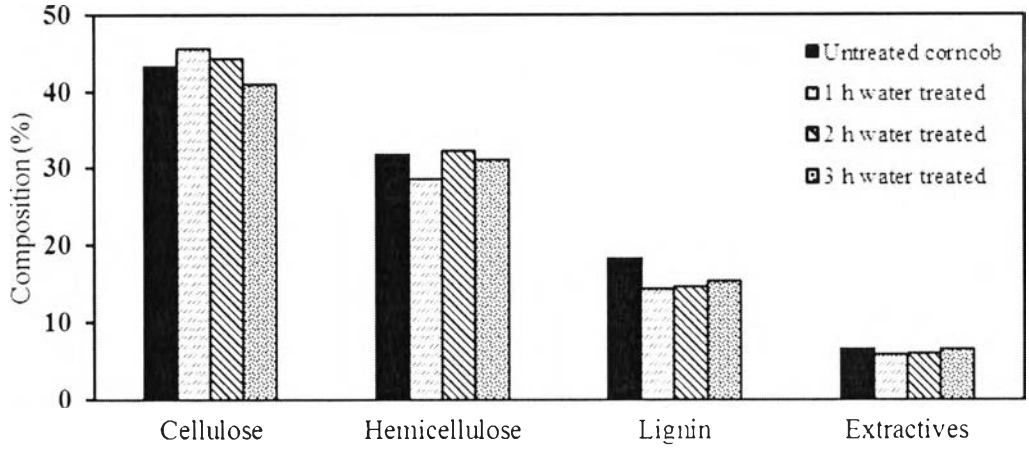

Figure 4.2 Chemical compositions of untreated corncob and steam-explosion pretreated corncob by using H_2O as a preimpregnation agent with difference time.

Table 4.3 Chemical compositions of untreated sugarcane bagasse and steam-explosion pretreated sugarcane bagasse with H_2O and H_2SO_4 as preimpregnation agents and 1, 2, and 3 h pretreated time

Pretreatment conditions		**Sugarcane bagasse compositions (%)**			
		Cellulose	**Hemicellulose**	**Lignin**	**Extractives**
Untreated		41.96	25.96	12.43	19.65
H_2O	1 h	42.46	22.05	15.29	20.48
	2 h	41.8	24.86	13.87	19.56
	3 h	46.02	27.19	13.52	14.23

Table 4.3 (cont.) Chemical compositions of untreated sugarcane bagasse and steam-explosion pretreated sugarcane bagasse with H_2O and H_2SO_4 as preimpregnation agents and 1, 2, and 3 h pretreated time

Pretreatment conditions		Sugarcane bagasse compositions (%)			
		Cellulose	Hemicellulose	Lignin	Extractives
H_2SO_4	1 h	44.78	23.43	14.37	17.76
	2 h	42.76	24.42	17.23	15.85
	3 h	34.61	28.83	18.6	18.17

The chemical compositions of sugarcane bagasse before and after steam-explosion pretreatment are summarized in Table 4.3. The extractive content for the H_2O steam-explosion pretreated sugarcane bagasse decreases for the 3 h pretreatment time, while the extractive content for the 1 h pretreated sugarcane bagasse increases. For the H_2SO_4 steam-explosion pretreated sugarcane bagasse, the extractive content is lower than the untreated sugarcane bagasse for all pretreatment times. This is because of the preimpregnation agents partially wash out some of the extractive during the pretreatment process. The H_2SO_4 pretreated sugarcane bagasse has higher cellulose content for the 1 and 2 h pretreatment conditions than the untreated sugarcane bagasse. For the H_2O steam-explosion pretreated sugarcane bagasse, the cellulose content increases at the 1 and 3 h pretreatment times, while the cellulose content for the 2 h pretreatment time is about the same as the untreated sugarcane bagasse. Furthermore, the hemicellulose fraction slightly increases for 3 h of the pretreatment by using both H_2O and H_2SO_4 as the preimpregnation agent. But the hemicellulose fraction for the 1 and 2 h pretreatment times with H_2O and H_2SO_4 as a preimpregnation agent slightly decreases.

This is due to the H_2SO_4 and H_2O steam-explosion pretreatment allows the deconstruction of the lignocellulosic material structure and the release of sugar monomers, mostly derived from the hemicelluloses. But for the 3 h steam-explosion pretreatment with H_2SO_4 gives the higher hemicelluloses content than using H_2O as a preimpregnation agent. This is due to the hemicelluloses is preferably removed and easily hydrolyzed by H_2SO_4than using H_2O because of its amorphous and branched structure (Wyman *et al.*, 2005). Furthermore, the lignin fraction for both steam-explosion pretreatment with H_2O and H_2SO_4 is slightly higher than the untreated sugarcane bagasse. This is due H_2O and H_2SO_4 soften the lignin structure under the heat before lignin is released from the cell wall and distributed evenly onto the raw material. Figures 4.3 and 4.4 also show the effects of the pretreatment on the chemical composition of sugarcane bagasse.

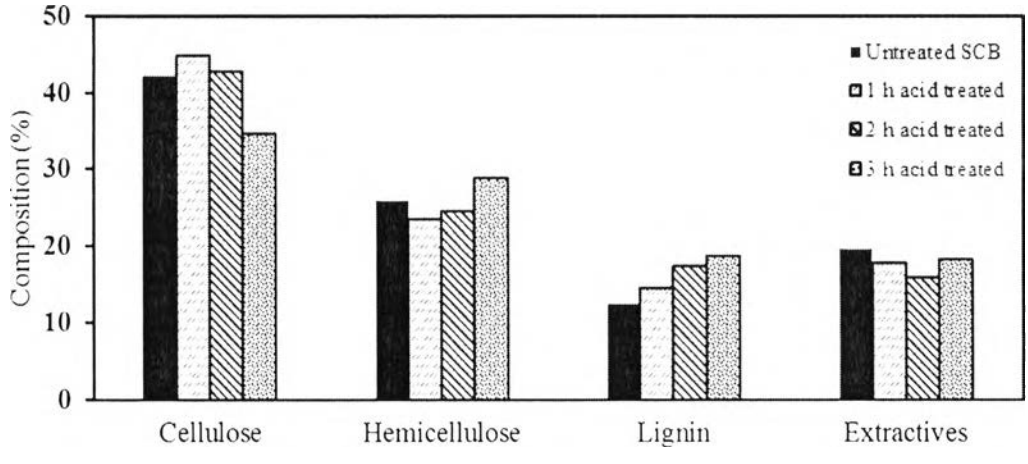

Figure 4.3 Chemical compositions of untreated sugarcane bagasse and steam-explosion pretreated sugarcane bagasse by using H_2SO_4 as a preimpregnation agent with difference times.

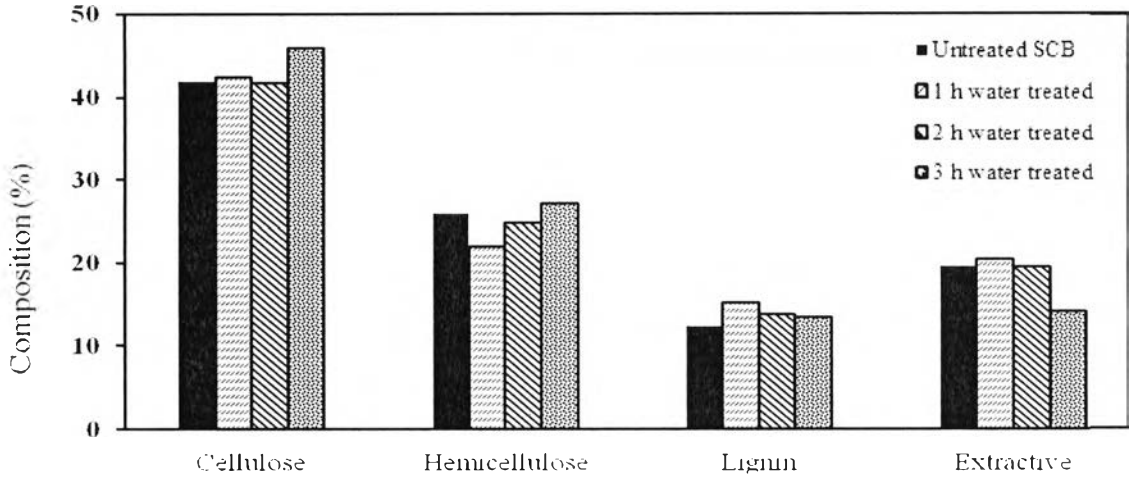

Figure 4.4 Chemical compositions of untreated sugarcane bagasse and steam-explosion pretreated sugarcane bagasse by using H_2O as a preimpregnation agent with difference time.

4.2 Glucose Production from Microbial Hydrolysis of Untreated Corncob and Sugarcane Bagasse

The glucose concentration profile from the microbial hydrolysis of untreated corncob and sugarcane bagasse is an important factor in an ethanol production process. The microbial hydrolysis of corncob and sugarcane bagasse was performed in the batch reactor, containing 1000 ml of the 65 modified DSMZ broth medium 2 without CMC and 1 g of corncob and sugarcane bagasse powder with pH adjusted to 7.2. In this experiment, the bacteria strain A002 obtained from the Thai Higher Termites, *Microcerotermes sp.*, was used to hydrolyze the untreated and steam-explosion pretreated corncob and sugarcane bagasse. The chemical composition of untreated corncob shows higher in cellulose fraction than the untreated sugarcane bagasse. The cellulose content is one of a major component in producing glucose from the microbial hydrolysis. Moreover, the elemental composition of the corncob and sugarcane bagasse especially the carbon content is essential for bacteria to grow and hydrolyze corncob and sugarcane bagasse to glucose.

Table 4.1 clearly shows that the carbon content in the untreated corncob is higher than sugarcane bagasse. From the chemical composition and elemental analysis, the untreated corncob has higher cellulose content than the untreated sugarcane bagasse, which is suitable for microbial hydrolysis and producing more glucose.

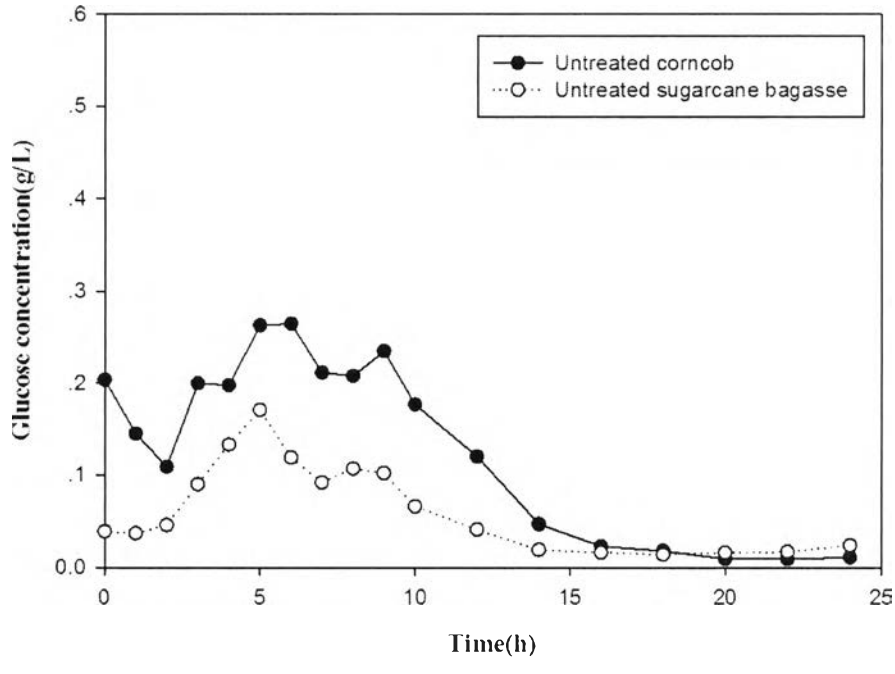

Figure 4.5 Comparison between glucose concentration from the microbial hydrolysis of untreated corncob and sugarcane bagasse at 37 °C with bacteria strain A002.

As shown in Figure 4.5, the amount of produced glucose from the microbial hydrolysis of untreated corncob at 37 °C with bacteria strain A002 increases with the increase in the operation time until it reaches the maximum glucose concentration. The maximum glucose concentration of untreated corncob is 0.265 g/L at 6 h. After that, the glucose concentration profile rapidly decreases and remains constant after 20 h. The same trend in the glucose concentration profile of untreated sugarcane bagasse at 37 °C with bacteria strain A002 can be obtained. The maximum glucose concentration of untreated sugarcane bagasse is 0.171 g/L at 5 h of the hydrolysis time. Comparison of glucose production between the untreated corncob and

sugarcane bagasse shows that the higher glucose concentration can be obtained from the untreated corncob than the untreated sugarcane bagass because the untreated corncob has higher cellulose contents than the untreated sugarcane bagasse, which can be supported by Kumar *et al.* (2009). The result is in agreement with the cellulose fraction of untreated corncob (43.35%), which is higher than the untreated sugarcane bagasse (41.96%) as shown in Tables 4.2 – 4.3.

4.3 Glucose Production from Microbial Hydrolysis of Steam-Explosion Pretreated Corncob and Sugarcane Bagasse

4.3.1 Steam-Explosion Pretreatment with H_2O

In order to determine the optimum condition for glucose production from microbial hydrolysis of steam-explosion pretreated corncob and sugarcane bagasse, the steam-explosion pretreatment time and preimpregnation agent were investigated. Figure 4.6 shows the amount of glucose production from the steam-explosion pretreated corncob by using H_2O as a preimpregnation agent. The maximum glucose concentration from the microbial hydrolysis of steam-explosion pretreated corncob at 1, 2, and 3 h pretreatment times are 0.248 g/L, 0.268 g/L, and 0.259 g/L, respectively. Figure 4.7 represents the glucose concentration profile of the steam-explosion pretreated sugarcane bagasse by using H_2O as a preimpregnation agent. The glucose concentration from the 1 h steam-explosion pretreated sugarcane bagasse dramatically increases until it reaches the maximum concentration about 0.369 g/L, and significantly decreases after 9 h. The same trend can be observed for the 2 and 3 h pretreatment times, but the glucose concentration slightly decreases to 0.364 g/L and 0.269 g/L, respectively. In addition, the glucose concentration profiles from the steam-explosion pretreated sugarcane bagasse with 1, 2, and 3 h by using H_2O as a preimpregnation agent show higher glucose concentration at the optimum conditions than the untreated sugarcane bagasse. On the contrary, the glucose concentration profiles from the steam-explosion pretreated corncob by using H_2O as a preimpregnation agent do not change significantly over the period of hydrolysis for all studied steam-explosion residence times.

The glucose production from the H_2O steam-explosion pretreatment also shows that a longer pretreatment time leads to lower glucose concentration, which is in agreement to a report by Amores *et al.* (2013). The glucose concentration results can also be supported by the chemical composition changes of the steam-explosion pretreated corncob by using H_2O as a preimpregnation agent, as shown in Table 4.2. The cellulose compositions of steam-explosion pretreated corncob at all pretreatment times are not much difference from the untreated corncob. But at the 2 h pretreatment time, the highest cellulose can be obtained, which can be supported by the highest glucose concentration from the microbial hydrolysis of the 2 h steam-explosion pretreatment of corncob, as shown in Figure 4.6. For the steam-explosion pretreated sugarcane bagasse, the highest glucose production can be obtained at the 2 h steam-explosion pretreatment where the cellulose content is 42.46%, which is higher than the untreated sugarcane bagasse.

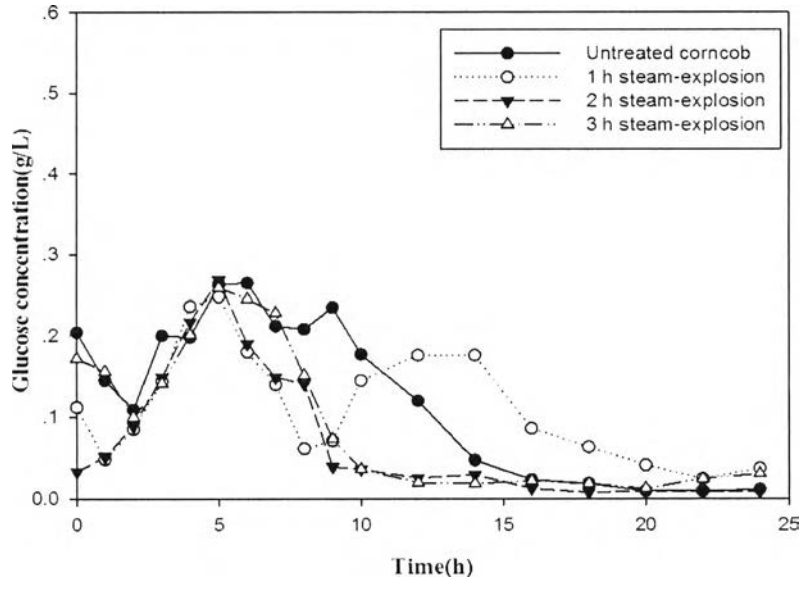

Figure 4.6 Effect of steam-explosion pretreatment time on glucose concentration from the microbial hydrolysis at 37 °C using bacteria strain A 002 of the pretreated corncob by using H_2O as a preimpregnation agent.

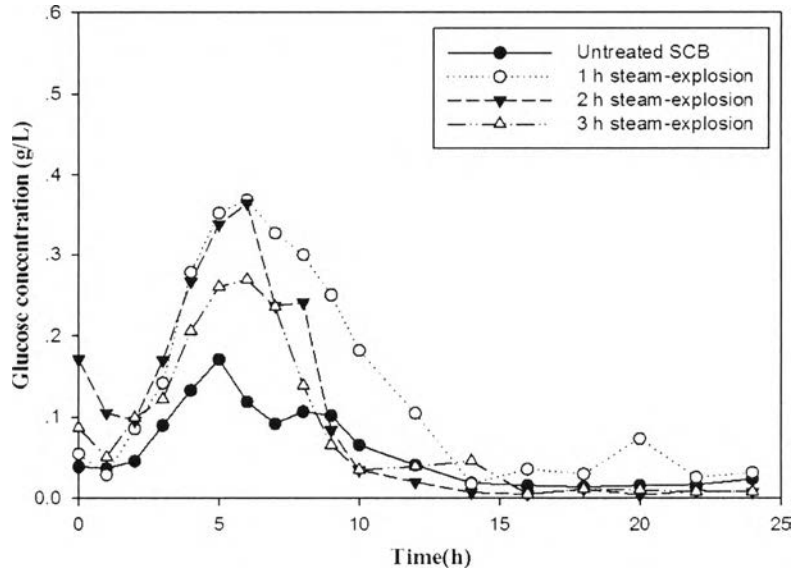

Figure 4.7 Effect of steam-explosion pretreatment time on the glucose concentration from the microbial hydrolysis at 37 °C using bacteria strain A 002 of the pretreated sugarcane bagasse by using H_2O as a preimpregnation agent.

4.3.2 Steam-Explosion Pretreatment with H_2SO_4

For the steam-explosion pretreatment of corncob and sugarcane bagasse by using H_2SO_4 as a preimpregnation agent, the glucose concentration profiles are shown in Figures 4.8 – 4.9. For the H_2SO_4 steam-explosion pretreated corncob, Figure 4.8, the glucose concentration slightly increases from the beginning and reaches the maximum concentration at 0.265 g/L at 5 h of the hydrolysis time, 0.245 g/L at 5 h of the hydrolysis time, and 0.251 g/L at 4 h of the hydrolysis time for the 1, 2, and 3 h steam-explosion pretreatment times, respectively. From Figure 4.9, the optimum condition for the highest glucose concentration from the steam-explosion pretreated sugarcance bagasse can be clearly obtained at 1 h of the steam-explosion pretreatment time with the glucose concentration of 0.6 g/L. The highest glucose concentrations from 2 and 3 h pretreatment times are 0.487 g/L at 4 h of the hydrolysis time, and 0.541 g/L at 5 h of the hydrolysis time, respectively. In comparison with the untreated corncob and sugarcane bagasse, the glucose concentration from the steam-explosion pretreated sugarcane bagasse by using H_2SO_4 is higher than the untreated sugarcane bagasse, while the amount of produced

glucose from the steam-explosion pretreated corncob slightly decreases. The results suggest that the structure of the sugarcane bagasse can be further break down especially when it is pretreated with the H_2SO_4 as a preimpregnation agent than the corncob. This helps to increase the accessibility between cellulose and enzyme. In comparison with H_2O and H_2SO_4 steam-explosion pretreated sugarcance bagasse, the highest glucose concentration from the H_2O pretreated sugarcane bagasse is 0.369 g/L at the 1 h pretreatement time, while the amount of produced glucose from the H_2SO_4 pretreated sugarcane bagasse is 0.6 g/L. This indicates that using H_2SO_4 as a preimpregnation agent for the steam-explosion pretreatment of sugarcane bagasse gives higher glucose concentration than using H_2O. Therefore, the preimpregnation agent and pretreatment time clearly affect the glucose production from the microbial hydrolysis of sugarcane bagasse.

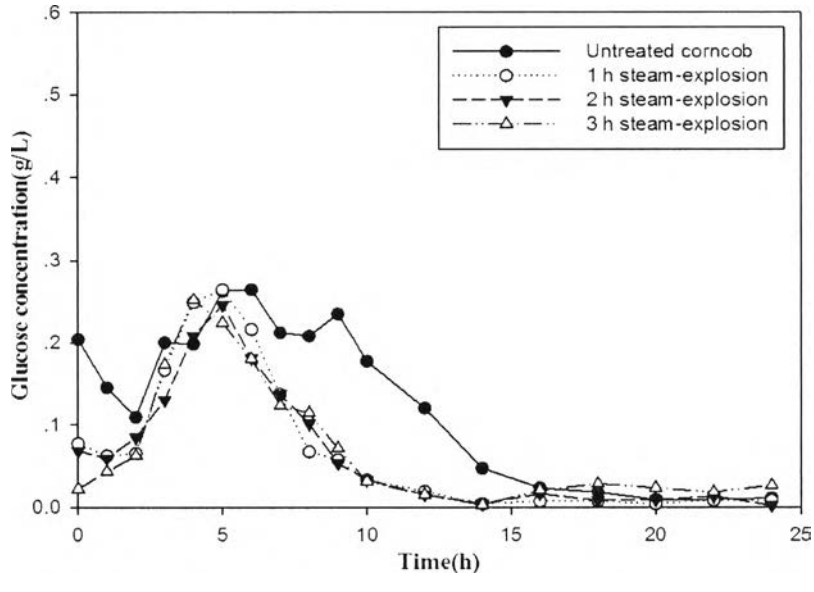

Figure 4.8 Effect of steam-explosion pretreatment time on glucose concentration from the microbial hydrolysis at 37 °C using bacteria strain A 002 of corncob by using H_2SO_4 as a preimpregnation agent.

The effects are more pronounced by using H_2SO_4 as a preimpregnation agent for the steam-explosion of sugarcane bagasse. The glucose concentration results can be related to the chemical composition of the steam-explosion pretreated corncob and

sugarcane bagasse. As shown in Table 4.2, the highest cellulose content (43.66%) can be obtained at the 1 h steam-explosion pretreatment of corncob by using H_2SO_4 as a preimpregnation agent with the highest glucose concentration 0.265 g/L from the microbial hydrolysis of corncob. Furthermore, the chemical composition changes of the steam-explosion pretreated sugarcane bagasse are shown in Table 4.3. The highest cellulose content for the 1 h steam-explosion of sugarcane bagasse by using H_2SO_4 is 44.78%, which is higher than the cellulose content in the untreated sugarcane bagasse (41.96%). The result is in agreement with the glucose production from the microbial hydrolysis of the 1 h steam-explosion pretreated sugarcane bagasse, which gives the highest glucose concentration at 0.6 g/L. The obtained results are in agreement with the results from Ballestero *et al.* (2006), who reported that diluted-acid impregnation of biomass is an efficient method to increase enzymatic hydrolysis of cellulose to ethanol of wheat straw, compared with water-impregnated biomass.

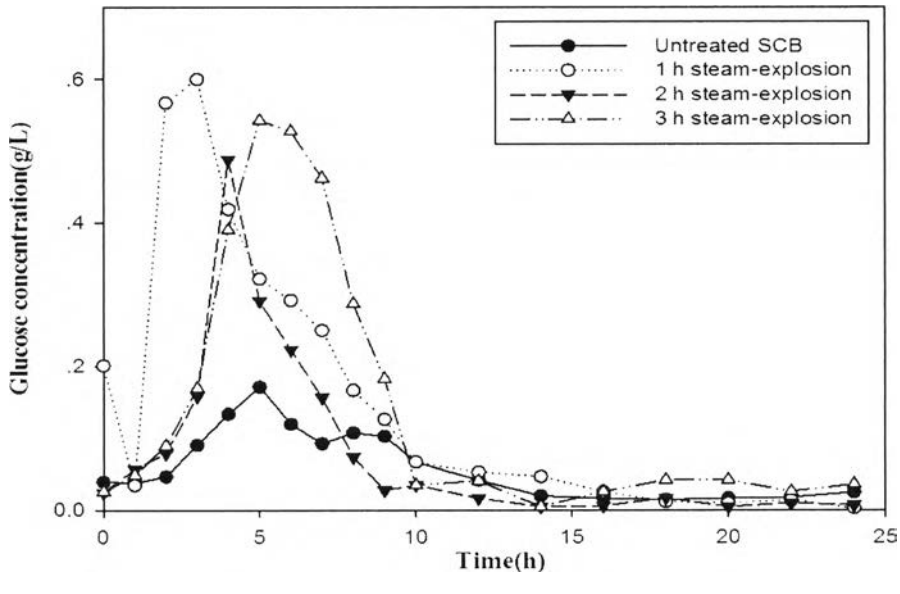

Figure 4.9 Effect of steam-explosion pretreatment time on glucose concentration from the microbial hydrolysis at 37 °C using bacteria strain A 002 of sugarcane bagasse by using H_2SO_4 as a preimpregnation agent.

4.4 Bacteria Concentration from Microbial Hydrolysis of Steam-Explosion Pretreated Corncob and Sugarcane Bagasse

The optimum glucose production obtained from the hydrolysis of untreated corncob and sugarcane bagasse with bacteria strain A002 at 37 ˚C are compared with the bacteria concentration, shown in Figures 4.10 and 4.11. In Figure 4.10, the glucose concentration from the enzymatic hydrolysis of untreated corncob dramatically increases after 2 h of the hydrolysis time until it reaches the maximum glucose concentration and continued to decrease until 24 h, while the bacteria concentration continuously increases. It can be observed that glucose is consumed by the bacteria after 6 h for the strain A002. The glucose and bacteria evolution from the hydrolysis of untreated sugarcane bagasse is shown in Figure 4.11. The glucose concentration slightly increases until it reaches the optimum condition and continued to decrease until 24 h. The bacteria concentration increases. It can be conclude that glucose is consumed by the bacteria after 4 h for the strain A002.

In addition, the optimum glucose concentration from the hydrolysis of steam-explosion pretreated corncob and sugarcane bagasse with difference times and the preimpregnation agents (H_2O and H_2SO_4) are also compared with the bacteria concentration, as shown in Figures 4.12 – 4.15. Figure 4.12 shows the glucose and bacteria evolutions from the hydrolysis of 2 h steam-explosion pretreated corncob by using H_2O as the preimpregnation agent. The glucose concentration increases until it reaches the maximum concentration, and then decreases over times until reaches the plateau around the same time period that the bacteria growth rates started to slow. The glucose is consumed by the bacteria after 4 h for the strain A002. For the glucose and bacteria evolutions from the hydrolysis of 1 h steam-explosion pretreated sugarcane bagasse by using H_2O as a preimpregnation agent is shown in Figure 4.13. The glucose concentration profile also increases until it reaches the maximum concentration, and then decreases. But the bacteria growth rates increases and it can be observed that the glucose is consumed by the bacteria after 6 h of the hydrolysis period.

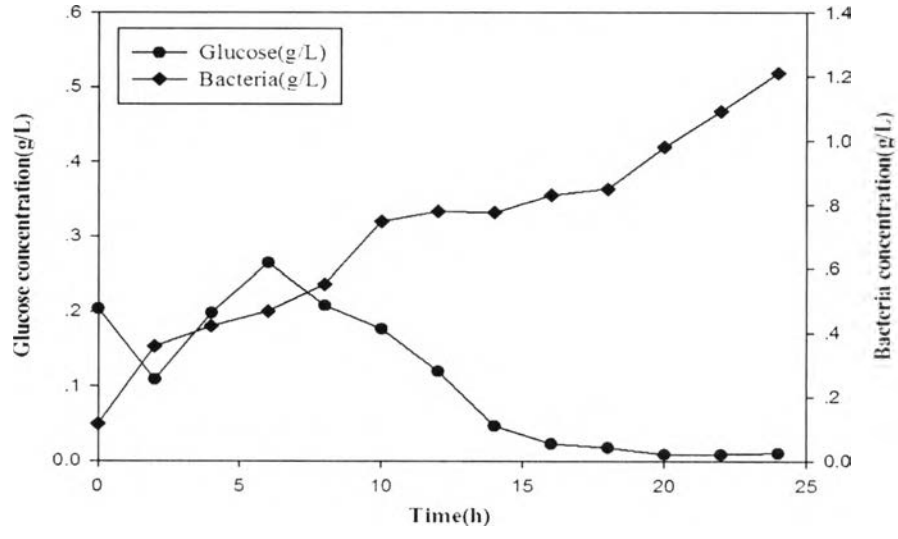

Figure 4.10 Glucose and bacteria evolutions from the hydrolysis of untreated corncob with bacteria strain A002 at 37 °C.

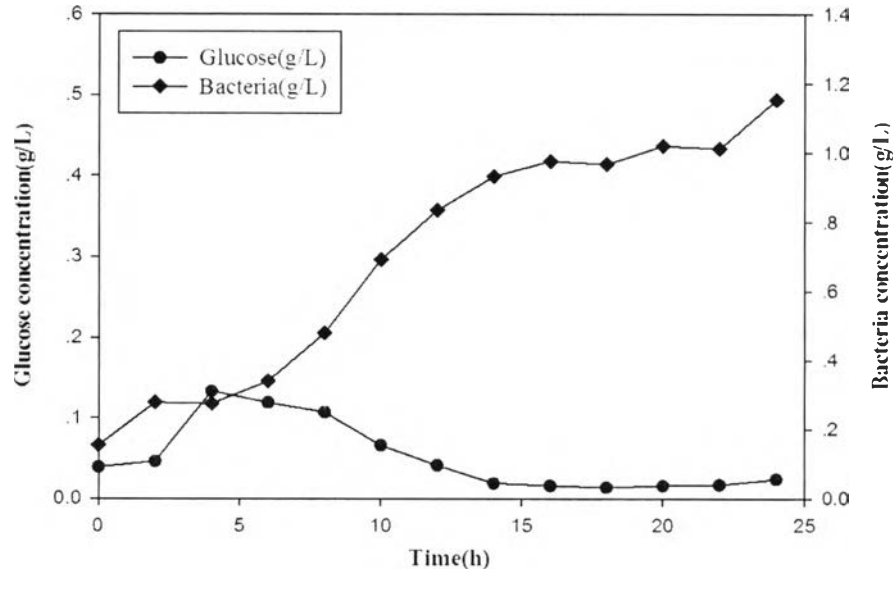

Figure 4.11 Glucose and bacteria evolutions from the hydrolysis of untreated sugarcane bagasse with bacteria strain A002 at 37 °C.

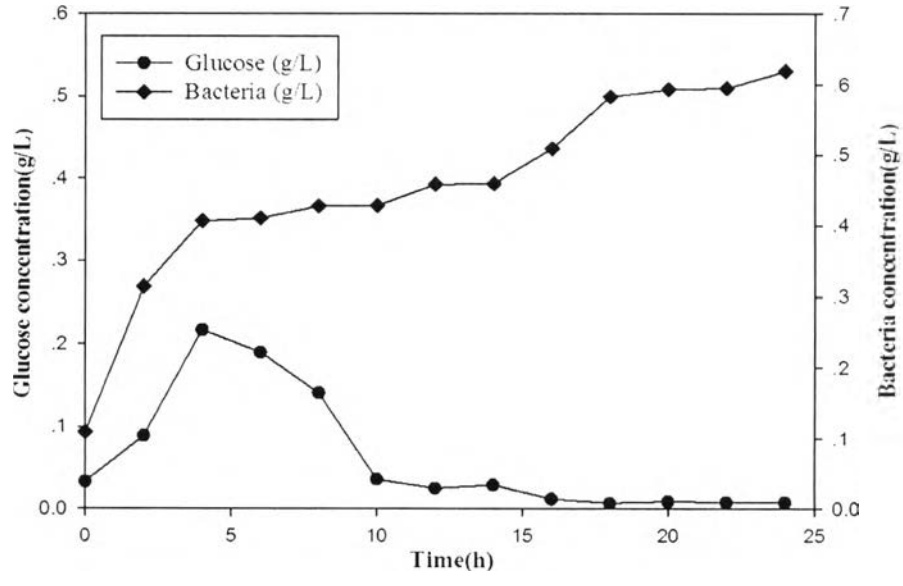

Figure 4.12 Glucose and bacteria evolutions from the hydrolysis of 2 h steam-explosion pretreated corncob by using H_2O as a preimpregnation agent with bacteria strain A002 at 37 °C.

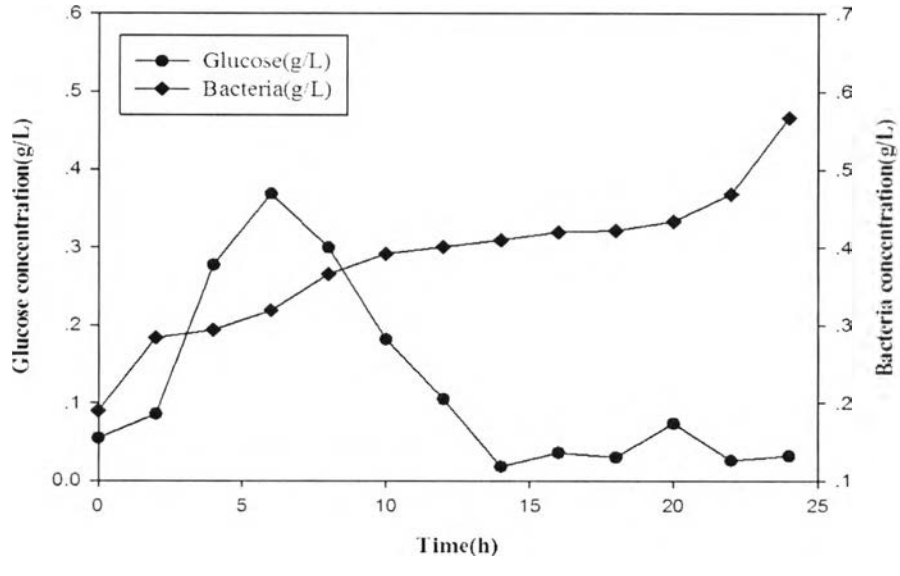

Figure 4.13 Glucose and bacteria evolutions from the hydrolysis of 1 h steam-explosion pretreated sugarcane bagasse by using H_2O as a preimpregnation agent with bacteria strain A002 at 37 °C.

The glucose and bacteria evolutions from the hydrolysis of corncob and sugarcane bagasse by using H_2SO_4 as the preimpregnation agents are shown in Figures 4.14 – 4.15. Figure 4.14 shows the glucose production from the 1 h steam-explosion pretreated corncob by using H_2SO_4 as a preimpregnation agent. The same trend of the glucose profile can be observed, while the bacteria concentration continuously increases and starts to slow down when the glucose concentration decreases because the glucose is consumed by bacteria after 4 h of the hydrolysis time. Figure 4.15 shows the glucose and bacteria evolutions from the hydrolysis of the 1 h steam-explosion pretreated sugarcane bagasse by using H_2SO_4 as the preimpregnation agent. It can be seen that the glucose concentration dramatically increases until it reaches the maximum concentration and then decreases. On the contrary, the bacteria concentration dramatically increases with little fluctuation. It can be observed that the glucose is consumed by the bacteria after 2 h of the hydrolysis time.

In conclusion, it clearly indicates that the bacteria growth rate is almost the same in all untreated and pretreatment conditions of corncob and sugarcane bagasse. A plausible explanation for this phenomenon is that the consumption of glucose by growing bacteria strain A002 populations causes the glucose level to drop to the level that they can no longer support continued growth of the bacteria populations (Siddique and Zalik, 2012). In addition, the results show that after the corncob and sugarcane bagasse are pretreated, the maximum glucose concentration can be obtained at a shorter hydrolysis time than the untreated corncob and sugarcane bagasse. Furthermore, the bacteria growth is slightly faster, corresponding to the faster hydrolysis of the pretreated corncob and sugarcane bagasse.

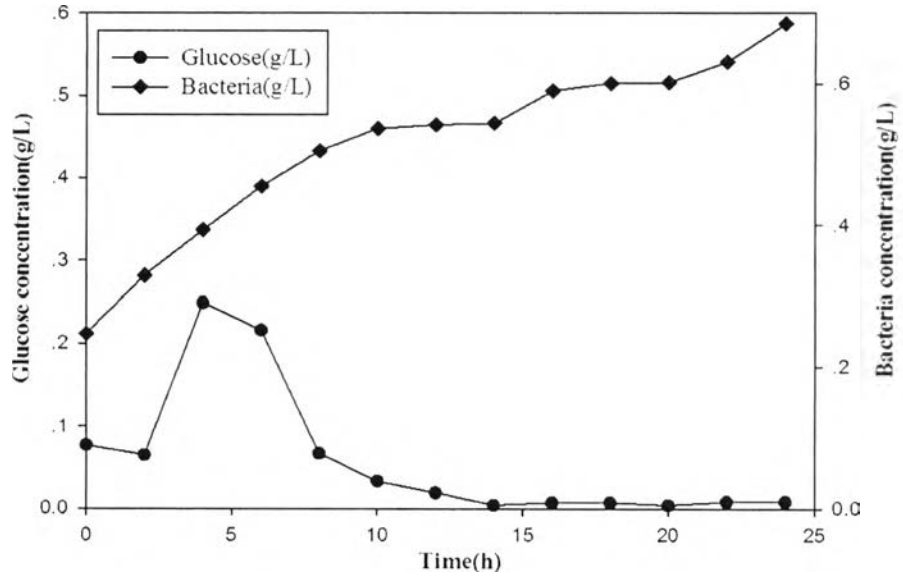

Figure 4.14 Glucose and bacteria evolutions from the hydrolysis of 1 h steam-explosion pretreated corncob by using H_2SO_4 as a preimpregnation agent with bacteria strain A002 at 37 °C.

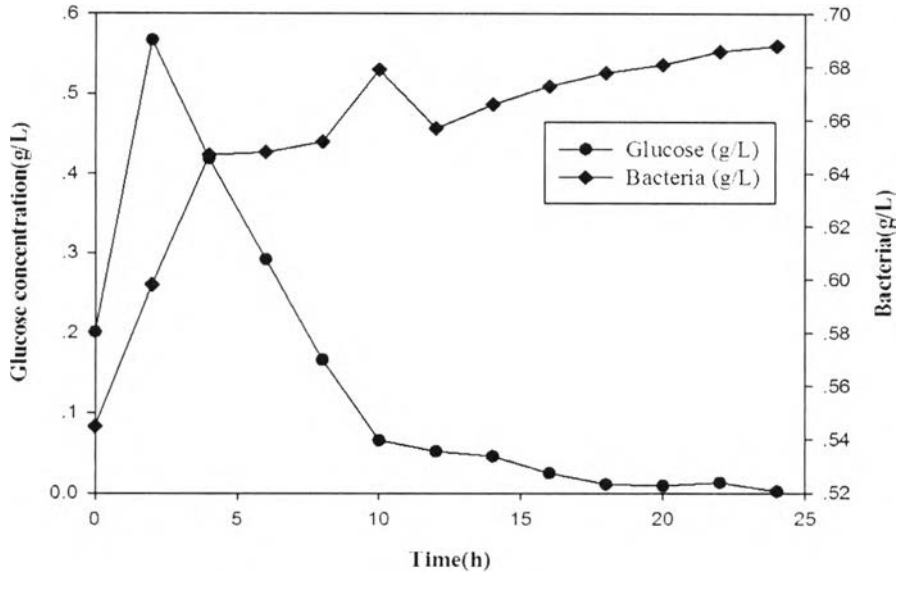

Figure 4.15 Glucose and bacteria evolutions from the hydrolysis of 1 h steam-explosion pretreated sugarcane bagasse by using H_2SO_4 as a preimpregnation agent with bacteria strain A002 at 37 °C.

4.5 Structure of Microbial Hydrolyzed Corncob and Sugarcane Bagasse

Physical changes in the untreated, the steam-explosion pretreated, and hydrolyzed corncob and sugarcane bagasse particles were analyzed by using Hitachi/S-4800 scanning electron microscope (SEM). A compact structures and smooth surfaces were obtained from the untreated corncob and sugarcane bagasse, as shown in Figure 4.16 (a,b). After the microbial hydrolysis by bacteria strain A002, the surface morphology of both corncob and sugarcane bagasse change to be rough and porous surfaces, as illustrated in Figure 4.16 (c,d).

For the steam-explosion pretreatment effect, the most apparent effect is a color change from yellow for corncob and brown for sugarcane bagasse into a darker color. The surface morphologies were investigated for all the optimum conditions of the steam-explosion pretreatment of corncob and sugarcane bagasse, as shown in Figure 4.17. The micrographs of H_2O and H_2SO_4 steam-explosion pretreated corncob and sugarcane bagasse show that the pretreatment induces the physical changes in both corncob and sugarcane bagasse surfaces. As shown in Figure 4.17(a) and (c), the steam-explosion pretreated corncob and sugarcane bagasse by using H_2SO_4 as a preimpregnation agent are disrupted, and the interior of the structures are exposed. Furthermore, the steam-explosion pretreated corncob and sugarcane bagasse by using H_2O as a preimpregnation agent, as shown in Figure 4.17 (b) and (d), also show some holes and rough surfaces. The results indicate that the steam-explosion pretreatment removed external fibers, thus the cellulose becomes more accessible to enzymes, and the yield of monosaccharides is increased. Similar structural changes were earlier reported for rice straw pretreated with electron beam irradiation (Sindhu *et al.*, 2010) and for rice straw pretreated with aqueous ammonia soaking pretreatment (Kim *et al.*, 2009). They showed that the use of H_2SO_4 and H_2O as the preimpregnation agent broke down the fibrous structure of corncob and sugarcane bagasse and then accelerate the microbial hydrolysis process resulting in the higher hydrolysis rate and higher glucose production than the untreated corncob and sugarcane bagasse.

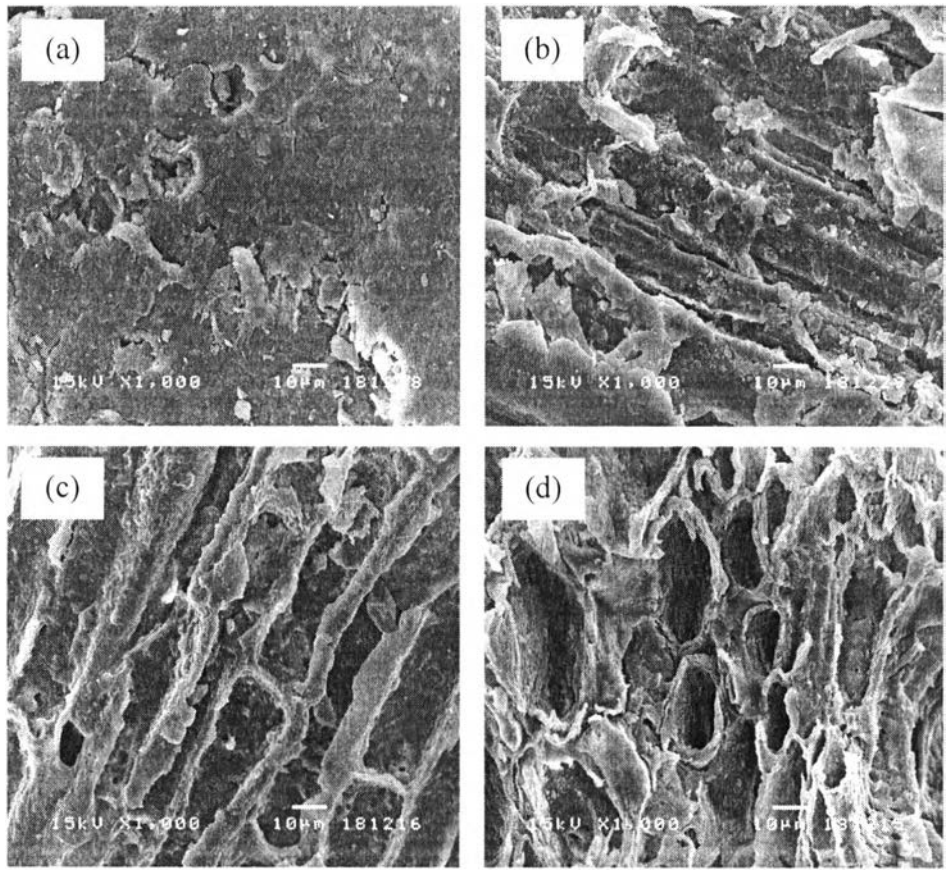

Figure 4.16 Scanning electron microscope of (a) untreated corncob; (b) untreated sugarcane bagasse; (c) hydrolyzed corncob; (d) hydrolyzed sugarcane bagasse.

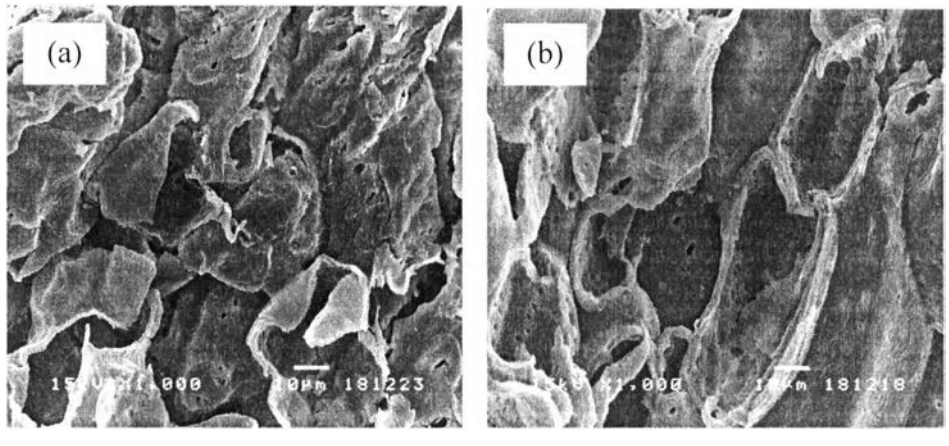

Figure 4.17 Scanning electron microscope of corncob surfaces (a) by using H_2SO_4 as a preimpregnation agent; (b) by using H_2O as a preimpregnation agent.

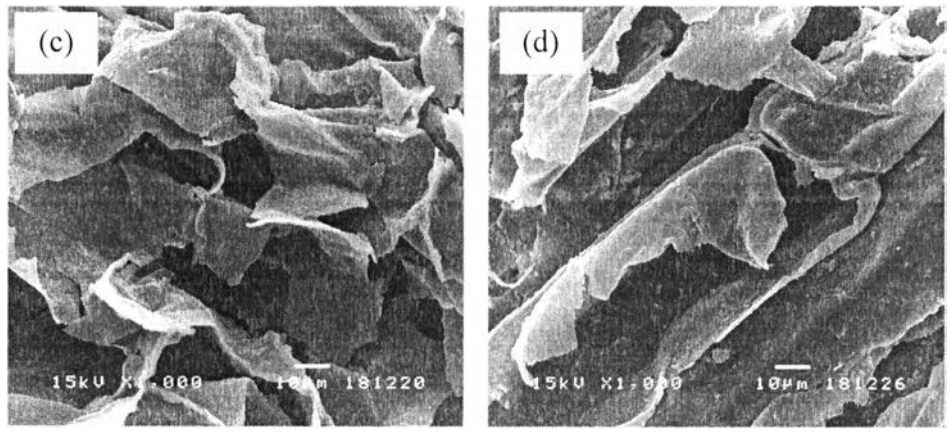

Figure 4.17 (cont.) (c) sugarcane bagasse surface by using H_2SO_4 as a preimpregnation agent; (d) by using H_2O as a preimpregnation agent.