# CHAPTER IV
# RESULTS AND DISCUSSION

## 4.1 Cellulose Pretreatment

After pretreatment with [BMIM]Cl, the pretreated cellulose was characterized by XRD to identify the crystallinity change. It was found that the crystallinity of cellulose was significantly reduced after the ionic liquid (IL) pretreatment, which can be clearly seen from the maximum peak near 2θ of 23°, as shown in Figure 4.1.

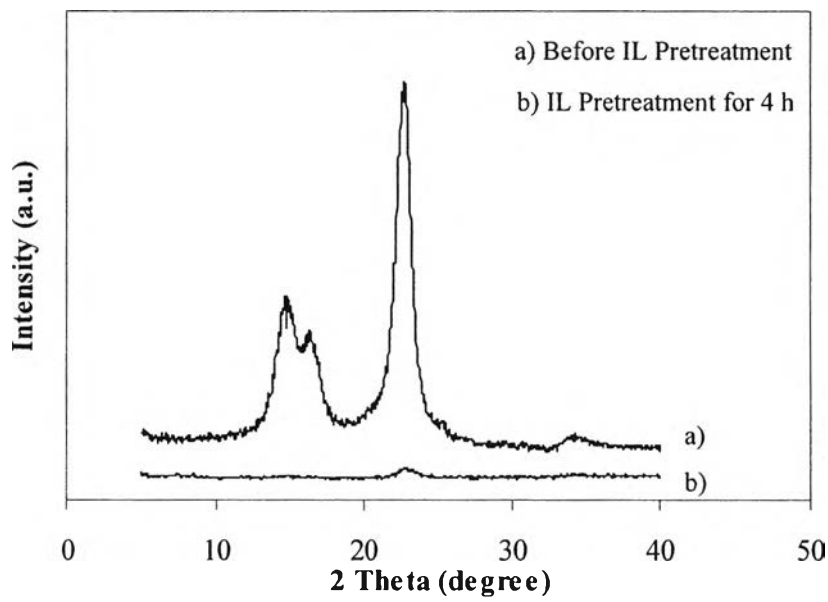


**Figure 4.1** Crystallinity of cellulose before and after IL pretreatment for 4 h (Cellullose-to-[BMIM]Cl ratio of 5:100, 100°C).

When varying the cellullose-to-[BMIM]Cl ratio from 2:100 to 5:100 and 7:100 within a temperature range from 80 to 120°C, it was found that at the cellullose-to-[BMIM]Cl ratio of 5:100, the crystallinity of cellulose decreased significantly after treating with [BMIM]Cl at 100 and 120°C, while the crystallinity decrease at 80°C was much lower than the ones treated with the higher temperatures.

On the other hand, the decrease in the crystallinity at 100 and 120°C was around 90% and there was only a small difference between both temperatures. In addition, the crystallinity of the pretreated cellulose remained relatively unchanged after 4 h of the pretreatment, as shown in Figure 4.2.

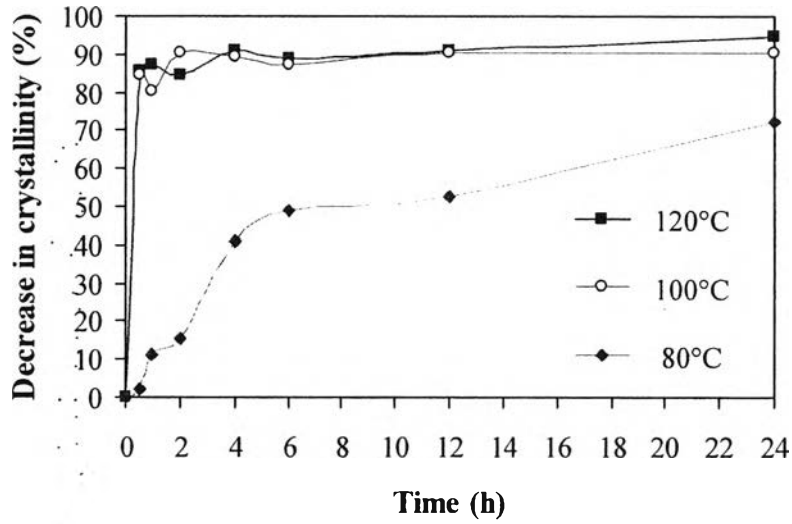


**Figure 4.2** Decrease in the crystallinity of cellulose treated with IL at different temperatures at the cellullose-to-[BMIM]Cl ratio of 5:100.

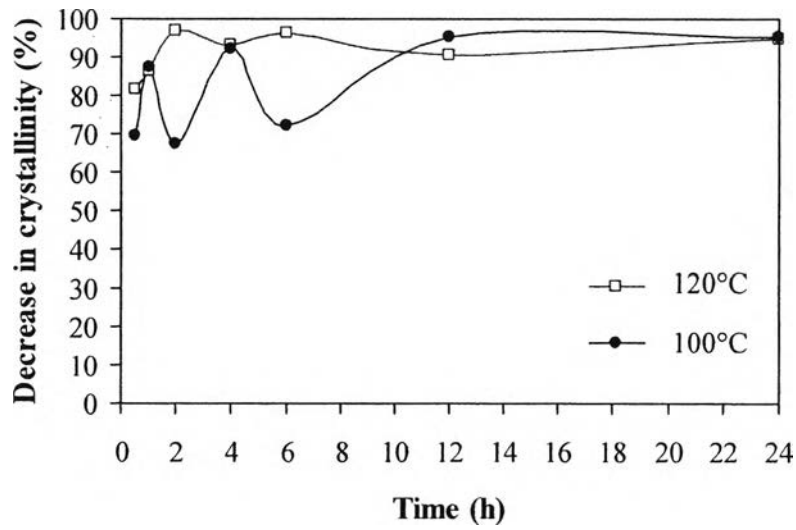


**Figure 4.3** Decrease in the crystallinity of cellulose treated with IL at different temperatures at the cellullose-to-[BMIM]Cl ratio of 7:100.

Furthermore, there is a fluctuation in the decrease in the crystllinity at the cellullose-to-[BMIM]Cl ratio of 7:100, as shown in Figure 4.3. This indicated the non-homogeneous accessibility of [BMIM]Cl into the cellulose.

It should be noted that the cellullose-to-[BMIM]Cl ratio of 2:100 is not an appropriate condition as a result of the difficulty in the complete regeneration of [BMIM]Cl from the cellulose. Moreover, it provided a comparable decrease in the crystallinity to the ratio of 5:100. As a consequence, the cellullose-to-[BMIM]Cl ratio of 5:100 with 4-h pretreatment was applied in the hydrolysis step. Furthermore, it was found thatunder these pretreatment conditions, surface areas of the unteated and pretreated cellulose were 0.71 and 1.20 $m^2/g$, respectively. After washing the pretreated cellulose by deionized water in order to recover the [BMIM]Cl, about 5wt% of [BMIM]Cl from elemental analysis in 1 g of cellulose still remained.

## 4.2 Enzymatic Hydrolysis

After the pretreatment step, the pretreated cellulose was further hydrolyzed for 24 h at 37°C by three effective isolates (strain A 002, M 015, and F 018). The three isolates were from Thai higher termites *Microcerotermes* sp., and found to be effective for cellulose hydrolysis (Taechapoempol, 2009). In the results, the amount of each bacteria weight was calculated based on the dry basis, which was approximatly 5% of the wet weight. The bacteria weight for each experiment was about 0.46 g. However, in the mixed strain experiments, the weight of each single strain was about 0.22 g.

### 4.2.1 Effect of Strains on Glucose Production

For strain A 002, the maximum amount of glucose was produced at 9 h for both untreated and pretreated celluloses about 0.25 and 0.29 g/L, respectively, Figure 4.4.

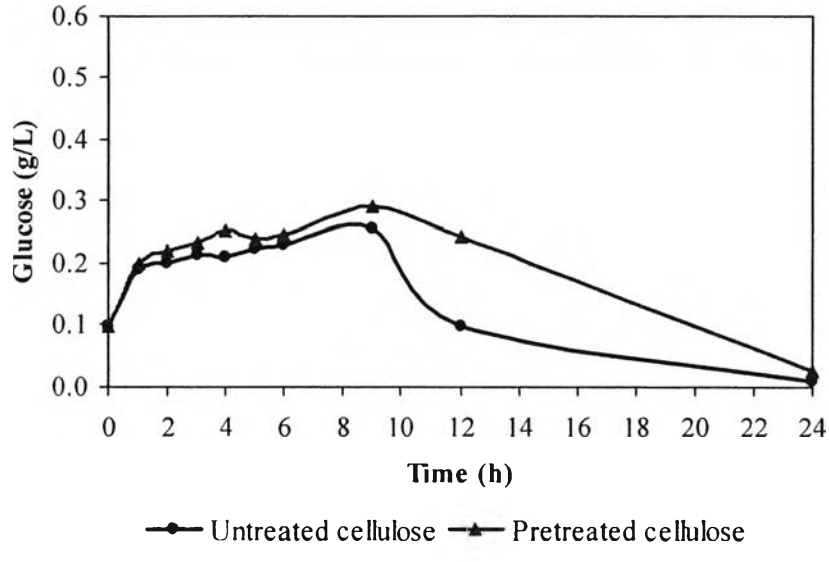


**Figure 4.4** Glucose evolution from the enzymatic hydrolysis using strain A 002.

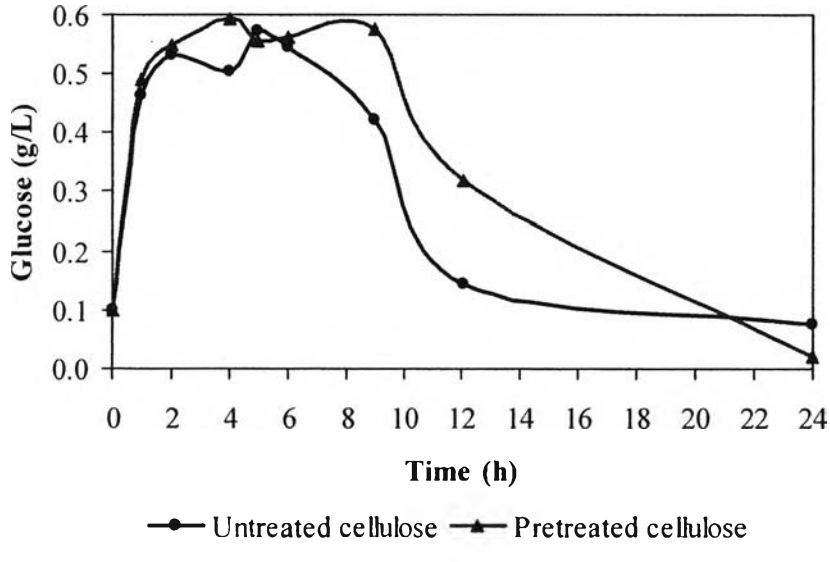


**Figure 4.5** Glucose evolution from the enzymatic hydrolysis using strain F 018.

Furthermore, the glucose concentration from the hydrolysis of the pretreated cellulose with strain F 018 increased sharply until it reached the maximum at 4 h of about 0.59 g/L, and significantly decreased after 9 h. The same pattern was obtained for the untreated one, but the hydrolysis time was shifted to 5 h with a slightly lower glucose concentration of 0.57 g/L, as shown in Figure 4.5.

The same pattern was also obtained for the untreated and pretreated celluloses using strain M 015. The glucose concentration slightly increased from the beginning until reached the maximum point at 9 h, about 0.18 g/L, for the untreated cellulose, while a higher glucose concentration, about 0.24 g/L, for the pretreated one was observed, as shown Figure 4.6.

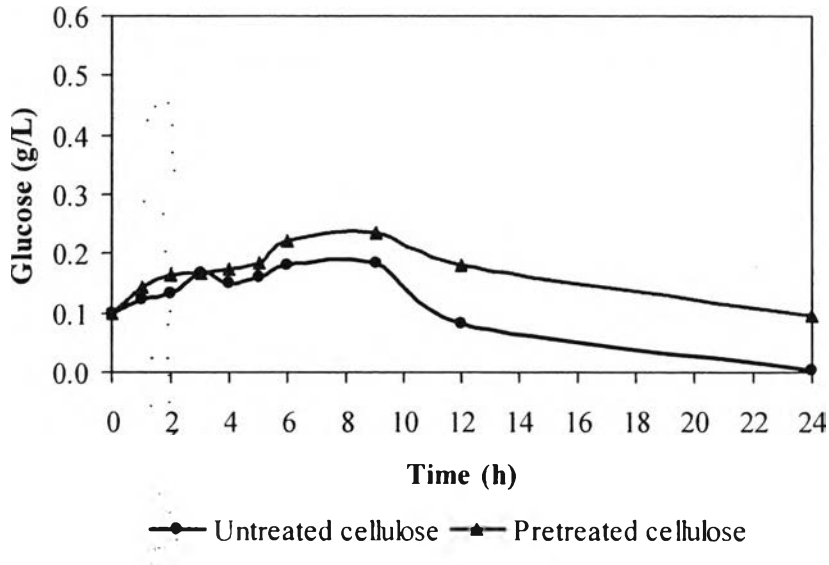


**Figure 4.6** Glucose evolution from the enzymatic hydrolysis using strain M 015 .

From the glucose evolution after the enzymatic hydrolysis by each strain, all pretrated celluloses gave higher glucose concentration than the untreated celluloses. Thus, [BMIM]Cl can be used to reduce the hydrolysis time and increase the cellulose conversion. After reaching the maximum value, the glucose concentration from the pretreated cellulose dropped at a slower rate than the untreated one. This pattern might be due to the trace of [BMIM]Cl left in the pretreated cellulose. Moreover, the enzymatic hydrolysis by strain F 018 gave the highest glucose concentration. The result corresponds to the highest FPase and β-glucosidase activities of strain F 018 (Taechapoempol, 2009).

4.2.2 Effect of Mixed Strains on Glucose Production

Glucose concentration of the untreated cellulose hydrolyzed by a mixed strains A 002 and M 015 reached the maximum value of 0.19 g/L at 9 h, while the concentration was 0.20 g/L at 4 h for the pretreated one, as shown in Figure 4.7.

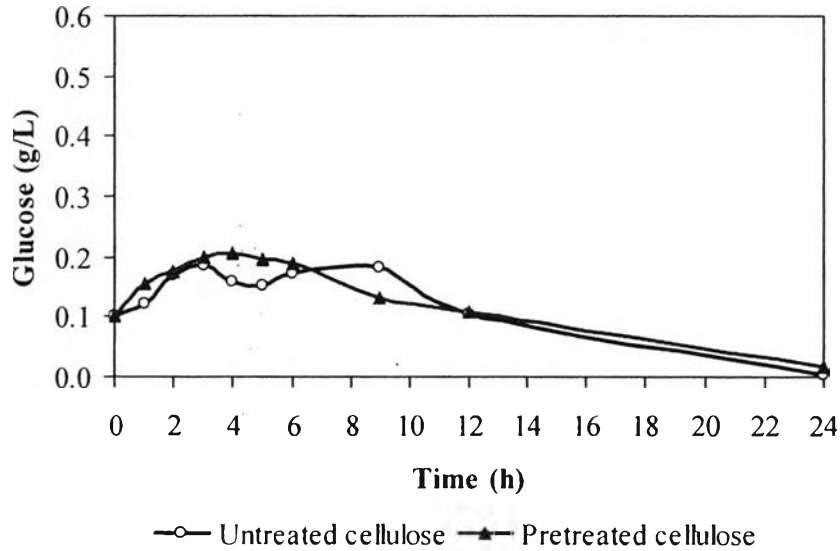


**Figure 4.7** Glucose evolution from the enzymatic hydrolysis using mixed strains A 002 and M 015.

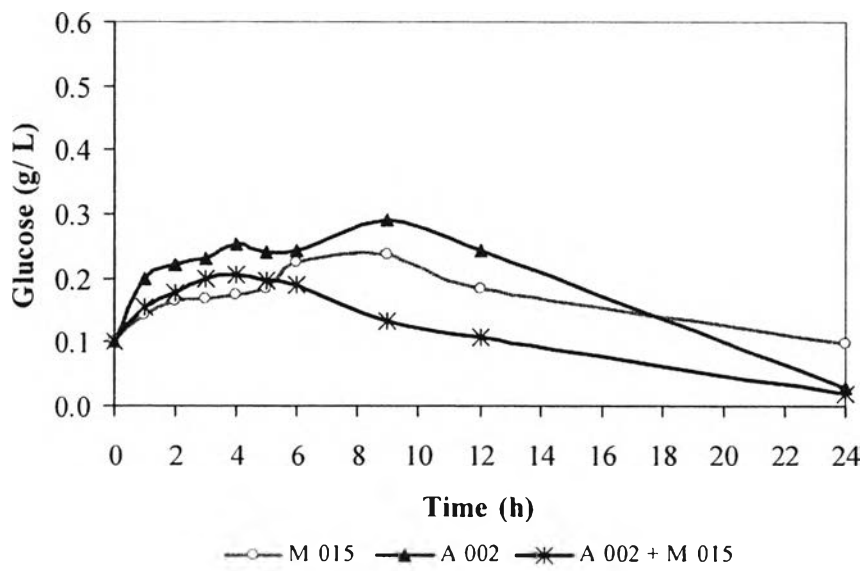


**Figure 4.8** Glucose evolution from the enzymatic hydrolysis of the pretreated cellulose using mixed strains A 002 and M 015, strain A 002, and strain M 015.

Figure 4.8 illustrates the comparison of glucose evolutions from the enzymatic hydrolysis of the mixed strains A 002 and M 015, strain A 002, and strain M 015, at the same starting concentration of bacteria. The glucose concentration from the enzymatic hydrolysis by strain A 002 or M 015 gave a higher concentration than the mixed strains.

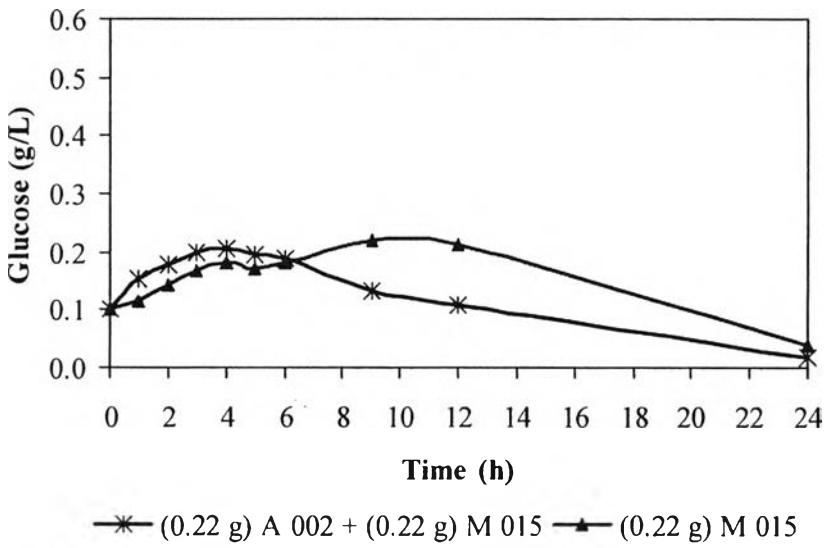


**Figure 4.9** Glucose evolution from the enzymatic hydrolysis of the pretreated cellulose using mixed strains A 002 and M 015, and strain M 015.

From Figure 4.9, glucose evolution from the enzymatic hydrolysis of the pretreated cellulose using strain M 015 (0.22 g of dry weight) and the mixed strains A 002 and M 015 (0.22 g of dry weight each) demonstrated the effect of the addition of strain A 002 on the M 015 performance. During the first few hours, the glucose evolution from the enzymatic hydrolysis by the mixed strains A 002 and M 015 was higher than the one hydrolyzed by M 015. Then, it gradually dropped. However, the one hydrolyzed by M 015 reached the highest value at 9 h with the glucose concentration higher than that from the mixed strains.

4.2.3 Effect of Bacteria Concentration on Glucose Production

The different intitial concentrations of strain M 015 (0.22 and 0.46 g of dry weight bacteria per liter) were used in order to compare glucose concentration. The glucose concentration from the enzymatic hydrolysis of the pretreated cellulose showed that the initial bacteria concentration hardly affected the glucose concentration, as shown in Figure 4.10.

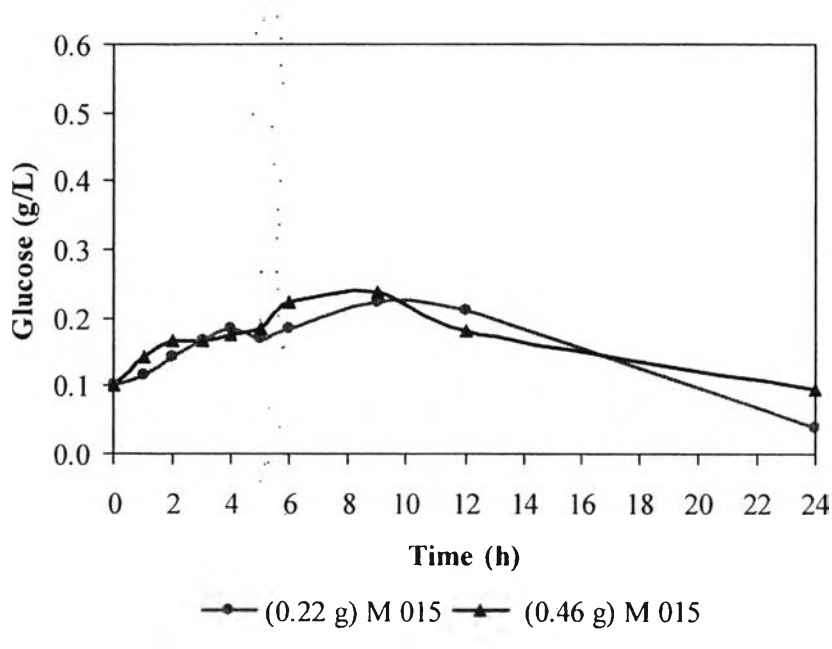


**Figure 4.10** Glucose evolution from the enzymatic hydrolysis of the pretreated cellulose using strain M 015 with different concentrations of bacteria.

4.2.4 Bacteria Concentration and Glucose Production vs. Time

Glucose production from the hydrolysis of the untreated cellulose by strain A 002 significantly increased in the first hour and slightly increased until reaching the highest value. At the same time, the bacteria concentration also gradually increased. After 9 h, the glucose concentration sharply dropped, while the bacteria concentration continually increased. Thus, it can be deduced that the produced glucose was consumed by the bacteria after 9 h. The results are shown in Figure 4.11.

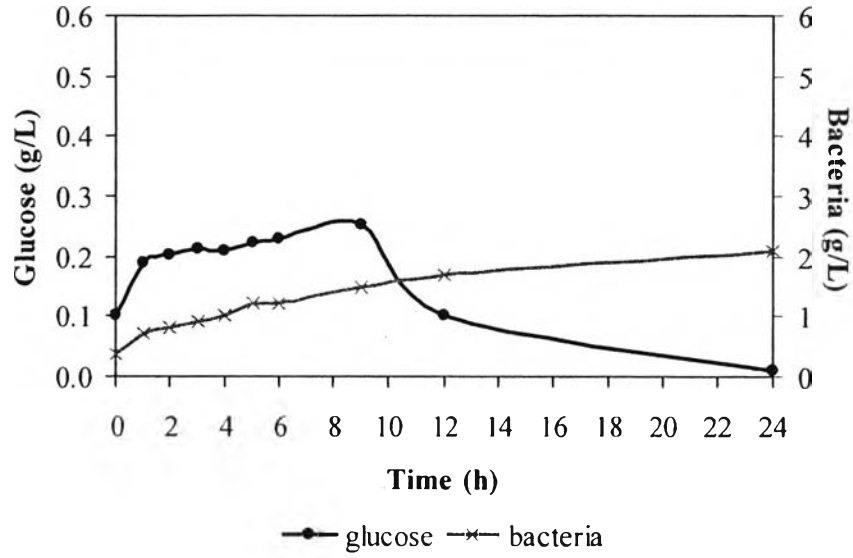


**Figure 4.11** Glucose evolution and bacteria growth from the enzymatic hydrolysis of the untreated cellulose using strain A 002.

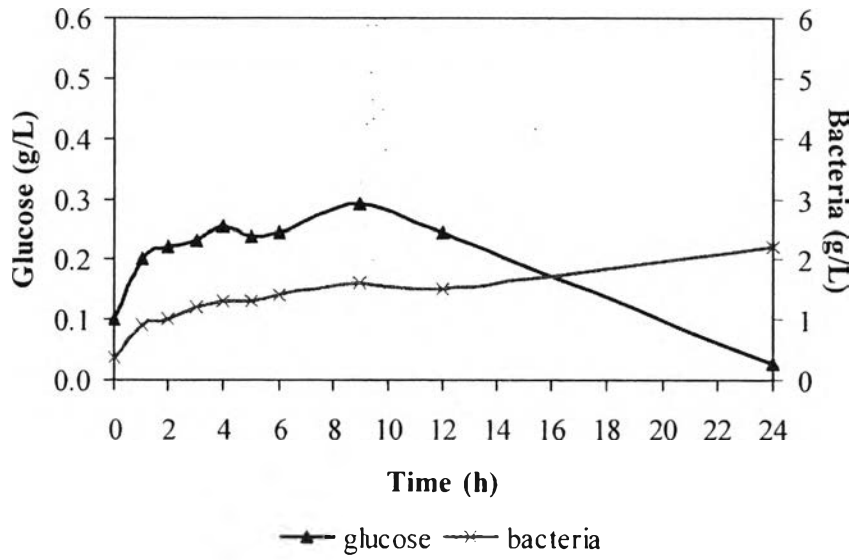


**Figure 4.12** Glucose evolution and bacteria growth from the enzymatic hydrolysis of the pretreated cellulose using strain A 002.

Figure 4.12 shows the glucose evolution and bacteria growth from the hydrolysis of the pretreated cellulose using strain A 002. Comparison to Figure 4.11 clarly indicates that the bacteria growth was slightly faster, corresponding to the

faster hydrolysis on the pretreated cellulose. Despite the higher bacteria growth rate, the decrease in the glucose concentration did not drop as fast as that of the untreated cellulose.

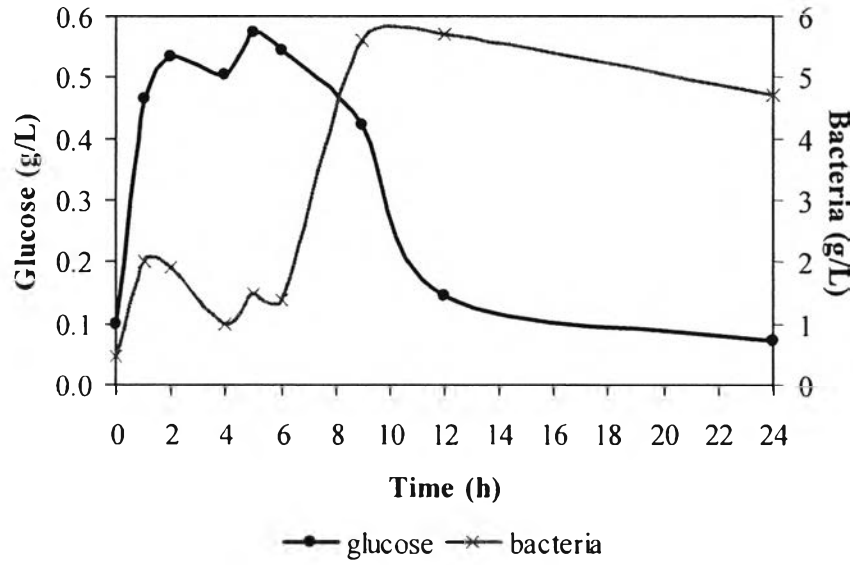


**Figure 4.13** Glucose evolution and bacteria growth from the enzymatic hydrolysis of the untreated cellulose using strain F 018.

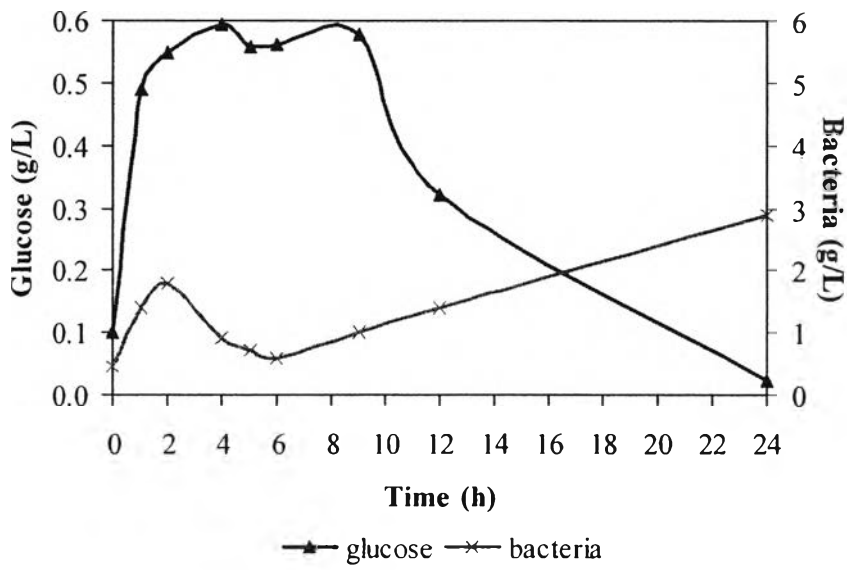


**Figure 4.14** Glucose evolution and bacteria growth from the enzymatic hydrolysis of the pretreated cellulose using strain F 018.

For the untreated cellulose conversion by strain F 018, the glucose concentration drastically increased in the first hour and reached the highest concentration at 5 h as shown in Figure 4.13. After that, the glucose concentration sharply decreased. Bacteria concentration increased in the first few hours. The bacteria concentration then dramatically increased. It can also be seen that the produced glucose was significantly consumed by bacteria after 6 h.

Figure 4.14 shows the pretreated cellulose conversion by strain F 018. The glucose concentration sharply increased and reached the highest concentration at 4 h. After that, the glucose concentration was quite stable and dropped after 9 h. The bacteria concentration increased during the first few hours. After 6 h, the bacteria concentration gradually increased but with at a much slower rate than the bacteria concentration of the untreated cellulose conversion by the same strain.

Figure 4.15 shows the untreated cellulose conversion by strain M015. The glucose concentration slightly increased, and reached the highest concentration at 9 h, and then sharply decreased. The bacteria concentration increased during the first few hours with little fluctuation. After that, the bacteria concentration gradually increased. Figure 4.16 shows the pretreated cellulose conversion by strain M 015. The trends of the glucose concentration and bacteria concentration were the same as untreated one; however, the glucose concentration was higher and decreased at a slower rate.

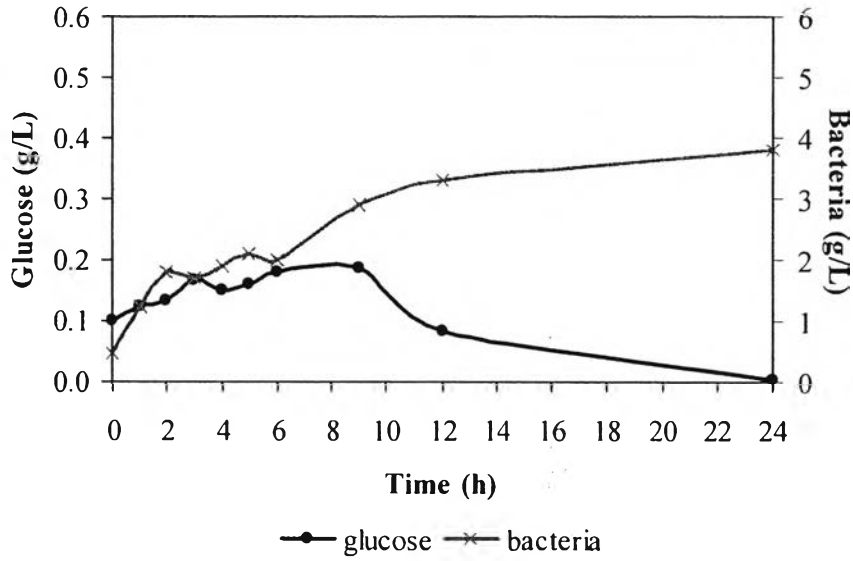


**Figure 4.15** Glucose evolution and bacteria growth from the enzymatic hydrolysis of the untreated cellulose using strain M 015.

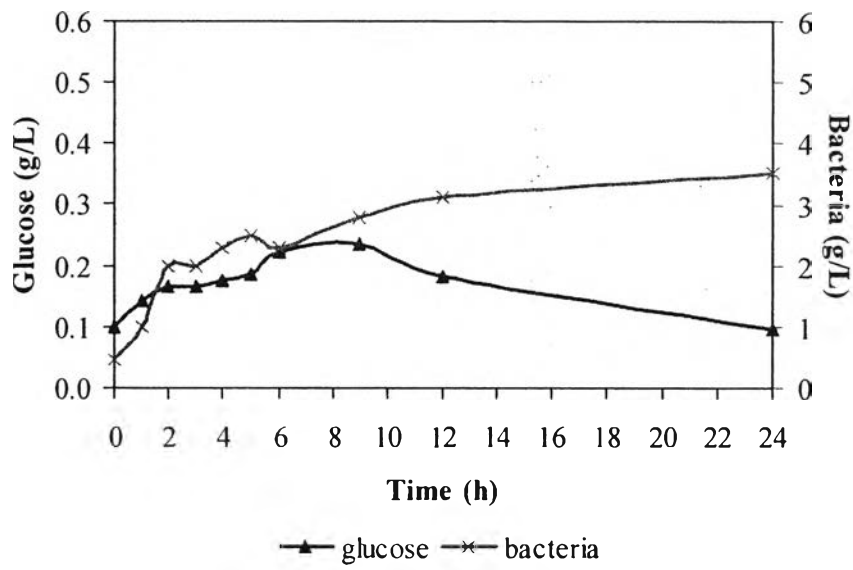


**Figure 4.16** Glucose evolution and bacteria growth from the enzymatic hydrolysis of the pretreated cellulose using strain M 015.

4.2.5 Cellulose Concentration and Glucose Production vs. Time

During the enzymatic hydrolysis, cellulose concentration was also determined in order to assure that celullose was hydrolyzed. Cellulose concentration from the enzymatic hydrolysis of the pretreated cellulose using strain A 002 is shown in Figure 4.17. The pretreated cellulose concentration gradually decreased during 9 h with the increase in the glucose concentration. Then, the pretreated cellulose concentration was constant, while the glucose concentration gradually dropped.

The pretreated cellulose concentration from the hydrolysis using strain F 018 is shown in Figure 4.18. The pretreated cellulose concentration sharply dropped in the first few hours. After 9 h, it was quite stable, while the glucose concentration sharply dropped.

Enzymatic hydrolysis of the pretreated cellulose by strain M 015 resulted in the decrease in the pretreated cellulose (Figure 4.19). Then, it remained stable. On the other hand, the glucose concentration reached the maximum at 9 h. Then, it slowly decreased.

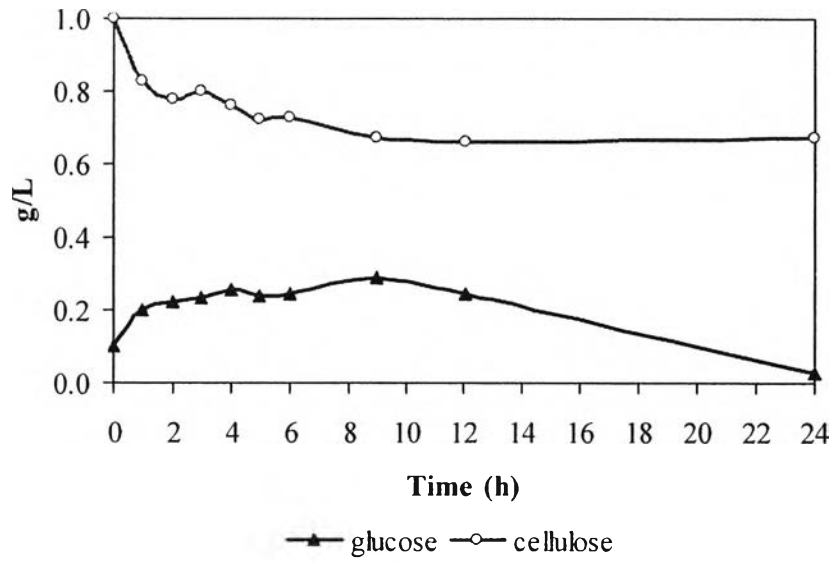


**Figure 4.17** Glucose evolution and pretreated cellulose concentration from the enzymatic hydrolysis using strain A 002.

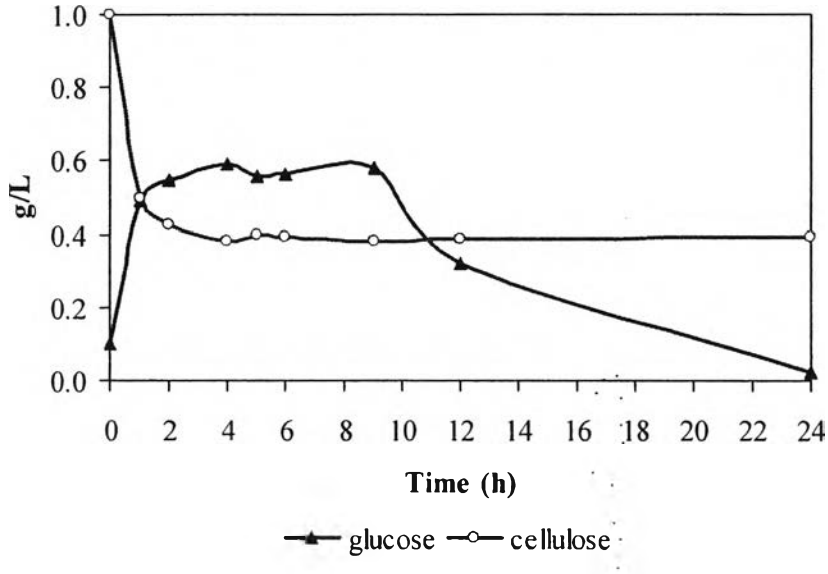


**Figure 4.18** Glucose evolution and pretreated cellulose concentration from the enzymatic hydrolysis using strain F 018.

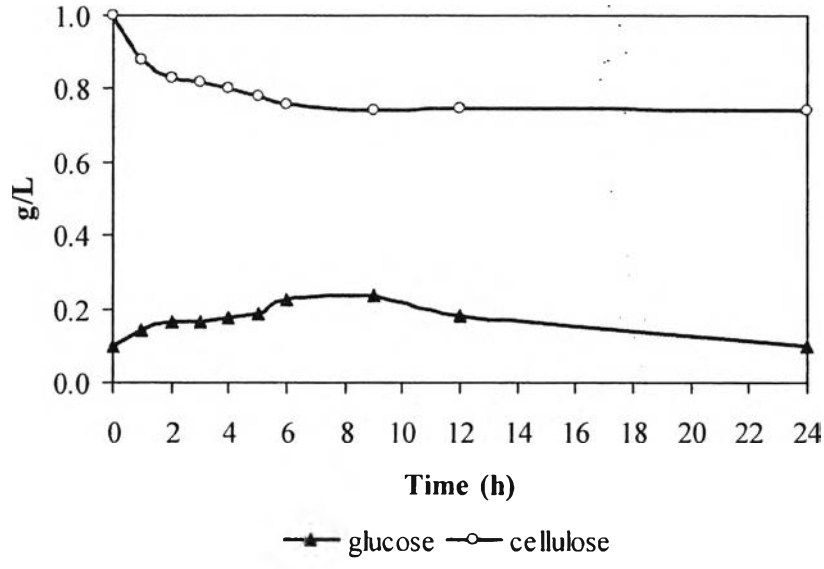


**Figure 4.19** Glucose evolution and pretreated cellulose concentration from the enzymatic hydrolysis using strain M 015.

From Figures 4.17, 4.18 and 4.19, the cellulose concentrations remained constant after the glucose concentration reached the maximum values.

These results impiled that the bacteria switched from using cellulose as a carbon source to glucose instead.

4.2.6 Effect of cellulose structure on the glucose production

Cellulose with different structures were employed in the enzymatic hydrolysis in order to prove that the structure had an effect on the cellulose conversion. Table 4.1 summarizes the crystallinity results obtained from XRD measurement of different cellulose filter papers, which were used in the enzymatic hydrolysis using strain A 002 at 9 h. The glucose concentration obtained from the hydrolysis is shown in Figure 4.20.

**Table 4.1** Crystallinity of Whatman filter paper

| **Whatman filter paper No.** | **Crystallinity specification** | **Crystallinity intensity of XRD peak at 2θ of 23°** |
|---|---|---|
| 1 | Medium crystalline | 1498 |
| 2 | Crystalline | 1573 |
| 4 | Coarse and gelatinous precipitate | 1223 |
| 5 | Fine crystalline | 2015 |

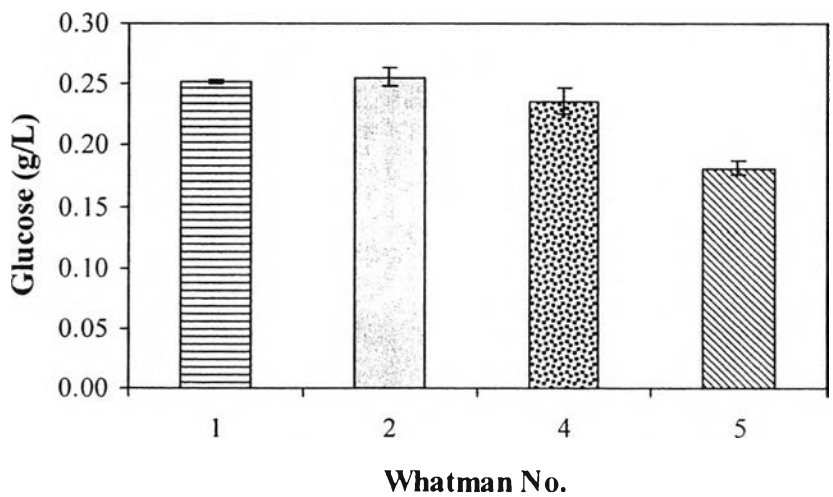


**Figure 4.20** Glucose concentration from the enzymatic hydrolysis of the untreated cellulose using isolated strain A 002 at 9 h.

The hydrolysis of the No.5 Whatman filter paper gave the lowest glucose concentration of 0.18 g/L. That is probably due to the fine crystalline structure of the cellulose. The glucose concentration from the enzymatic hydrolysis of the No. 1, 2, and 4 were about 0.25 g/L. The higher glucose concentration may be due to their lower crystallinity, resulting in the better accessibility of enzyme.