CHAPTER IV
RESULTS AND DISCUSSION

4.1 Material Characterization

The properties of activated carbon are influenced by chemical treatment. The activated carbon (DARCO®) was treated with nitric acid, sodium hydroxide, and 3-aminopropyltriethoxysilane (APTES) by shaking and refluxing. The physical properties of the untreated and treated activated carbon are shown in Table 4.1. The carbon pH in P2 medium, surface area, pore volume and pore size were detected by pH meter and surface area measurement (SAA) with BET (Brunauer-Emmett-Teller), respectively.

For acid-base treated activated carbon, the BET surface area slightly increased when these compared to fresh activated carbon because the chemical treatment affected the surface morphology of activated carbon and some cleavages were eroded, which confirmed by SEM images, as shown in Figure 4.1(a)-(f). It is seen that the activated carbon prepared by a reflux method with APTES had the lowest surface area because APTES is a large molecule and long chain chemical that might block some pores, while the molecules of nitric acid and sodium hydroxide are smaller than APTES.

Moreover, the carbon pH plays an important role in controlling the efficiency of immobilized fermentation. The chemical treatment with nitric acid and sodium hydroxide slightly increased the carbon pH from 5.71 to 5.96 and 6.01, respectively. The treatment with both nitric acid and sodium hydroxide (NASH-AC) provided a lower carbon pH, indicating that the carbon surface still has some acid functional groups from nitric acid.

The material treated with chemicals prepared by a reflux method provides a higher carbon pH than traditional method (shaking). It is probably due to physisorption on the activated carbon surface, while the reflux method could provide a chemical adsorption (chemisorption). From Table 4.1, the carbon pH of activated carbon refluxed with sodium hydroxide (SH-AC(R)) and APTES (AS-AC(R)) were drastically increased from 5.71 to 6.46 and 6.50, respectively. Therefore, the refluxed

activated carbons are suitable to be used as an immobilized material for ABE fermentation.

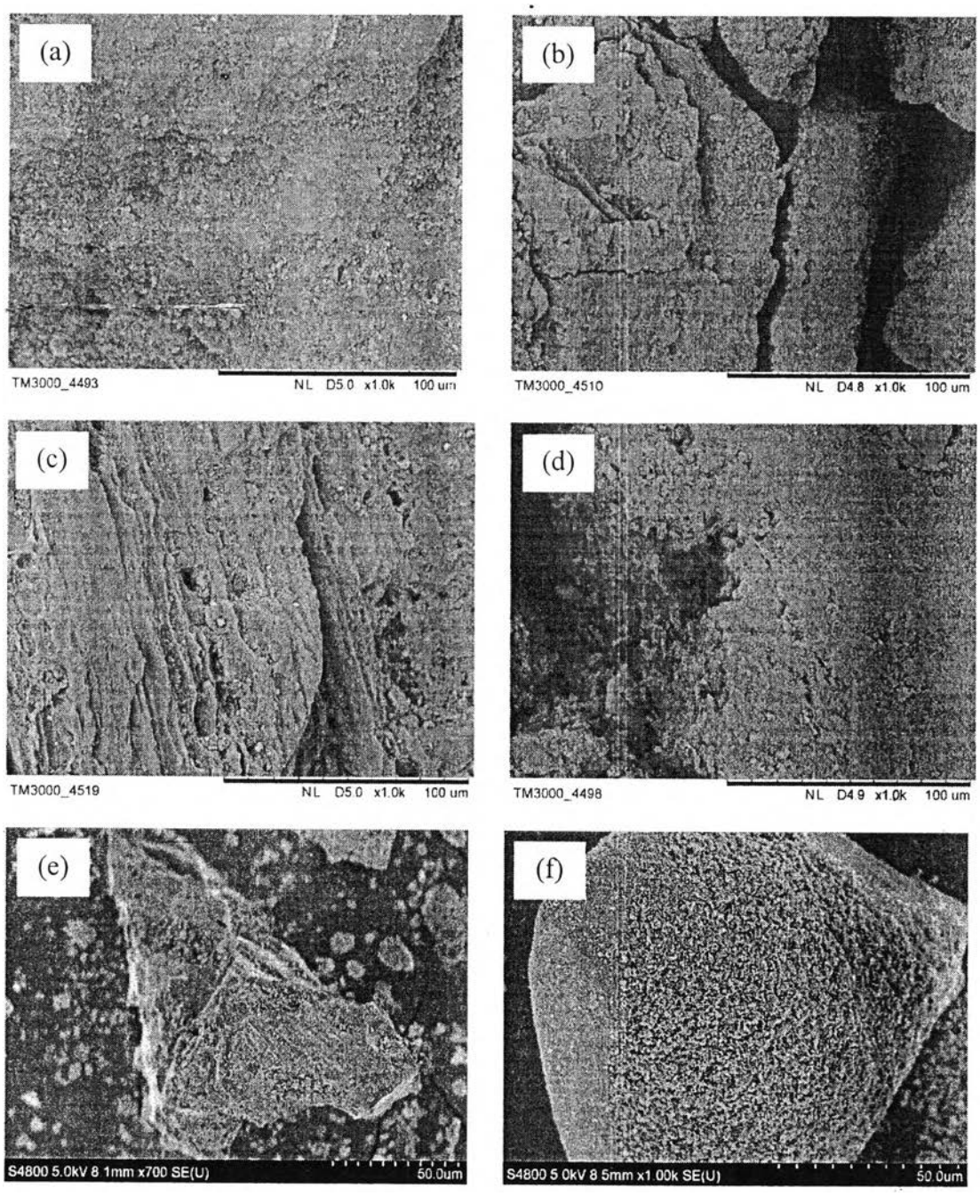

Figure 4.1 SEM photographs of activated carbon samples (a) DI-AC; (b) NA-AC; (c) NASH-AC; (d) SH-AC; (e) AS-AC(R); and (f) SH-AC(R) at 700x and 1000x magnification.

Table 4.1 Physical properties of untreated and treated activated carbons.

Sample	Carbon pH in P2 medium	Surface area (m^2/g)	Pore volume (ml/g)	Pore size (nm)
DI-AC	5.71	490.5	0.535	4.366
NASH-AC	5.96	573.9	0.664	4.625
SH-AC	6.01	470.0	0.545	4.636
AS-AC(R)	6.50	305.6	0.395	5.175
SH-AC(R)	6.46	557.2	0.566	4.063

The treated activated carbons were characterized by Fourier transform infrared spectroscopy (FTIR). FTIR is used to measure the absorption of infrared radiation, which indentifies molecular components and structures, as shown in Figure 4.3. A small peak at 780 cm^{-1} is the characteristic of strong bending vibrations of C-C bonds (Alkene), which is considered as reference of treatment. The effect of treatment was mainly considered at 1570 and 3400 cm^{-1}, which intensity was integrated at these two peaks, as shown in Figure 4.4. The intensity of shaking treatment provided similar results, while the reflux treatment with APTES (AS-AC(R)) showed clearly difference with the highest intensity, confirming that the chemisorption with amine functional group occurs on the activated carbon surface.The peaks of amine functional group at 1570 and 3400 cm^{-1} attributed to the bending and stretching vibrations of N-H. These forming bonds confirmed the surface modification of AS-AC(R) on the activated carbon. The O-H bond at 3400 cm^{-1} is stretching vibration of hydroxyl group. As a result, the carbon treated by reflux treatment with sodium hydroxide (SH-AC(R)) did not show the intensity peaks as good as carbon treated by reflux with APTES (AS-AC(R)). This is because the physisorption of hydroxyl group (-OH) might be removed when the surface was washed with deionized water after the treatment. The interaction machanism between APTES and activated carbon is shown in Figure 4.2(b) (Metwalli *et al.*, 2006) (modified from Shanmugharaj *et al.*,2007).

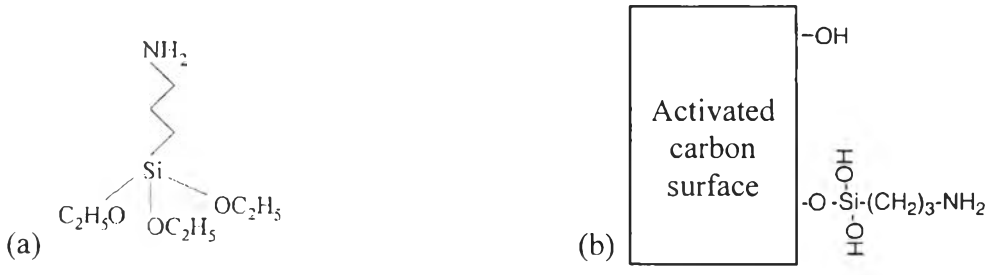

Figure 4.2 3-aminopropyltriethoxysilane (APTES) (a) Schematic structure; and (b) Mechanism of APTES on activated carbon surface.

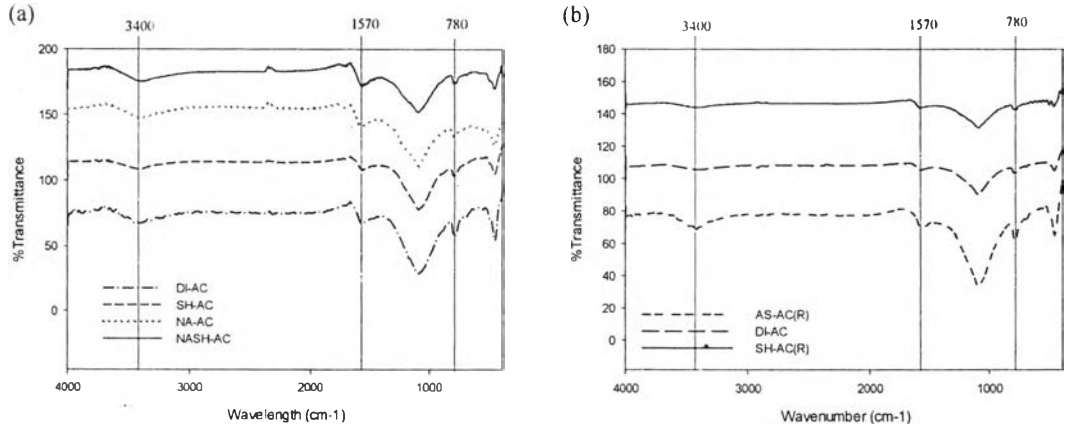

Figure 4.3 FTIR spectra of various activated carbons (a) Acid-base treatment; and (b) Amine-base treatment.

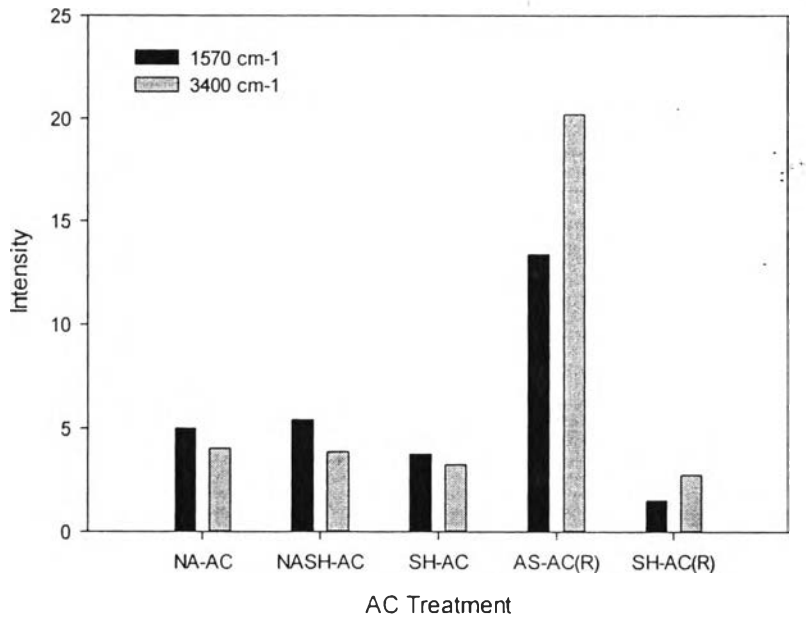

Figure 4.4 FTIR intensity at wavenumber of 1570 and 3400 cm^{-1} obtained from various types of treated activated carbons.

The molecule of APTES was hydrolyzed in chemical treatment to an alkylammonium ion, hydroxide ion, and carboxylate ion, as shown in equation 1. Then, these ions were bonded on activated carbon surface that exhibited the chemisorption, as shown in FTIR results. All of the isotherms (DI-AC, NASH-AC, and AS-AC(R)) is an adsorption isotherm type II, as shown in Figure 4.5(a)-(c). As a result, the hysteresis loop of NASH-AC showed a smaller loop than both DI-AC and AS-AC(R), which the acid-base treatment can desorp the gaseous molecule faster than others. In addition, the AS-AC(R) had a larger hysteresis loop than others because the desorption was interferred by APTES.

$$R - NH_2 + C_2H_5OH \overset{hydrolysis}{\Longleftrightarrow} R' - NH_3^+ + OH^- \qquad \text{eq (1)}$$

Amine — EtOH (excess) — Alkylammonium ion — Hydroxide ion

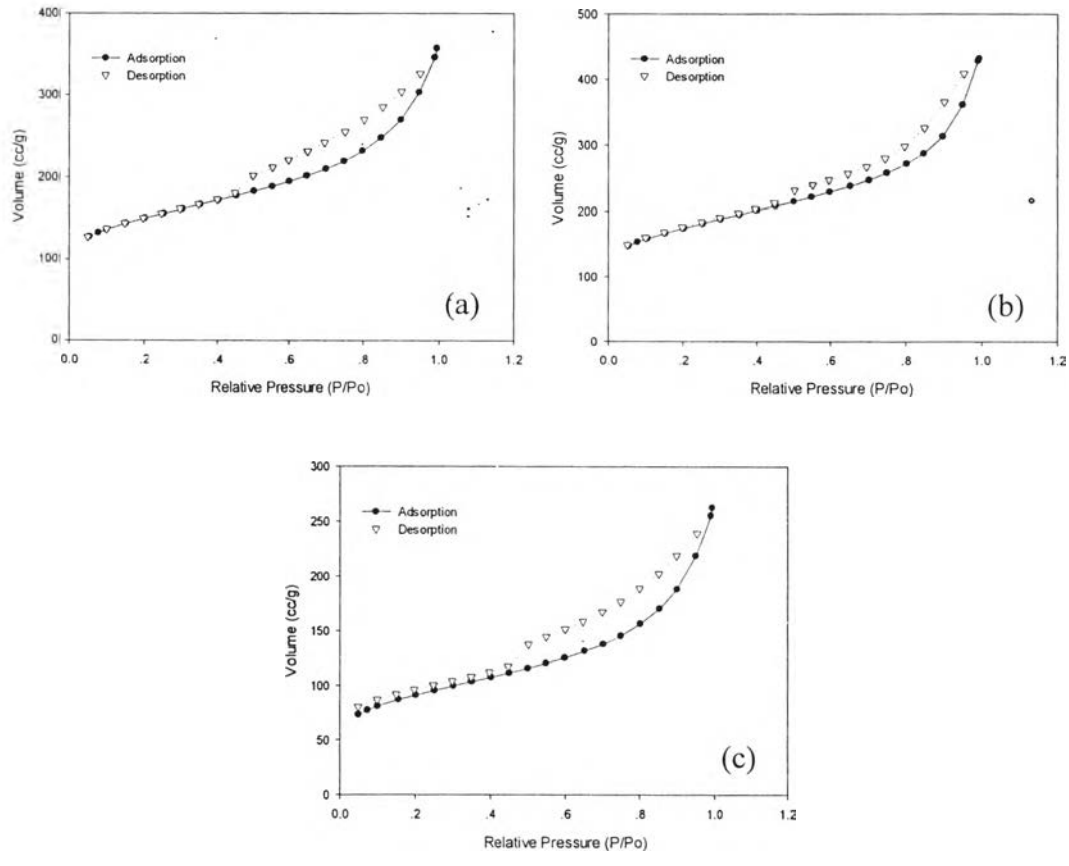

Figure 4.5 Adsorption-desorption isotherms of untreated and treated activated carbons (a) DI-AC; (b) NASH-AC; and (c) AS-AC(R).

Moreover, the chemical treatment also affected an atomic composition, as shown in Table 4.2. Further treatment of the activated carbon using chemicals resulted in a significant increase in oxygen contents, whereas the percentage atomic of carbon obviously decreased in all treated samples. Especially, the activated carbon treated with sodium hydroxide and APTES obviously increased the percentage atomic of oxygen. It is interesting to note that the oxygen content on untreated sample increased upon the surface modification with chemicals, as enchanced by C-O (absorbance at 1000-1300 cm^{-1}) and N-O (absorbance 1500 cm^{-1}), as evidenced by FTIR spectra. In nitric acid treatment, we observed that the system had a brown gas and pungent smelling. This observation directly suggestes that nitrogen oxides (NO_x)

and hydrogen sulfide (H_2S) were propably produced. These gases related to the EDX results that treated samples of both NA-AC and NASH-AC had no sulfur element.

Table 4.2 EDX atomic percentage of various activated carbons

Sample	Atomic Percentage			
	C	O	S	Others
DI-AC	88.24	6.24	1.08	4.44
NA-AC	81.36	9.49	0	9.15
NASH-AC	78.99	12.25	0	8.76
SH-AC	78.54	10.18	1.35	9.93
AS-AC(R)	78.27	11.96	1.66	8.11
SH-AC(R)	79.86	12.07	1.10	6.97

4.2 ABE Fermentation by *Clostridium beijerinckii* TISTR1461

4.2.1 ABE Fermentation with Acid-Base Treatment

Clostridium beijerinckii TISTR 1461 was used in the ABE fermentation to compare between free cell and immobilization systems. Immobilized materials from different chemical treatments (NASH-AC, SH-AC, AS-AC(R) and SH-AC(R)) were compared in this study. Another system was a control system which had a fermentation broth without bacteria. The medium was glucose concentration of 60 g/l, mixing with buffer, mineral and vitamin, called P2 medium. The first phase of this bacteria is called acidogenesis phase or log phase. In log phase, the birth rate of bacteria rapidly increases and a huge amount of glucose is consumed, which occurred at first 48 h fermentation, as shown in Figures 4.6 and 4.7. The cell growth of bacteria was examined by light absorbance at 600 nm. The free cell system provided the highest absorbance, while the immobilization system exhibited lower absorbance. Because the bacteria was adsorbed on the activated carbon surface, the number of bacteria in broth should be smaller than in the free cell system. In Figures 4.8, acidogenesis phase, which is the acids-forming pathway, are activated and the organic acids were produced. The pH broth immediately dropped

from pH 6.5 to 5.28 for NASH-AC system and 4.81, 4.91 for free cell and SH-AC, respectively. Then, these organic acid were consumed to produce acetone, butanol, and ethanol in the next phase called Solventogenesis phase. The acetic acid was rapidly decreased in the first 24 h and NASH-AC still had the highest acid concentration. When, acetic acid was decreased, the butyric acid increased and then changed to butanol via *C. beijerinckii*. However, either glucose or cell growth profile showed the constant results because the control system did not have any bacteria. Therefore, this system did not have any metabolism, resulting in none solvents and organic acids producing.

After 48 h fermentation, the systems were shifted to solventogenesis phase, in which the glucose and cell growth profile were steady. The free cell system gave the highest value of both glucose concentration and cell growth. The highest pH of immobilized system with NASH-AC was around 5.47, while other systems had a lower pH of 4.97, 4.82 for free cell and SH-AC system, respectively. In solventogenesis, the NASH-AC system (after 72 h) provided the highest butanol concentration of 7.54 g/l, while free cell and SH-AC system provided 7.50 and 7.22 g/l, respectively. The different chemical treatment due to the different results both surface area and acidity. However, these treatment has no significant effect on butanol production.

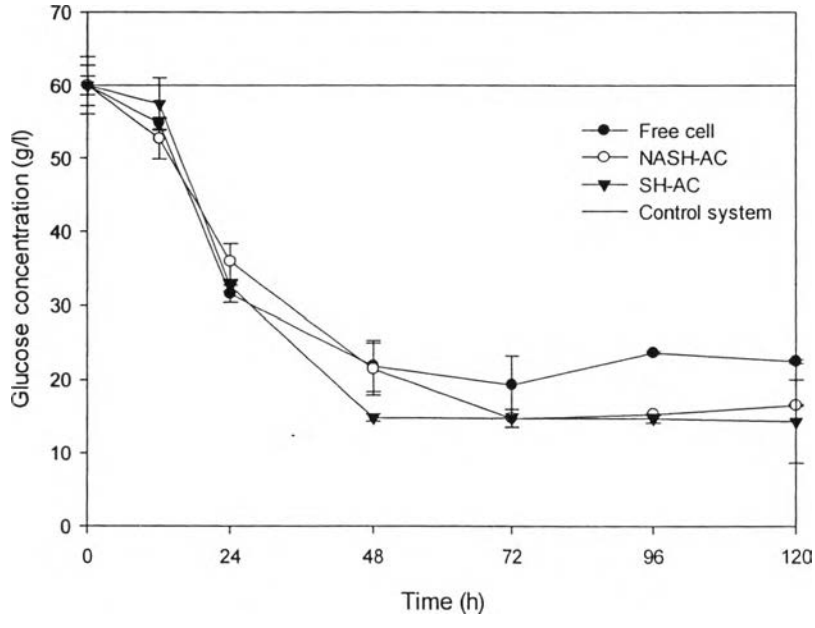

Figure 4.6 Glucose profiles of acid-base immobilized activated carbon fermentation.

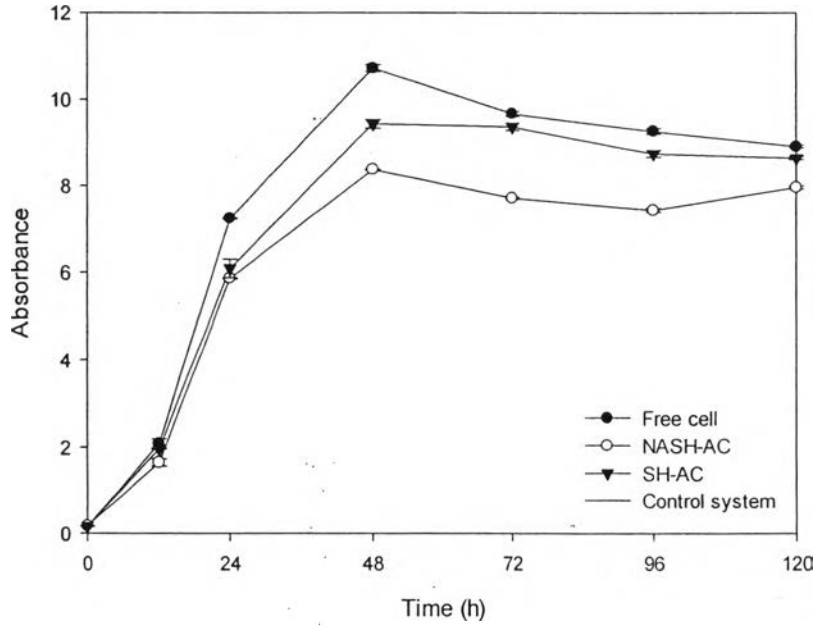

Figure 4.7 Cell growth profiles of acid-base immobilized activated carbon fermentation.

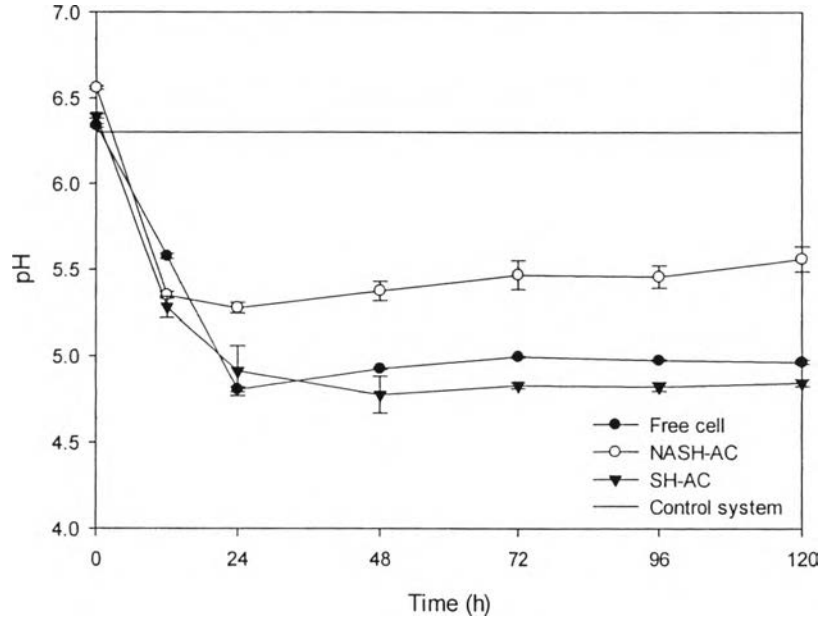

Figure 4.8 pH profiles of acid-base immobilized activated carbon fermentation.

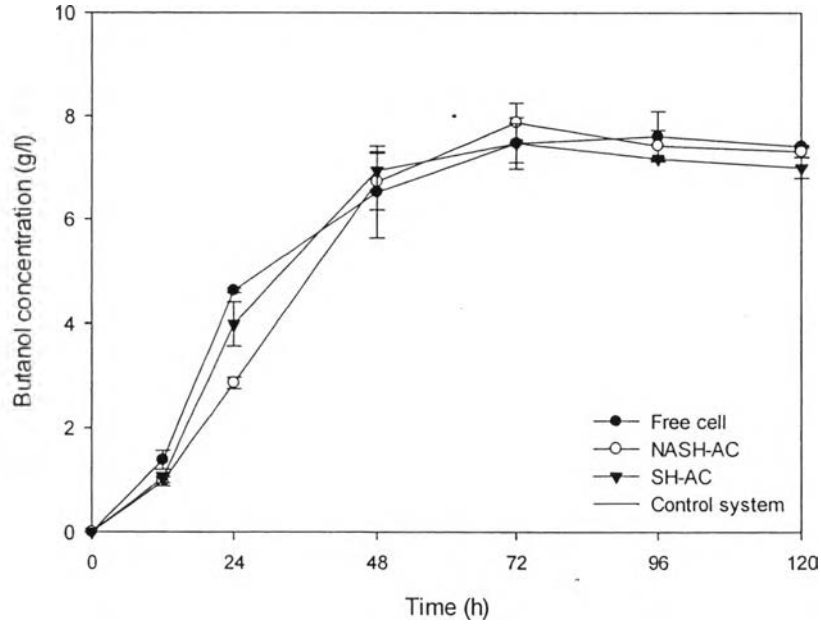

Figure 4.9 Butanol profiles of acid-base immobilized activated carbon fermentation.

4.2.2 ABE Fermentation with Amine-Base Treatment

To study the effect of chemical treatment on immobilized material for ABE fermentation, the system was fermented with AS-AC(R) and SH-AC(R). At first 48 h, glucose concentration of free cell and immobilization of AS-AC(R) and SH-AC(R) rapidly decreased from 60 to 27 g/l, 18.64, and 42.04 g/l, respectively. In log phase, the absorbance in all systems rapidly increased, as shown in Figure 4.11. The free cell system provided the absorbance in a range of 0.10 to 7.13 abs, while immobilization with AS-AC(R) and SH-AC(R) had absorbance range of 4.12 to 14.69 abs and 4.13 to 14.23 abs, respectively. Then, the cell growth in free cell system seemed steady after 48 h, while another system slightly increased. However, the absorbance indicates a cell growth in each system which cannot be used to compare with others. Because the immobilization with activated carbon generated a darker solution which is a suspension of fine activated carbon particles.

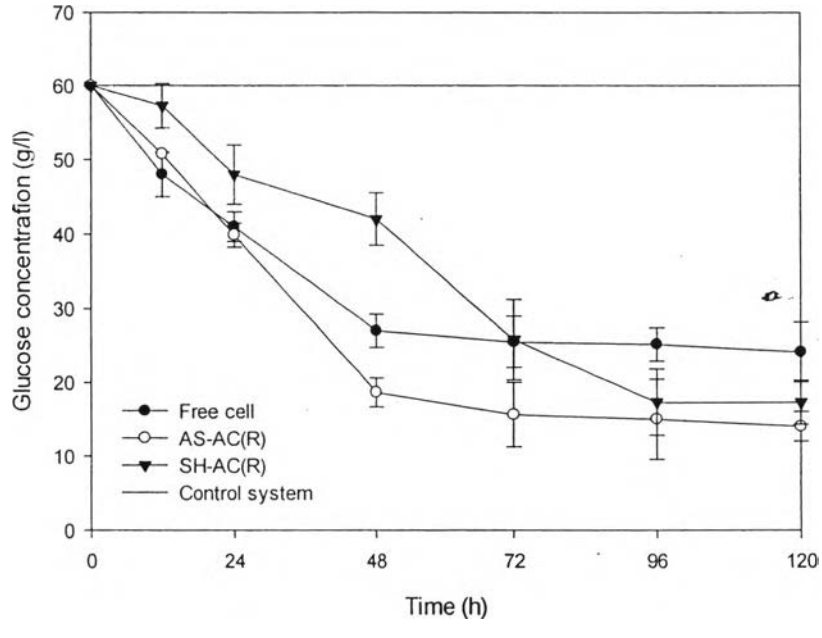

Figure 4.10 Glucose profiles of amine-base immobilized activated carbon fermentation.

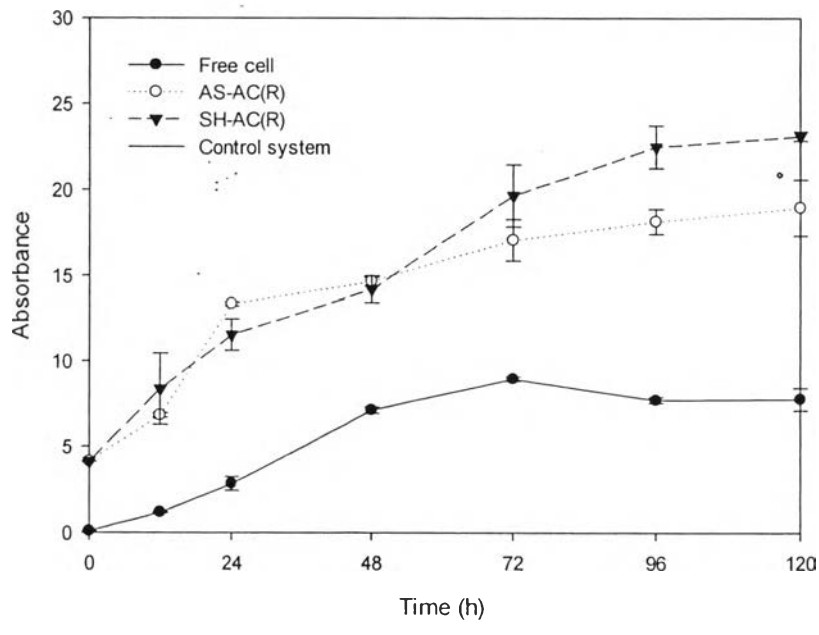

Figure 4.11 Cell growth profiles of amine-base immobilized activated carbon fermentation.

At first 48 h, during acidogenesis phase, glucose was rapidly consumed as shown in Figure 4.10 and organic acids in all immobilized system showed a higher acid concentration than free cell system. The highest acid productivity of free cell, AS-AC(R), and SH-AC(R) were 0.95, 3.50, and 3.35 g/l, respectively. The acid production affected the pH broth that immediately decreased from 6.4 to 5, as shown in Figure 4.12. Then, those total acids continuously decreased after 12 h, while solvents concentration were increased. At first 24 h, the butanol was produced with productivity rate of 0.13, 0.18 and 0.03 g/l/h for free cell, AS-AC(R), and SH-AC(R), respectively. The pH broth of free cell, AS-AC(R), and SH-AC(R) system at stationary phase was approximately 4.85, 4.32 and 4.60. The pH profile related the glucose profile which glucose concentration at 72 h was constant.

The acid profiles related to the solvent profiles because an increase of solvent was resulted from the consumption of organic acids. At 12-24 h, the butyric acid had the highest concentration, and AS-AC(R) can produce to the maximum of

concentration at 2.34 g/l, whereas free cell and SH-AC(R) produced 0.38 and 1.31 g/l (as shown in Figure C2). The concentration of butanol depends upon the butyric acid concentration. Therefore, immobilization with AS-AC(R) provided the highest butanol concentration of 11.16 g/l and free cell could produce butanol concentration of 5.41 g/l. In solventogenesis phase, the butanol concentration remained constant concentration after 72 h, which free cell, AS-AC(R) and SH-AC(R) accounted approximately for 10.66, 5.64 and 4.48 g/l, respectively (as shown in Table B1).

The immobilization provided the highest butanol concentration for activated carbon treated with 3-aminopropyltriethoxysilane. This treatment generated a chemical adsorption of amine functional groups on the activated carbon surface and the acidity on the activated carbon surface was decreased. Whereas, other treatments formed the physical adsorption which is easier to remove after washing with deionized water (Referring to FTIR results). Thus, it could be concluded that amine treatment modified the activated carbon surface and bacteria can be active at the appropriate condition. The immobilization of microbial cell in treated activated carbon are clearly seen in Figure 4.14.

The present section report results on the butanol adsorption of the treated activated carbon. The experiments were carried out in a batch reactor. The reator was kept under steady state condition for 72 h and initial concentration of butanol was 10 g/l. As a result, these activated carbons had a similar adsorption trend which butanol concentration was decreasing approximately 21% at first 48 h. The system which has the highest adsorption was NASH-AC, SH-AC(R), SH-AC, and AS-AC(R), respectively. Furthermore, equilibrium was reached after 48 h which slightly changed the butanol concentration. Therefore, the products of ABE fermentation was also adsorbed on the immobilized material.

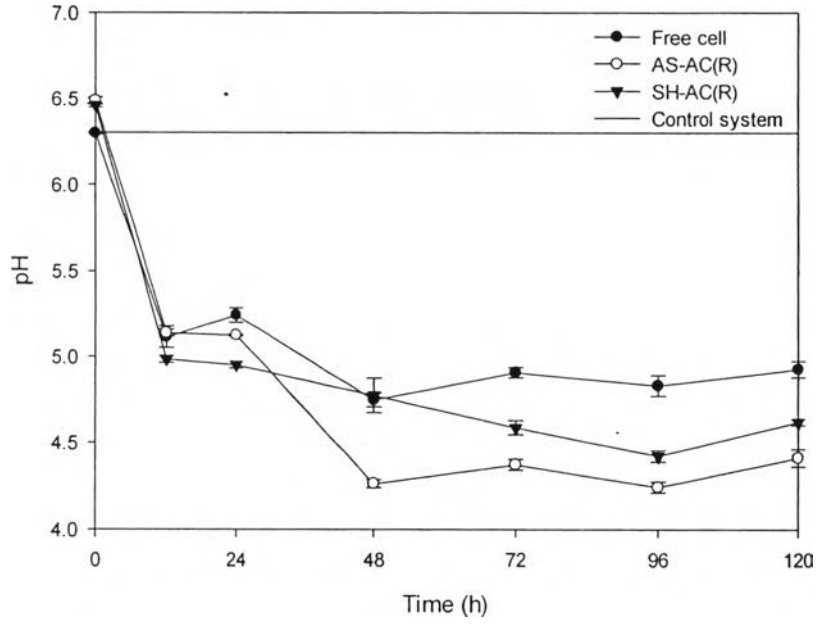

Figure 4.12 pH profiles of amine-base immobilized activated carbon fermentation.

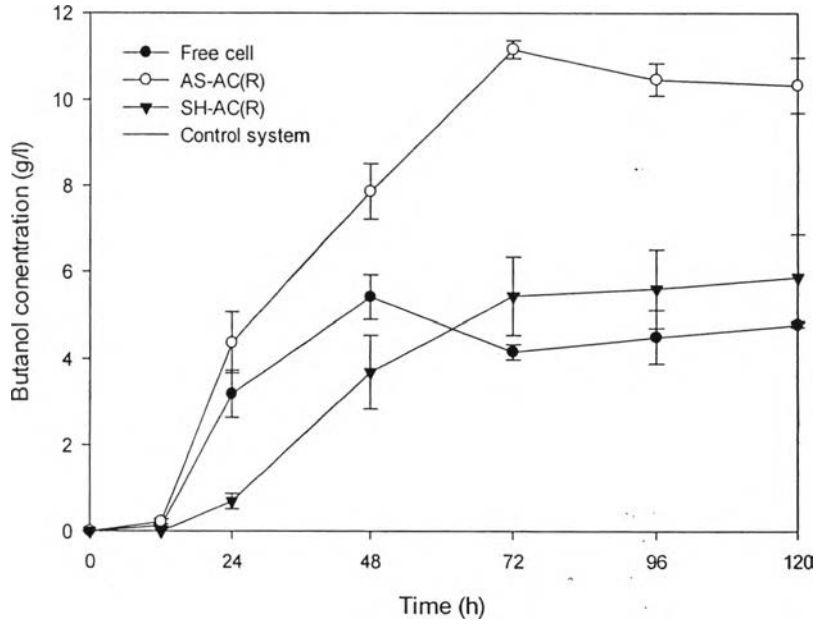

Figure 4.13 Butanol profiles of amine-base immobilized activated carbon fermentation.

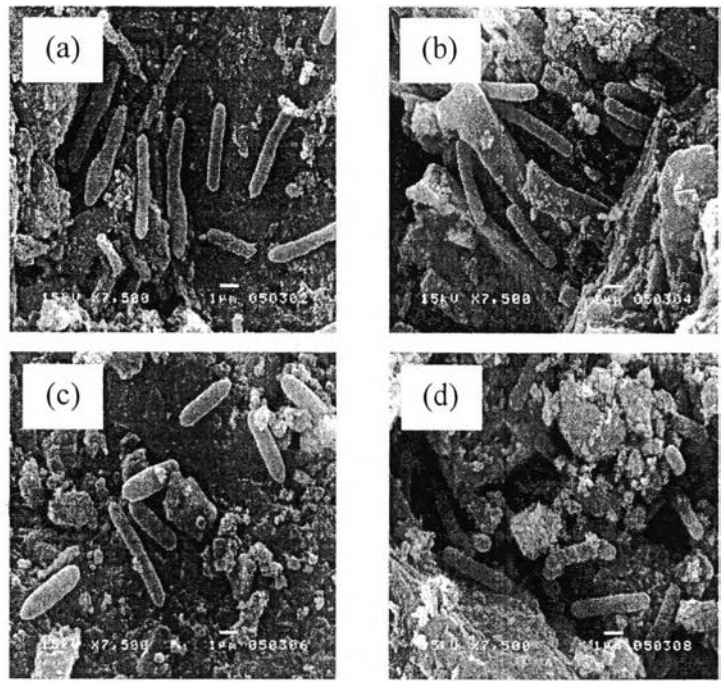

Figure 4.14 SEM photographs of *Clostridium beijerinckii* TISTR1461 immobilized on treated activated carbons (a) NASH-AC; (b) SH-AC; (c) AS-AC(R); and (d) SH-AC(R) at 7500x magnification.

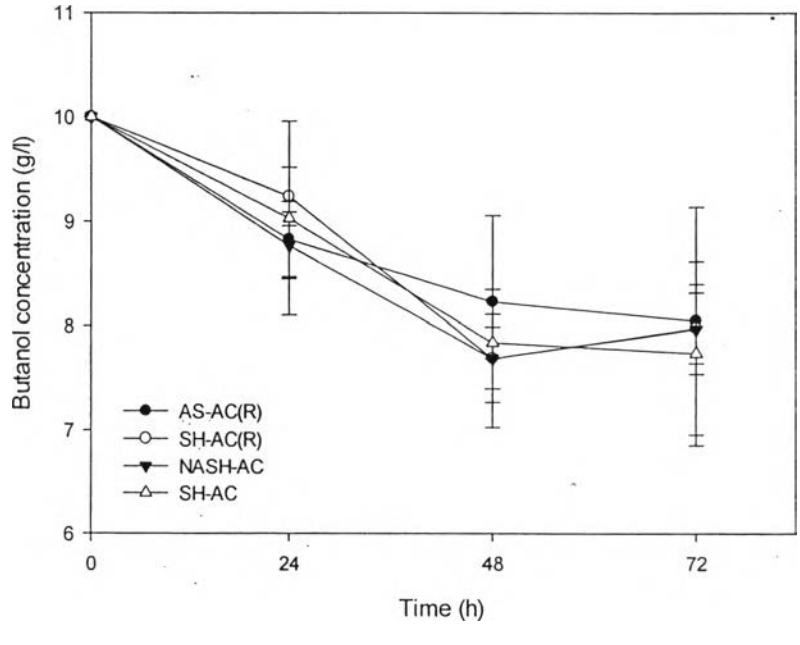

Figure 4.15 Butanol concentration adsorption of immobilized materials adsorption.