CHAPTER IV
RESULTS AND DISCUSSION

4.1 Materials Characterization

4.1.1 Materials Composition

4.1.1.1 Brick (XRF)

For chemically clay brick consist mainly of silicon and aluminium or since these elements usually occur as oxides, as silica and alumina with smaller amounts of iron, calcium, sodium and other elements. Table 4.1 shows the composition of element in raw material for brick. It shows that the main composition of brick compose of silicon dioxide, aluminium oxide and iron oxide subsequently in percentage of 63.87, 19.99, and 9.1, respectively. Traces elements of brick are potassium, magnesium, titanium calcium sodium, and manganese oxide. The silicon dioxide make brick more strength. For aluminium oxide and iron, they change the brick pigment to red after sintering (Sutas *et al.*, 2012).

Table 4.1 XRF analysis for composition of brick

Substance	Proportion (%)
SiO_2	63.87
Al_2O_3	19.99
Fe_2O_3	9.10
K_2O	2.19
MgO	1.29
TiO_2	1.01
CaO	0.85
Na_2O	0.79
P_2O_5	0.20
MnO	0.16
Others	0.55

4.1.1.2 Activated Carbon (SEM/EDS)

For activated carbon Darco grade with granular size derived from Sigma Aldrich. Table 4.2 shows the composition of activated carbon characterized by SEM and EDS elemental analysis. The results of EDS analysis shown the major elemental composition of activated carbon are carbon and oxygen in percentage of 75.25 and 15.95, respectively. Some Si was found might be from the lignite compound of activated carbon because Darco activated carbon was modified from lignite carbon (Sigma-Aldrich, 2012).The EDS result the small amount of S was observed might be the effect of acidic treatment (H_2SO_4) in the chemical modification process of activated carbon (Yin *et al.*, 2007; Inagaki, 2013).

Table 4.2 EDS analysis for composition of activated carbon

Element	Proportion (%)
C	75.25
O	15.95
Si	6.45
S	1.43
Others	0.92

4.1.1.3 Zeolite 13X (XRF)

Zeolite molecular sieve type 13X were purchased from Sigma Aldrich. Zeolite 13X's composition is 1 Na_2O: 1 Al_2O_3 : 2.8 ± 0.2 SiO_2 : xH_2O. The sodium form represents the basic structure of the type X family, with an effective pore opening in the 910¼ range. Table 4.3 shows the results of zeolite 13X analyzed by XRF. The major compositions of treated and untreated zeolite 13X are silicon dioxide, aluminium oxide, and sodium oxide, respectively. Others trace elements are magnesium, calcium, and iron oxide respectively. The results shows the decreased of sodium oxide proportion. This might be explained by the proton from acid and dissociate of water molecule attack the frame work Si-O-Al bonds and the Na^+ cation can dissociation of water molecule by formation of soluable hydroxide. Thus the cation decomposition could be demonstrated cation exchanged aluminosilicate zeolites according to the chemical reactions in equation 4.1. (Buhl *et al.*, 2004; Storch *et al.*, 2008).

$$H^+ + OH^- + Na^+ \equiv Al^- - O - Si \equiv \leftrightarrow OH^- + Na^+ + \equiv Al + HO - Si \equiv \quad (4.1)$$

Table 4.3 XRF analysis for composition of treated and untreated zeolite 13X

Substance	Treated zeolite (%)	Untreated zeolite (%)
SiO_2	50.72	48.69
Al_2O_3	28.88	29.76
Na_2O	13.58	16.77
MgO	2.49	2.15
CaO	1.76	1.23
Fe_2O_3	0.85	0.73
Others	1.71	0.67

4.1.2 X-ray Diffraction

4.1.2.1 Brick

Figure 4.1 shows the XRD pattern of brick, when 2 theta range, 5-80 degree, and speed distribution equal to 2 degree/min. The result show the most crystalline minerals content is quartz (SiO_2), aluminium oxide, iron oxide and the other is titanium oxide, as shown in Figure 4.1 which the main components agree with the XRF results. Normally, the phase composition of raw brick clays are complex due to use of highly impure soil; however, the major phases found in the raw soil materials was quartz (Ahmed *et al.*, 2008). The highest peak intensity at 26.6° (2 theta degree) was indicated to quartz (SiO_2), which showed the same XRD pattern as the previous study (Ahmed *et al.*, 2008; Gredmaier *et al.*, 2011). The crystal size (D) for quartz was calculated by Debye-Scherrer equation.

$$D = \frac{K\lambda}{\beta\cos\theta} \tag{4.2}$$

Where D is the crystallite size, k is shape factor (equal to 0.89 as a constant assuming that the particles are spherical), λ is the X-ray wavelength (0.15418 nm), β is the full width at half-maximum (FWHM) of diffraction peak (Liang and Lee, 2012). Thus by calculating the D value of quartz at 2θ = 26.2° (the strongest peak) is 76.2 nm.

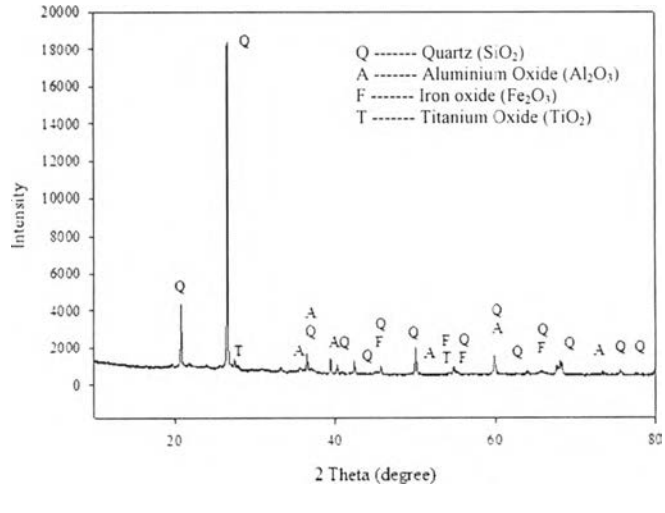

Figure 4.1 X-Ray diffraction pattern of brick.

4.1.2.2 Activated Carbon

The XRD pattern of activated carbon is shown in Figure 4.2. The XRD analysis shows the reflection at 22.6, 42.3, 54.8, and 59.9° (diffraction angle corresponding to 2 theta degree) for graphitic (0 0 2), (1 0 0), (0 0 4), and (1 0 3) planes, respectively, corresponding to graphite pattern (Inagaki, 2013). The X-ray diffraction pattern of granular activated carbon had demonstrated a high crystallinity of activated carbon for graphite (0 0 2) plane with high XRD peak intensity. The XRD pattern was also similar to the granular activated carbon from previous study (Tseng *et al.*, 2011; Liang and Lee, 2012). The lignite crystallites forms were observed at 20.9, 36.5, 45.8, 50.1, and 68.8° (2 theta degree). The lignite was observed might be because Darco activated carbon was modified from lignite carbon (Sigma-Aldrich, 2012). The crystal size (D) was calculated from full width at half maximum (FWHM) data by Debye –Scherrer equation (Equation 4.2). The crystal size of C (0 0 2) (the strongest peak) is 46.6 nm.

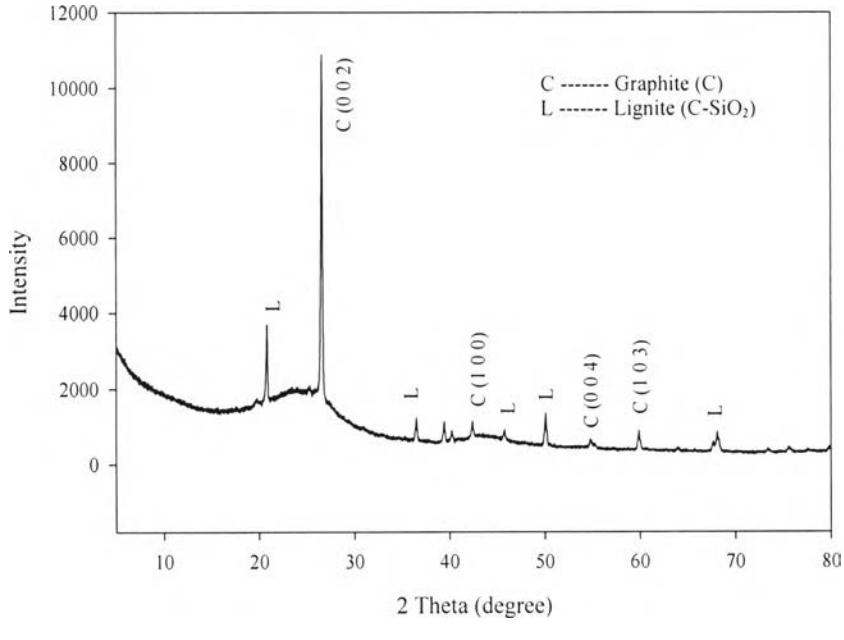

Figure 4.2 X-Ray diffraction pattern of activated carbon.

4.1.2.3 Zeolite

Figure 4.3 shows the XRD patterns of zeolite 13X compared between zeolite before treated and treated zeolite with 0.5%wt of H_2SO_4 for 2 theta range (5-80 degree) and speed distribution equal to 2 degree/min. The result indicated sodium aluminum silicon oxide hydrate in different plane for both zeolites which agrees to the XRF result and Sigma Aldrich information (zeolite 13X's composition is 1 Na_2O: 1 Al_2O_3 : 2.8 ± 0.2 SiO_2 : xH_2O). The XRD pattern and plane observed corresponded to the XRD pattern from Zheng *et al.* (2008) study. To compare the XRD pattern between treated zeolite and untreated zeolite, the same XRD pattern were observed; however, the reduction of XRD peak intensity was observed in treated zeolite. The reduction peak intensity might be related with the acidity from sulfuric which led to a collapse and destructive effect to the zeolite crystal lattice (Pifferi *et al.*, 1982). The crystallite sizes for both treated and untreated zeolite were calculated from the FWHM data at two theta equal to 6.1 degree corresponding to the (1 1 1) diffraction plane of sodium aluminum silicon oxide hydrate. The crystal sizes with using Debye –Scherrer equation (Equation 4.2) are 67.0° and 68.5° for treated and untreated zeolite, respectively, implying that the destructive effect from acid to crystal lattice occurred (Pifferi *et al.*, 1982).

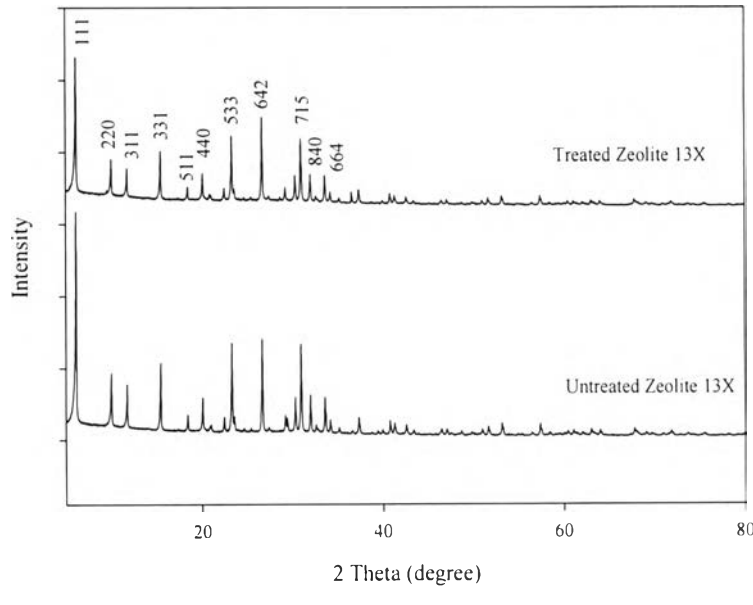

Figure 4.3 X-Ray diffraction patterns of treated and untreated zeolite 13X.

4.1.3 Surface Area Measurement (SAA)

Surface area, pore volume, and pore diameters of carrier materials (brick, activated carbon and zeolite) were measured by N_2 adsorption at 77 K (Brunauer-Emmett-Teller) method.and the results are shown in Table 4.4.

Table 4.4 BET surface area, total pore volume, and average pore diameter of materials

Materials	BET surface area (m^2/g)	Total pore volume (cm^3/g)	Average pore diameter (Å)
Brick	7.08	0.02	113.10
Activated Carbon	595	0.65	43.36
Zeolite (Treated)	531	0.43	32.11

From Table 4.4, activated carbon has high surface area of 595 m^2/g with total pore volume and average pore diameter of 0.65 cm^3/g and 43.46 Å, respectively. This agrees with Sigma Aldrich product information for Darco grade (Sigma-Aldrich, 2012) and properties definition of activated carbon which is indicated to a highly porous structure, high sorption capacity, and high surface area material produced from carbon-rich precursor (Sing, 2014). The zeolite had also high surface area of 531 m^2/g and 0.43 cm^3/g and 32.11 Å for total pore volume and average pore diameter. Normally, zeolites are aluminosilicates with well-define crystalline with high porous and large surface area structure (Payne and Abdel-Fattah, 2005). For brick, it has the lowest surface area of 7.08 m^2/g compared with other materials and low total pore volume of 0.02 cm^3/g which agrees with high average pore diameter of 113.10 Å.

4.1.4 Initial pH of Materials Measurement

Table 4.5 shows the initial pH of material which was measured by grinding the solid with adding drop of water to the surface of material to enable pH measurement with pH electrode. The materials initial pH are 5.91, 2.56, 10.38, and 7.06 for brick, activated carbon, untreated zeolite, and treated zeolite, respectively. The high acidity was observed in activated carbon. Normally, activated carbon has bifunctional acidic and basic properties. The acidic character of activated carbons is primarily attributed to surface oxygen groups for example phenolic and carboxylic. The acidic of activated carbon express at relative low temperature or high temperature with oxygen content (Knappe, 2006). Moreover, by a process to modify the activated carbon might use acid activation or acid washing that led to acidity exhibit of activated carbon (Inagaki, 2013). According to production information of Sigma Aldrich, acid washed granular activated carbon was produced by steam activation of lignite coal (Sigma-Aldrich, 2012). For zeolite 13X which are hydrated aluminosilicates minerals containing with exchangeable alkaline cations (Na) as well as water in their structural frame work that alkaline cations can easily be exchanged by surrounding positive ions (Payne and Abdel-Fattah, 2005). Thus the high basicity that observed for zeolite might be explained by Na^+ cation dissociation of water molecule by formation of soluable hydroxide (Lutz *et al.*, 2005), as shown in equation (4.1) and form to sodium hydroxide which is a strong basic. For treated zeolite, it was found the lower basicity that might be the effect of H^+ from sulfuric acid that could reduce the basicity of zeolite. This result agrees with the decrease of sodium oxide proportion in treated zeolite observed from XRF result.

Table 4.5 Initial pH of materials

Materials	**Initial pH**
Brick	5.91
Activated Carbon	2.56
Untreated Zeolite 13X	10.38
Treated Zeolite 13X	7.06

4.1.5 Scanning Electron Microscope (SEM)

4.1.5.1 Materials without cell immobilization

Figure 4.4 demonstrates the surfaces of material by scanning electron microscope at magnification x3000. The rough surface and porous was observed in brick surface. For activated carbon, small cavities, cracks particles, and complicated pore network on the surface were found. The untreated zeolite indicated the well uniform spherical crytalline structure with porous structure. However, treated zeolite showed less crystallite uniform and demonstrated collapse and destructive structure that might be from the acid treatment.

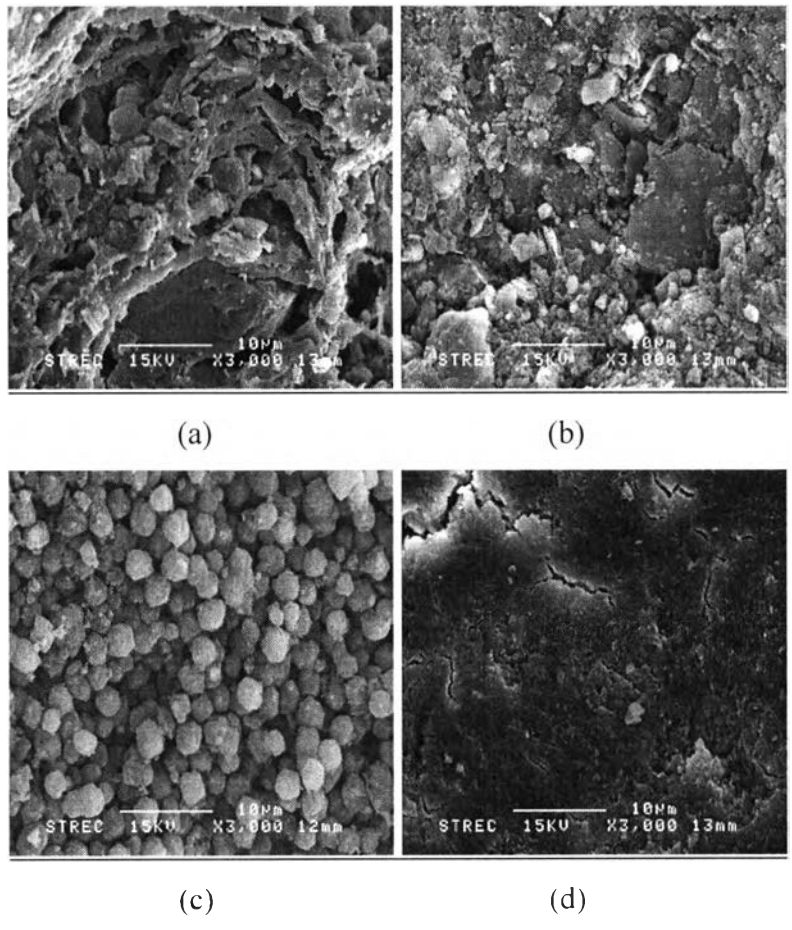

Figure 4.4 Scanning electron microscope images of materials before the immobilization; (a) brick, (b) activated carbon, (c) untreated zeolite, (d) treated zeolite.

4.1.5.1 Materials with cell immobilization

The materials surfaces with cell immobilization by SEM at a resolution of x3500 are shown in Figure 4.5. It is clearly seen that the cells were adsorbed not only the materials surfaces but also in the porous of all materials.

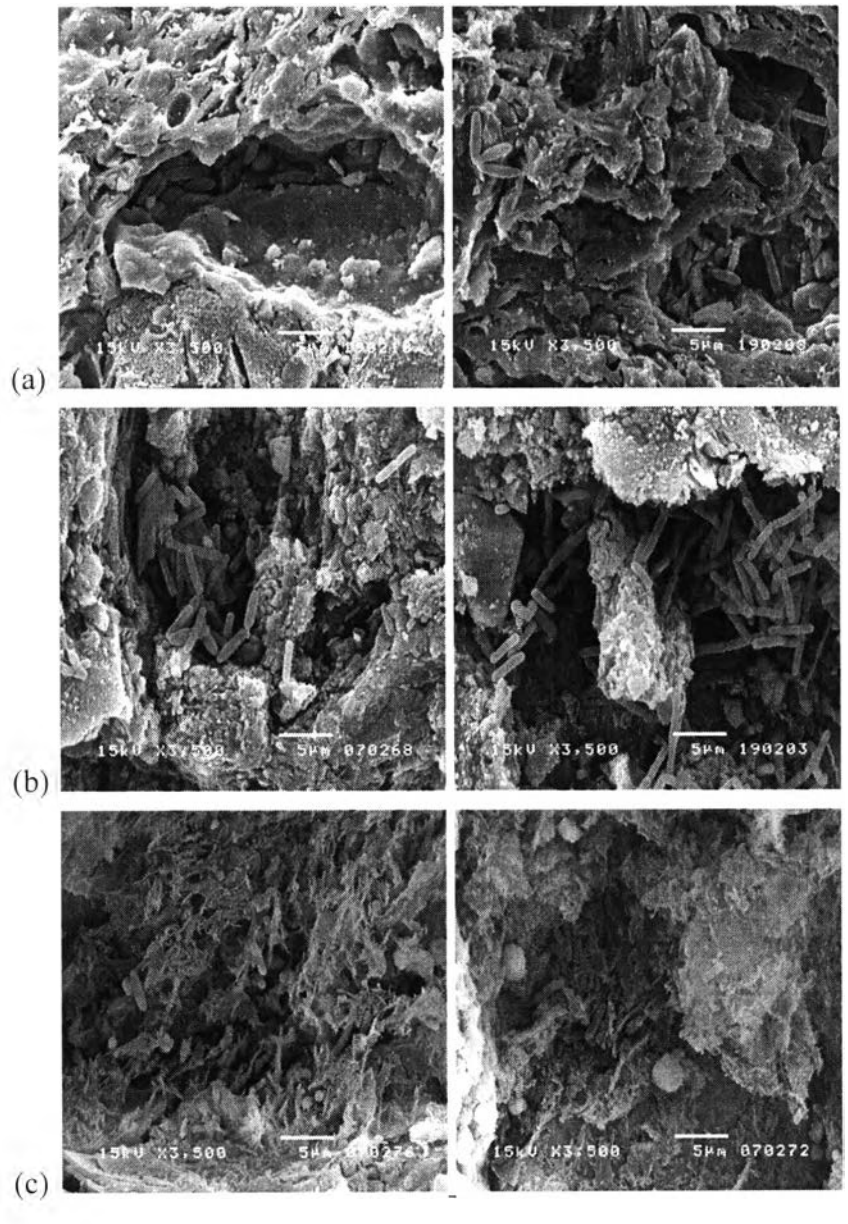

Figure 4.5 Scanning electron microscope images of materials after the immobilization; (a) brick, (b) activated carbon, and (c) zeolite.

4.2 Fermentation

4.2.1 Free Mobilized Cells Fermentation

After inoculate microorganism to production medium, the ABE fermentation was started and products were collected at 0, 12, 24, 48, 72, 96, and 120 hours of fermentation to measure pH and analyze product concentration compared between free cells mobilized batch and immobilized cells fermentation. Figure 4.6 shows pH profiles of free mobilized cells fermentation. At 0^{th} hour, pH of solution was about 6.5 that is in the range of proper initial pH for optimal organism growth rate (Khamaiseh *et al.*, 2013; Kumar *et al.*, 2013). The pH was significantly decreased after 12 hours of fermentation. This is indicating acidogenic stage which is the cell growth phase. Acetic acid and butyric acid were produced in this stage as a result the pH was obviously dropped from 6.5 to about 4.8 in this phase (Rao and Mutharasan, 1987). After 24 hours of fermentation the pH value was slightly increased because of the reutilized of acid and steadily constant at about 5.0, indicating the organism shift metabolic to produce solvents (Tashiro *et al.*, 2004). Solventogenic stage is slower growth phase and solvents (ABE) were formed with a consumption of the acids that formed during the first stage.

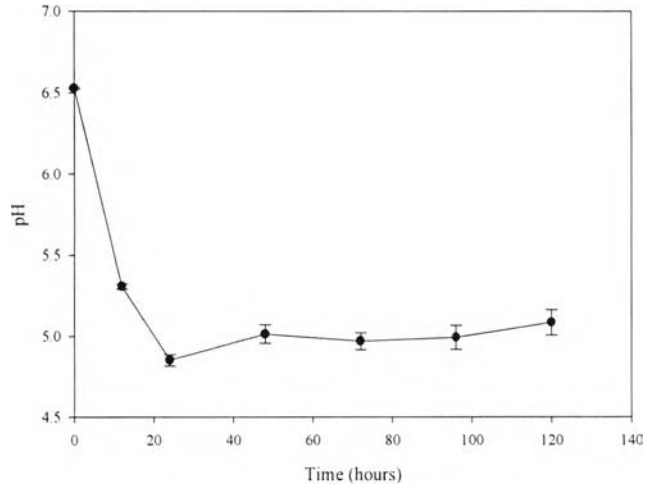

Figure 4.6 pH profile of free mobilized cells fermentation.

Samples of fermentation were collected after centrifuge and filtrated at different times to analyze the product concentration. At the beginning of the fermentation, 60 g/l of glucose was present in the medium, as shown in Figure 4.7. The glucose in production medium is the raw material that microorganisms uptake as energy source for convert to the fermentation product (Benson, 2001). The glucose consumption has significantly decreased after 12th hours and slightly constant after 48th hours for free cell batch, indicating the glucose present in the media was used up and leading to exhaustion of the carbon source in the media (Kumar *et al.*, 2013). The glucose was rapid consumed and converted to acids in the acidogenesis phase after 12th which correspond to the pH profile result. After 48th hours, there was hardly glucose consumption in the solventogenesis phase. When the fermentation was run for 120 hours the glucose concentration was maintained about 22 g/l, resulting in 38 g/l of glucose was utilized.

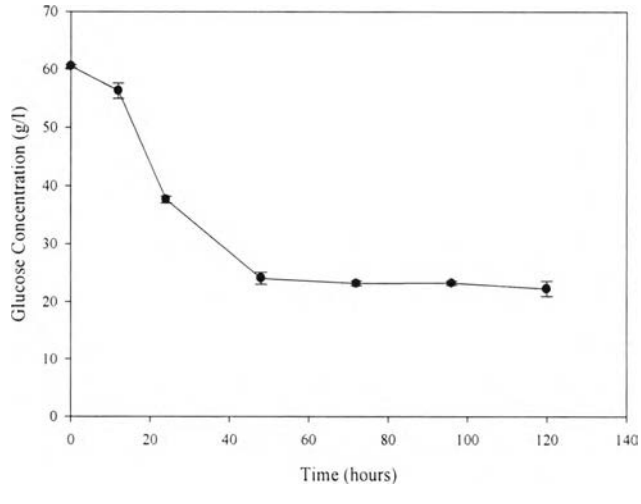

Figure 4.7 Glucose profile of free mobilized cells fermentation.

Figure 4.8 and 4.9 show acid and solvent production for free cells fermentation. For acid production, the concentration of acetic acid and butyric acid are shown in Figure 4.8. At first 12th hours, the result indicates the high acid production for both batches which agree with pH profile. This can be demonstrated the acidogenic stage for metabolic path way (Rao and Mutharasan, 1987). Before 12th hours the maximum acid production was about 1.7 g/l and 0.42 g/l for acetic

acid and butyric acid. After 12th hours acid concentration dramatically decreased during operation, suggesting that the cells successfully shifted to the solventogenic stage from the acidogenic stage. This result is corresponding to the solvent production after 12th hours acids were uptake by organism and solvent ABE were produced, as shown in Figure 4.9. The maximum total solvents from free mobilized cells fermentation was 9.85 g/l for total ABE which consist of 4.08 g/l, 5.29 g/l, 0.47 g/l of acetone, butanol, and ethanol, respectively.

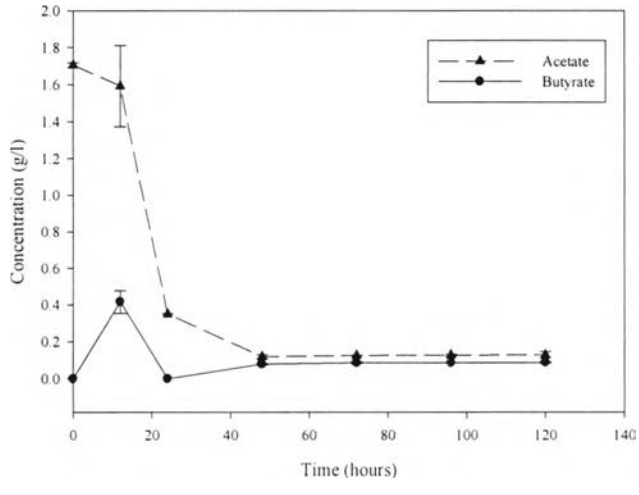

Figure 4.8 Acids production of free mobilized cells fermentation.

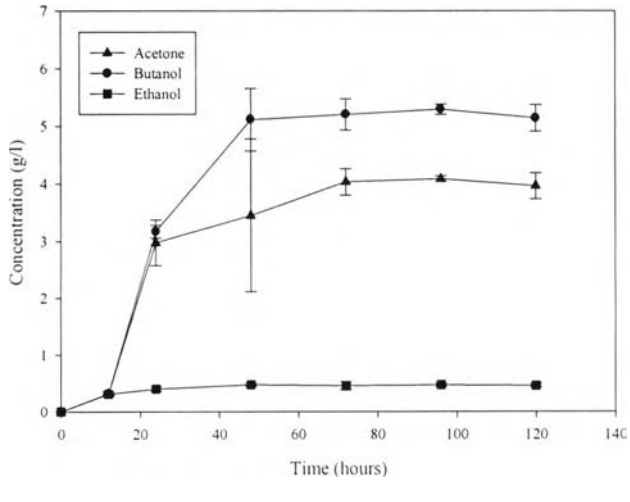

Figure 4.9 Solvents production of free mobilized cells fermentation.

Figure 4.10 shows the growth curve of *Clostridium beijerinckii* TISTR 1461 by optical density at 600 nm (OD600). The cell growth showed almost no lag phase and followed by log phase with cells exponential growth curve for acidogenic stage after first 12^{th} hours with acid production (pH decrease). Then the slower growth phase that cells entering to stationary phase after 48 hours which indicated the solventogenic stage of cells with solvent production (Mathews and Wang, 2009). These results are reasonable to the pH profile, acid, and solvent production results that was descripted from previous results.

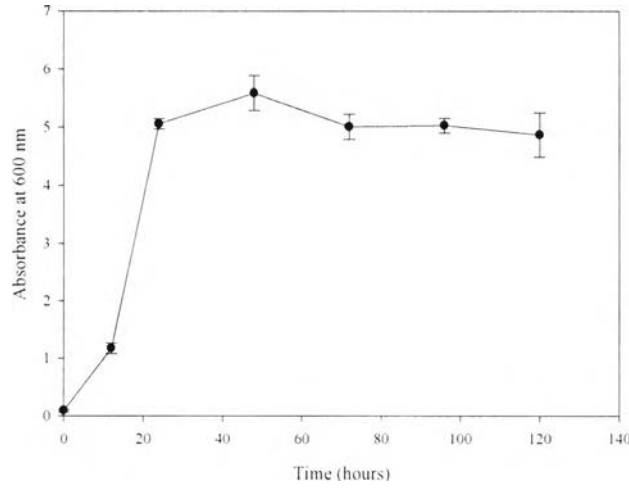

Figure 4.10 Growth curve of *Clostridium beijerinckii* TISTR 1461.

4.2.2 Immobilized Cells Fermentation on Brick

For pH profile, as shown in Figure 4.11, the pH profile of immobilized cells fermentation on brick batch express the similar pH profile result as free cells fermentation batch. The initial pH of medium of 6.5 was significantly decreased after 12 hours of fermentation. After that, the pH of solution rather constant at 4.8 after 24 hours of fermentation which is similar to free cells batch. This pH profile suggested that the switch from acidogenesis to solventogenesis occurred (Tashiro *et al.*, 2004). The cell growth enters the stationary phase during solventogenesis, and the organic acids are uptaked and acetone, butanol, and ethanol are produced.

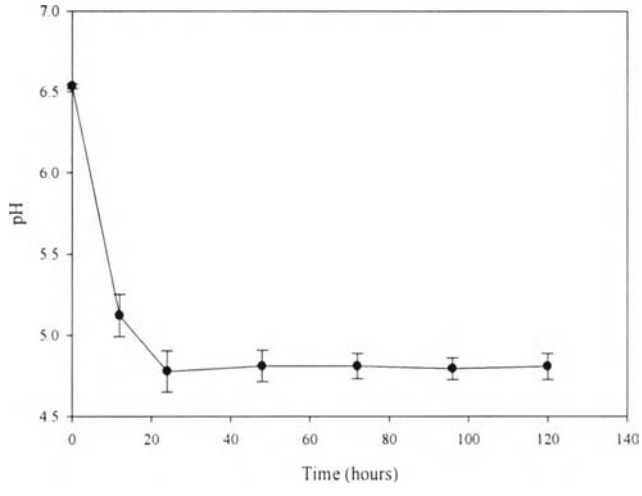

Figure 4.11 pH profile of immobilized cells fermentation on brick.

For glucose consumption of immobilized cells on brick batch compared with free cells fermentation are presented in Figure 4.12. During immobilized cells fermentation, utilization of glucose was about 37 g/l compared with utilized glucose of free cells of 34 g/l. The final glucose concentration of immobilized and free cells batches were 23 g/l and 26 g/l respectively. The glucose utilization for cell immobilization shows slightly faster and higher than free cells did.

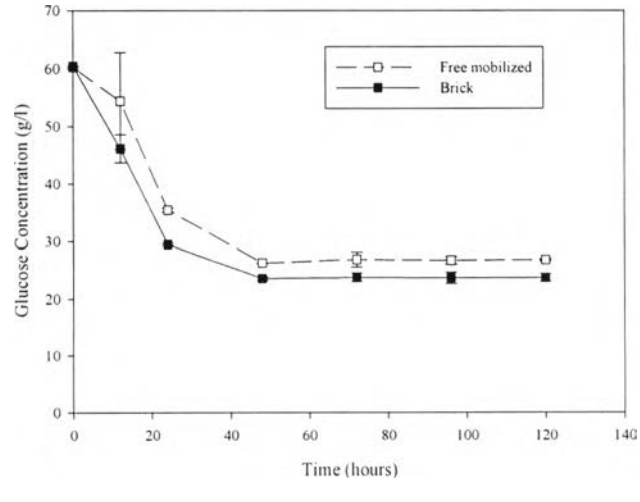

Figure 4.12 Glucose profiles of immobilized cells fermentation on brick compared with free cell system.

Acids and solvents production for cell immobilization on brick were shown in Figures 4.13 and 4.14. The acids production for immobilized cells fermentation on brick shows similar profile result as free mobilized cells batch. It shows cell shifting stage from acidogenesis to solventogenesis similar to free cells batch. For solvents production, the maximum concentration of butanol for cells immobilized on bricks was 5.80 g/l and compared to free cells the maximum concentration of butanol was 5.29 g/l. Therefore, the butanol production with immobilized cells was about 9.5 percentages higher than those with free cells. From this result indicated that the immobilized cells on bricks could improve the butanol production. This result agrees well with the results from previous study (Qureshi *et al.*, 2000; Yen and Li, 2011) that cells immobilization on brick could enhance butanol production. For acetone and ethanol production of immobilized cell batch were 4.42 g/l and 0.49 compared with 4.08 g/l and 0.47g/l from free cells batch. The cell immobilization on brick is also higher acetone and ethanol production. The higher solvents production result from cell immobilization batch might be explained by the increasing of cell density in immobilized batch compared with free cells (Yen and Li, 2011). Moreover, from previous literature (Liu *et al.*, 2013) indicated that the immobilized cells showed higher butanol torelance toxicity than free cells thus the higher ABE production was observed in immobilized cells batch.

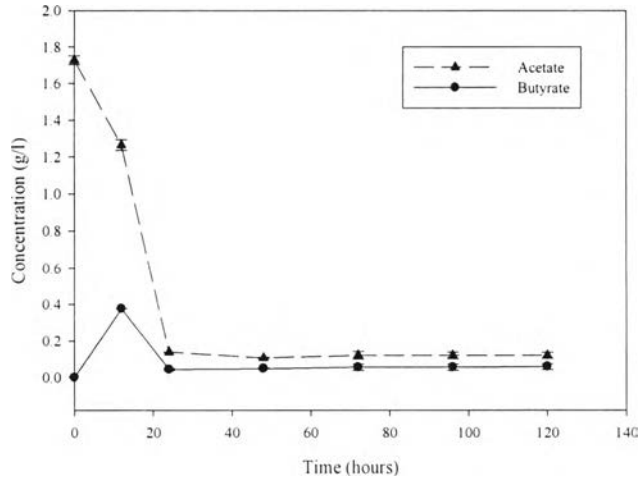

Figure 4.13 Acids production of immobilized cells fermentation on brick.

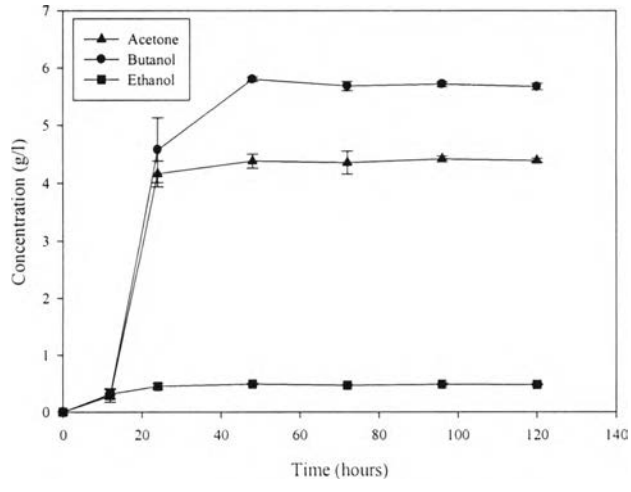

Figure 4.14 Solvents production of immobilized cells fermentation on brick.

4.2.3 Immobilized Cells Fermentation on Activated Carbon

Figure 4.15 shows the pH profile of fermentation by immobilized *C. beijerinckii* onto an activated carbon.

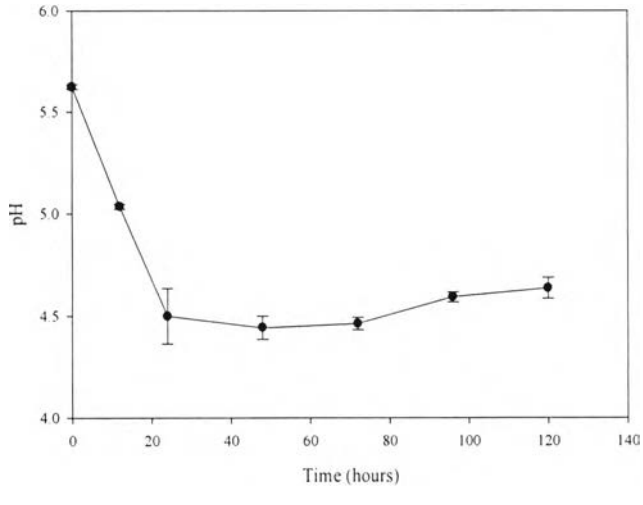

Figure 4.15 pH profile of immobilized cells fermentation on activated carbon.

For this batch, the initial pH value of medium was 5.6 and significantly decreased after 12 hours of fermentation. The pH value profile was rather constant at 4.5 after 24th hours of fermentation. For these pH profile could

indicate the acidogenic and solventogenic stage of organism (Tashiro *et al.*, 2004). Normally, the initial pH of medium without material adding was 6.5 and constant at 5.0 for stationary phase. However, comparing to free mobilized cell fermentation, the pH profile for this batch shows lower pH profile all of fermentation time of stream. This low pH result might be affected by the acidity of activated carbon that was added in to medium as a carrier. The initial pH value of activated carbon material was 2.56 (Table 4.5) thus adding activated carbon could affect to the decreasing of medium pH.

Glucose utilization of this batch compared with free cells batch is shown in Figure 4.16. The glucose concentration was significantly dropped after 12th hours and rather constant after 48 hours. This result is reasonable with the pH profile result that indicated the metabolic pathway of organism. However, comparing with free cells batch, the glucose utilized were 26 g/l and 35 g/l for immobilized and free mobilized cell batch, respectively with an initial glucose concentration of 60 g/l. Thus glucose remaining for both batches were 34 g/l and 25 g/l for immobilized and free mobilized cell batch, respectively. Glucose profile of immobilized cells fermentation on activated carbon shows the lower consumption than free cells did. This result effects to the acids and solvents production that will be explained in next part.

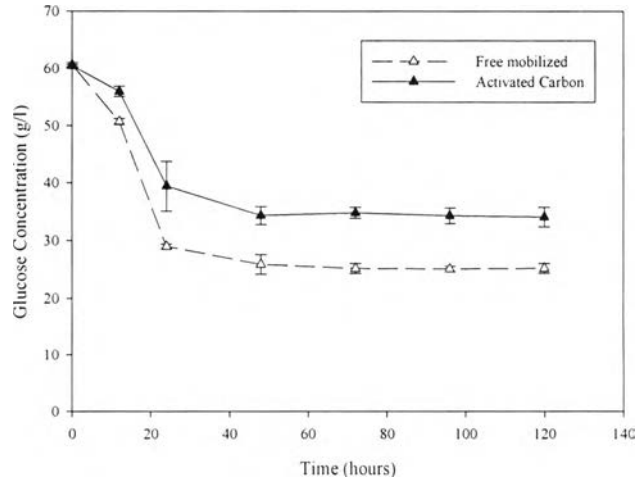

Figure 4.16 Glucose profile of immobilized cells fermentation on activated carbon compared with free cell system.

Figure 4.17 and 4.18 show acids and solvents production profile for immobilized cells fermentation on activated carbon. The acids and solvents production profile could be demonstrated the organism metabolic phase which had been explained for previous results. Before 12^{th} hours the maximum acids production were 1.4 g/l and 0.45 g/l for acetate and butyrate, respectively. This acid production results shows the lower acid production compared with free cells batch. The maximum butanol production was 2.93 g/l, which is 45 percentages lower than free mobilized cell fermentation. For maximum acetone and ethanol production were 3.22 g/l and 0.46 g/l, respectively which are also lower than free cells fermentation batch. By comparing with free cells fermentation, the immobilized cell fermentation on activated carbon show the lower concentration for both acids and solvents concentration. These results might be explained by the effect of medium pH. The proper pH control is important for the fermentation to shift to solventogenic stage and increase yield of butanol (Jones and Woods, 1986). Normally, the optimum initial pH of medium should be adjusted in the range of 6.5-6.8 which be optimized the growth rates of *Clostridium* genus (Tashiro *et al.*, 2004; Schmidt and Weuster-Botz, 2012; Kumar *et al.*, 2013). However, in this fermentation the initial pH of medium was affected by acidity of activated carbon to about 5.6 that is not the optimal condition for cell growth. Thus, this inappropriate initial pH might be affected to poor of cell growth in this batch. This is reasonable to low glucose consumption and acids production results because of poor cell growth from inappropriate initial pH. Moreover, after 24^{th} hours in the stationary phase the pH of medium was decreased to about 4.5 and keep continuing in this pH value. It indicated that the pH less than 4.5, leading to the cell death because of the diffusion and dissociation of undissociate acid into the cytoplasm of cell (Dürre, 2008). This might be the reason for low solvents production in this batch because of cells death during the stationary phase from the effect of low pH medium. This low solvents production results agree with the previous studies (Tashiro *et al.*, 2004; Wang and Blaschek, 2011) which indicated the low ABE production with pH of 4.5 condition because of solventogenesis terminatation from insufficient acids production by poor culture growth.

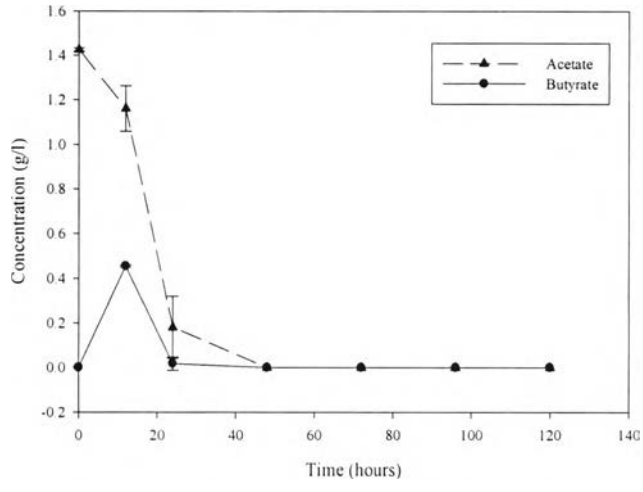

Figure 4.17 Acids production of immobilized cells fermentation on activated carbon.

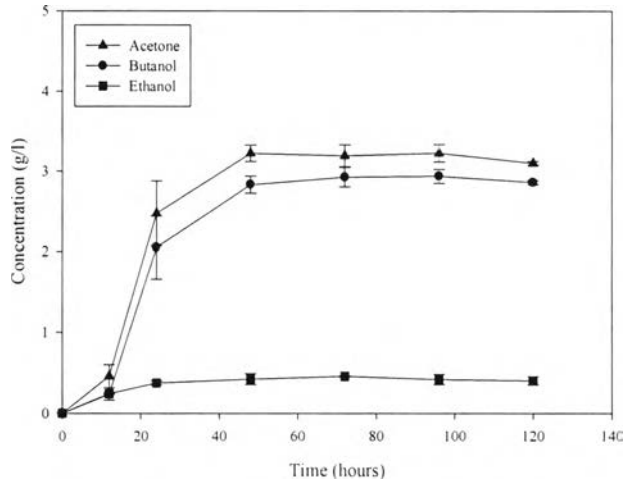

Figure 4.18 Solvents production of immobilized cells fermentation on activated carbon.

4.2.4 Immobilized Cells Fermentation on Zeolite

The pH profile during immobilized cells fermentation on zeolite is shown in Figure 4.19. The initial pH value of medium was 6.5. The pH was significantly dropped at 12^{th} hours and kept constant after 24^{th} hours. That would be indicated the acidogenesis and solventogenesis of microorganism phase before and

after 24th hours (Rao and Mutharasan, 1987). The pH at stationary phase was dropped to about 5.5, which was higher than free cells batch fermentation. The reason for low pH drop might be the buffering capacity of zeolite 13X that could control pH dropped during fermentation (Djukić-Vuković *et al.*, 2013). This result is corresponding to the initial pH of treated zeolite in Table 4.5 which was about 7.06. The increase in the initial pH of treated zeolite may be due to the basicity of zeolite, which has higher basicity than brick and activated carbon.

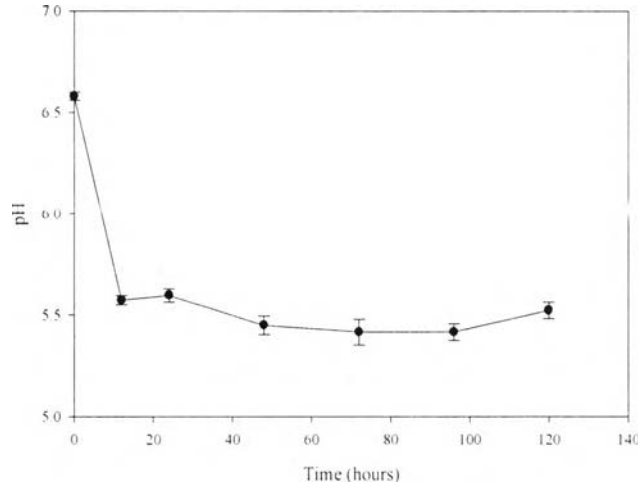

Figure 4.19 pH profile of immobilized cells fermentation on zeolite.

Figure 4.20 shows the glucose profile during fermentation with cell immobilization by zeolite compared with free mobilized cell fermentation. At the beginning of the fermentation, 60 g/l total glucose was presented in the medium for both batches. The glucose concentration was dramatically decreased after 24 hours of fermentation. This result agrees with acidogenesis which the microorganism consume glucose as a carbon source for convert to acids; therefore, the glucose consumption was decreased in the stationary phase (Kumar *et al.*, 2013). The immobilized cells fermentation showed almost complete glucose utilized with 54 g/l compared with 37 g/l of free cell batch. The final glucose concentrations were about 6 g/l and 23 g/l for immobilized cell and free mobilized cell, respectively. There could be demonstrated that immobilized cell enhanced the activities of cells and

increased the butanol tolerance (Chen *et al.*, 2013) hence contributing to higher glucose consumption and utilization efficiency.

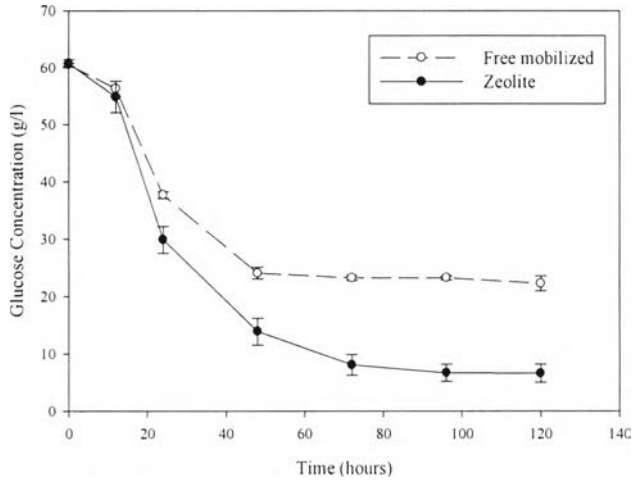

Figure 4.20 Glucose profile of immobilized cells fermentation on zeolite compared with free cell system .

Figure 4.21 and 4.22 show the acids and solvents production profiles of immobilized cells fermentation on zeolite. The maximum acids production were 2.03 g/l and 1.06 g/l for butyrate and acetate, which were higher than free cells batch. For acids production profile, before 12^{th} hours, the high concentration of acetate and butyrate was obtained this could be indicated the acidogenesis. The decreasing of acids were found between 12^{th} and 24^{th} hours, this result could be demonstrated that the cells shifted to solventogenesis from the acidogenesis (Yen and Li, 2011). However, after 24^{th} hours, the increasing of acids production were observed and the concentrations of acids were constant after 96^{th} hours. This might be demonstrated the cells were in both solventogenesis (stationary) phase and acidogenesis (exponential phase) of cell growth. These results were reasonable to the previous study (Kumar *et al.*, 2013) that it is possible that in the stationary phase of cells growth some cells might be in the exponential phase. The reason for cell express two phases is the solvent production do not restricted to a specific growth phase.

For the solvents (ABE) production, the solvents concentrations were significantly increased after 12^{th} hours and continued increasing until 72^{th} hours. The

solvents production was slightly constant after 72th hours. In this batch, 8.58 g/l butanol with immobilized cells was 62 percentages higher than 5.29 g/l butanol from free cells mobilized. The acetone and ethanol concentrations from immobilized cells on zeolite were higher than free cells which were 5.48 g/l and 0.57 g/l from immobilized cells and 4.08 g/l and 0.47 g/l from free cells. The increasing of solvents production could be explained by the increasing of cell density in the reactor from immobilization technique (Yen and Li, 2011), the immobilized cells could enhance cellular activities and improve butanol resistance of growing cells (Liu *et al.*, 2013).

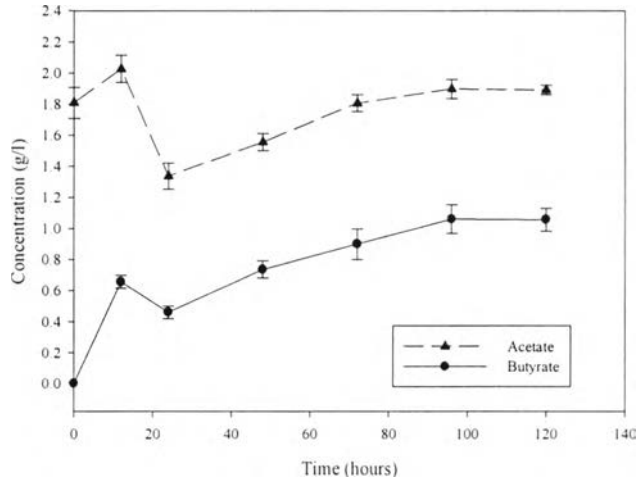

Figure 4.21 Acids production of immobilized cells fermentation on zeolite.

Moreover, the butanol production from immobilized cells on zeolite showed a significant number higher than brick did. This might be explained by the good properties of zeolite which did not express in brick. Zeolite 13X could increase the adsorptive binding forces between bacterial cells and zeolite surface from electrostatic interaction with a strong bonding. The X type zeolite has basic structure with sodium ion exchange that been buffering capacity which could control the pH during fermentation (Djukić-Vuković *et al.*, 2013) to enhance the ABE production. This is reasonable to pH profile and initial pH as shown in Figure 4.19 and Table 4.5. Nevertheless, the surface area analysis (Table 4.4) showed the higher surface area and pore volume of zeolite than brick. There could be indicated that zeolite had higher surface area for cell adsorption which is one of properties need for cell carrier

(Hrenovic *et al.*, 2011). Thus from all of these reasons, the immobilized cells fermentation on zeolite could significantly improve for ABE fermentation, as shown in these results.

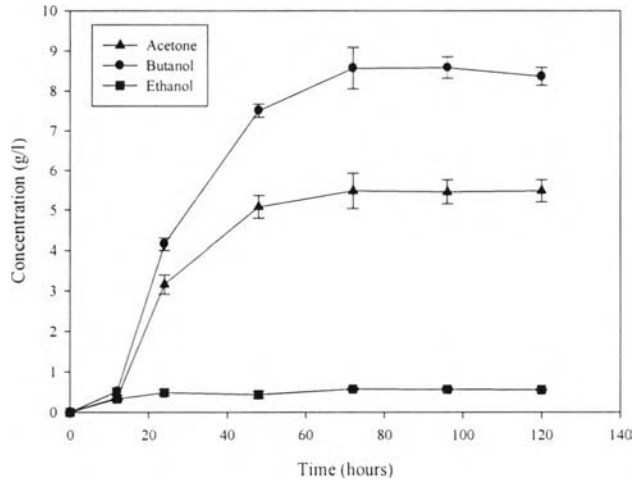

Figure 4.22 Solvents production of immobilized cells fermentation on zeolite.

4.2.5 Comparison of ABE Production with Different Ferment Operation

Table 4.6 shows the comparison of solvents production between free mobilized cells fermentation and immobilized cells fermentation on different materials.

Table 4.6 ABE production from different fermentation operations

Opration	Acetone (g/l)	Butanol (g/l)	Ethanol (g/l)	Total ABE (g/l)	Butanol yield* (g/g)	Solvents yield** (g/g)
Free cell	4.08	5.29	0.47	9.85	0.14	0.26
Brick	4.42	5.80	0.49	10.71	0.16	0.29
Activated carbon	3.22	2.93	0.46	6.61	0.11	0.25
Zeolite	5.48	8.58	0.57	14.63	0.16	0.27

* Butanol yield was based on butanol concentration per utilized glucose.

** Solvents yield was based on total solvents (ABE) produced per utilized glucose.

For cell immobilization on brick and zeolite showed the enhancement of all solvents production (acetone, butanol, and ethanol) especially butanol. For free mobilized cells fermentation, the highest butanol production was 5.29 g/l (yield 0.14) and for immobilized cell fermentation on brick and zeolite were 5.80 g/l (yield 0.16) and 8.58 g/l (yield 0.16), respectively. Therefore, the butanol production with immobilized cells on brick and zeolite were about 9.5 percentages and 62 percentages higher than free cells batch, respectively. Total solvents production was 10.71 g/l (yield 0.29) and 14.63 g/l (yield 0.27) for brick and zeolite batch which were also higher than 9.85 g/l (yield 0.26) from free cells batch. However, butanol production from immobilized cell fermentation on the activated carbon showed 45 percentages lower than free mobilized cells fermentation with 2.93 g/l (yield 0.11). The total solvents production was 6.61 g/l (yield 0.25) which was also lower than that of free cells batch. The reasons for solvents improvement and diminishment of each batch had already described in the previous results.

4.2.6 Repeated Batch Fermentation with Immobilized *Clostridium beijerinckii* on Zeolite

The cells immobilization on zeolite fermentation was chosen for further study in the repeated batch fermentation because of the high solvent production compared with other materials. The repeated batch fermentations were perform to evaluate the stability and long term performance of immobilized *Clostridium beijerinckii* on zeolite. This study investigated the seven reuse cycles of cell immobilization effect on ABE production with the initial glucose concentration of 60 g/l for each cycle. Butanol concentration from all 8 batches varied from 7.81 g/l to 5.55 g/l with an average concentration of 6.47 g/l. For acetone and ethanol production were varied from 3.90 g/l to 3.07 g/l and 0.55 g/l to 0.48 g/l with an average concentration of 3.34 g/l and 0.51 g/l, respectively. The results indicated that the immobilized cells on zeolite fermentation had high cell efficiency and maintained after seven sequential reuse cycles. There is almost no dramatically drop in acetone, butanol, and ethanol was observed during 384 hours of fermentation (48 hours for each cycle). The results for long term fermentation capability could be demonstrated by the enhancement of cells tolerance for butanol toxicity by the cell immobilization

technique (Yen and Li, 2011; Chen *et al.*, 2013) that the stable solvents production was observed with 7 repeat fermentation cycles.

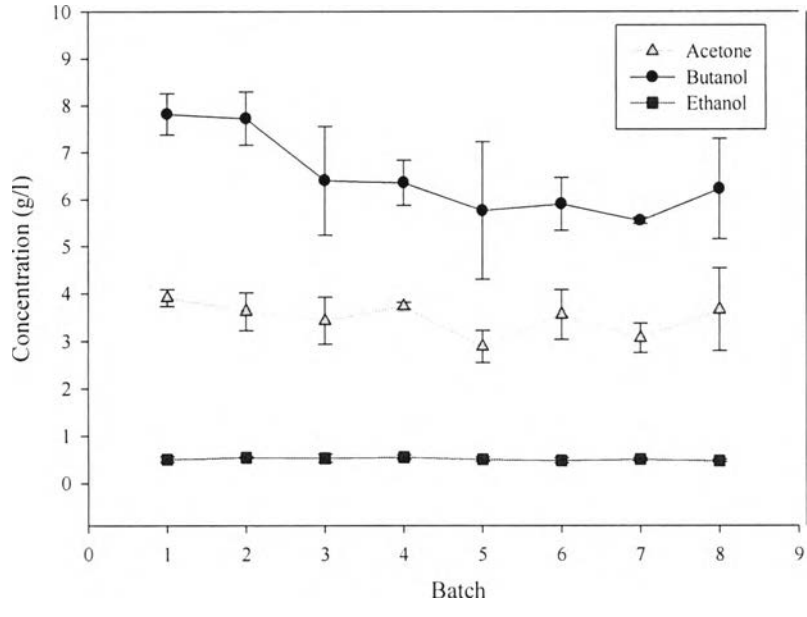

Figure 4.23 The repeated batch fermentation with immobilized cells on zeolite.

4.3 Economic discussion

From the repeat batch fermentation result shows that not only the immobilize cell fermentation on zeolite has high butanol production compare with brick and activated carbon but it also has the capacity to long term fermentation with stable cell efficiency production with seven recycles process.

In this research the cell immobilization on brick and zeolite could enhance butanol production. Brick for this research derived from construction site which has many advantages for example easily to applied for cell immobilization, readily available, and inexpensive material (Yen and Li, 2011). To compare price of material, brick construction grade has the lowest cost compare with commercial activated carbon and zeolite from Sigma Aldrich which are US$92.20/kg and US$113.50 /kg for activated carbon and zeolite, respectively (Sigma-Aldrich, 2012). Although zeolite has the higher cost than brick but the percentage of butanol

production improvement from the immobilized cells fermentation on zeolite was about 6 times higher than immobilized cells fermentation on brick (9.5 for brick and 62 for zeolite) that is significantly enhanced.

Butanol could be used for biofueles with high energy content per volume compare with ethanol (Pfromm *et al.*, 2010). Not only butanol could be used for biofuels but butanol is also an essential feedstock for the production of paint, solvents and plasticizers in the chemical industry (Carvalho *et al.*, 2012). The butanol price is US$1.03/kg for fuel n-butanol and US$1.65/kg for chemical n-butanol (Pereira *et al.*). Byproducts acetone and ethanol from ABE fermentation are also the valuable products (US$0.66/l for anhydrous ethanol and 0.91 US$/l) and could be used as chemical in industry (Pereira *et al.*). Nowadays, most of n-butanol is produced from oil-based sources with petrochemical route due to butanol from ABE fermentation still not economically competitive because of low butanol yield productivity (Xue *et al.*, 2013). The cell immobilization is the technique that could enhance ABE fermentation (Qureshi *et al.*, 2000). From previous study (Qureshi and Blaschek, 2001), to compare four different processes for butanol production; batch fermentation and distillative recovery, batch fermentation and pervaporative recovery, fed-batch fermentation and pervaporative recovery, and immobilized cell continuous fermentation and pervaporative recovery were evaluated for economic. The immobilized cell fermentation process showed the lowest butanol production cost and most cost effective technology for producing butanol. For this technique, butanol can be produce at US$ 0.11-0.36/kg, depending on byproduct credit which is the lowest cost range in compare with other processes. Therefore, the immobilized cells fermentation on zeolite 13X might be one of the attractive alternative choices that could be worth for butanol production investment. And it should be consider for investigate optimal condition to enhanced and developed butanol production with sustainable process in the future.