# CHAPTER IV

# RESULTS AND DISCUSSION

This research has the interest of using agricultural materials as immobilization carriers to immobilize cells and comparing with suspended cell culture in batch fermentation. In the construction of the immobilization carriers, agricultural residues and chitosan were selected based on their high potential as cell carriers. All materials chosen for the construction of these carriers are biodegradable because of their natural origin. By doing so, environmental concern related to the use of these materials can be eliminated. The organic nature of agricultural residues are expected to have positive effects on cell attachment to the supports as organic materials are suggested to possess rich functional groups suitable for cell adsorption [11]. Much saving in term of work and cost are obtainable if the immobilized cell carriers can be used repeatedly. To evaluate this possibility, reusability of the carriers was evaluated by using repeated batch operation. Agricultural residues used in this study were loofa sponge, bagasse, tamarind fruit fibre, and coconut fibre. They were selected because of the ease and large supply of the materials. Their utilization thus adds value to them.

This study attempts to develop the immobilization carriers for cell adsorption by combining the natural fibre and chitosan, the most abundant biopolymer in the world. These two abundant polymers are used in L(+)lactic acid fermentation. L(+)lactic acid productivity and yield of the system are considered as the key values to be evaluated.

The experiments in this research were divided into 3 parts. Firstly, lactic acid fermentation using *L.salivarius* and kinetic parameters were studied. The effects of $CaCO_3$ percentages and initial glucose concentrations on lactic acid production were examined. Next, the immobilization materials were evaluated for the possibility to use as immobilization carriers. Finally, cells were immobilized on agricultural materials and repeated batch fermentation by using these immobilized cells were performed.

## 4.1 Lactic acid fermentation by *L. salivarius*

### 4.1.1 Growth curve of *L. salivarius*

The experiment was set up in batch fermentation process by using 10 g $L^{-1}$ meat extract, 5 g $L^{-1}$ yeast extract, 10 g $L^{-1}$ peptone, 50 g $L^{-1}$ D-glucose, 1 g $L^{-1}$ Tween 80, 2 g $L^{-1}$ $K_2HPO_4$, 0.2 g $L^{-1}$ $MgSO_4 \cdot 7H_2O$ and 0.2 g $L^{-1}$ $MnSO_4 \cdot H_2O$ as fermentation medium. It was carried out in batch fermentor with stirring speed of 100 rpm, pH controlled at 5.5 by using 4.0 N NaOH to adjust pH, and the temperature of 37°C.

It can be seen from Figure 4.1 that the increase of cell and lactic acid increased concomitantly. Therefore, the product formation was considered as growth-associated. The lag period was 2 hours and the logarithmic phase was during the hour of 2 to 8. There was no stationary phase. Cell decreased immediately after reaching the maximum cell concentration at the hour of 8. This might be caused by cell lysis as according to Anatkatat [44]. Focusing on the lactic acid production, lactic acid formation started at the same time as the beginning of logarithmic phase. It then increased continuously until the end of fermentation at 24 hours. Glucose consumption started at the hour 2 and then depleted until running out at the hour of 24, fermentation ended.

The kinetic parameters of this growth curve was calculated and compared with the previous work of Anatkatat [44] as in the following table.

Table 4.1 Kinetic parameters of lactic production by *L. salivarius*

| Kinetic Parameters | Anatkatat [44] Glucose 70 g $L^{-1}$ | This research Glucose 50 g $L^{-1}$ |
|---|---|---|
| Xmax, g $L^{-1}$ | 3.02 | 3.02 |
| Pmax, g $L^{-1}$ | 60.79 | 46 |
| $\mu_{max}$, $h^{-1}$ | 0.39 | 0.62 |
| Maximum Yx/s | 0.17 | 0.15 |
| Maximum Yp/s | 1.73 | 1.12 |
| Maximum productivity, g $L^{-1}$ $h^{-1}$ | 1.64 | 2.86 |
| Maximum Qp | 8.31 | 1.83 |
| Maximum Qs | 5.96 | 2.08 |

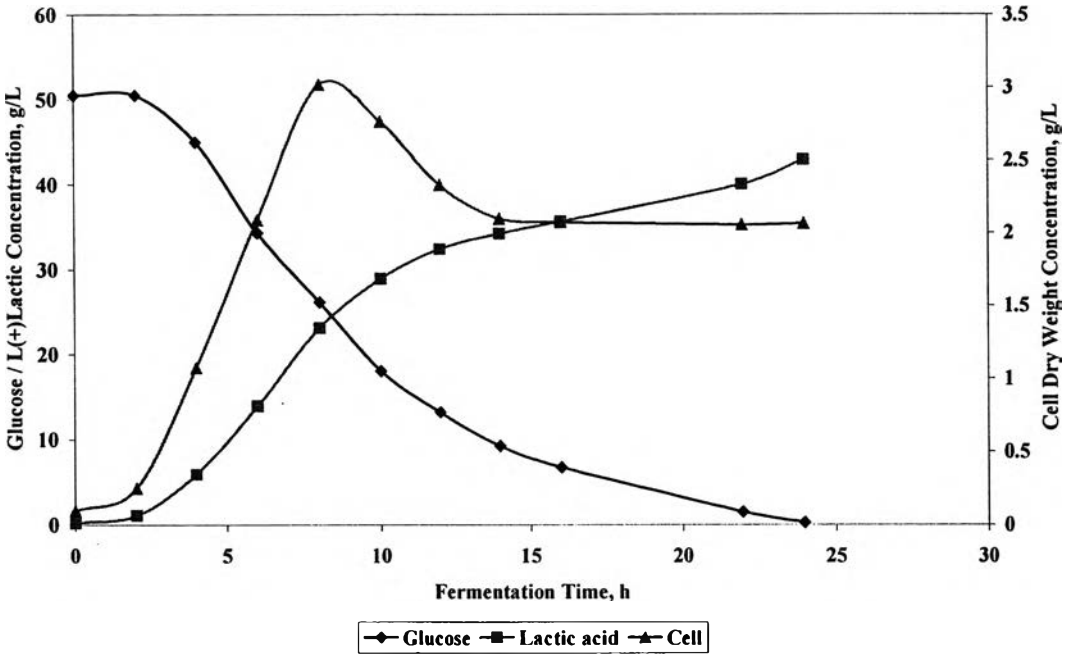



Figure 4.1 Growth curve of *Lactobacillus salivarius* ATCC 11741 at initial glucose concentration 50 g $L^{-1}$

4.1.2 Effect of $CaCO_3$ percentages on lactic acid production

Generally, NaOH, a strong base, was used as pH adjusting agent. It was necessary to shake the fermentation broth to evenly distribute the base in order to eliminate the pH gradient in the system. In the case of immobilization, rather vigorous shaking may reduce the amount of cells adhered on immobilized material surface. Therefore, $CaCO_3$ which was a slow reaction pH adjusting agent was selected to use in this experiment. The experiments were conducted in shaking flask operations at 100 rpm rotating speed and temperature was controlled at 37°C. pH was controlled by using different percentage of $CaCO_3$ at 0, 2.5, and 5.0% with the intention of evaluating the suitable concentration for lactic acid production.

When adding $CaCO_3$ to the fermentation broth, the lactic acid concentrations were significantly higher than the system without $CaCO_3$. Comparing between different percentages of $CaCO_3$ systems, the 5% $CaCO_3$ produced higher lactic concentration than 2.5% $CaCO_3$. The glucose consumption of 5% $CaCO_3$ system

decreased slightly more rapid than that of the 2.5% $CaCO_3$ system. Consequently, 5% $CaCO_3$ was chosen to use in further experiments.

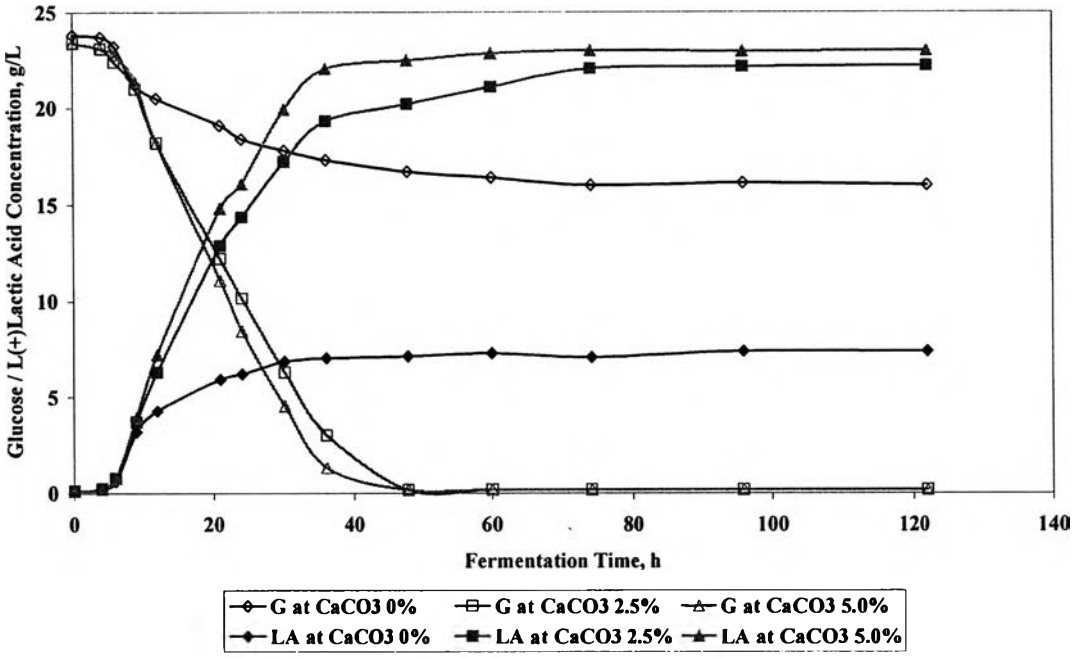



Figure 4.2 Glucose and L(+)lactic acid concentration profiles at various %$CaCO_3$.

4.1.3 Effect of initial glucose concentration on lactic acid production

To evaluate the optimum initial glucose concentration for $CaCO_3$ system, the experiments were conducted under the conditions of shaking flask at 100 rpm rotating speed, 37°C, and 5% $CaCO_3$. The glucose concentrations used were varied from 20 to 100 g $L^{-1}$. The controlled experiment was done at initial glucose concentration 20 g $L^{-1}$ without $CaCO_3$.

At initial glucose concentration 20 g $L^{-1}$ without $CaCO_3$, the lactic acid concentration was the lowest. When 5% $CaCO_3$ was introduced to the system, the lactic acid production was higher until the hour 16 and then leveled off because of substrate depletion. Considering on the $CaCO_3$ system, Figure 4.3 indicated that the lactic acid concentration increased with increases in the initial glucose concentration up to 50 g $L^{-1}$ and then decreased with further increases in the initial glucose concentration (at 70, 90, and 100 g $L^{-1}$). This decrease in the lactic acid concentration was due to the substrate inhibition phenomena. Higher substrate concentrations may have increased the osmotic pressure, which in turn affected the cell growth by either

removal of water from the microbial cells or restricting the normal diffusion process of water into these cells [59]. Hence, the initial glucose concentration of 50 g $L^{-1}$ was selected as the optimal condition for further experiments.

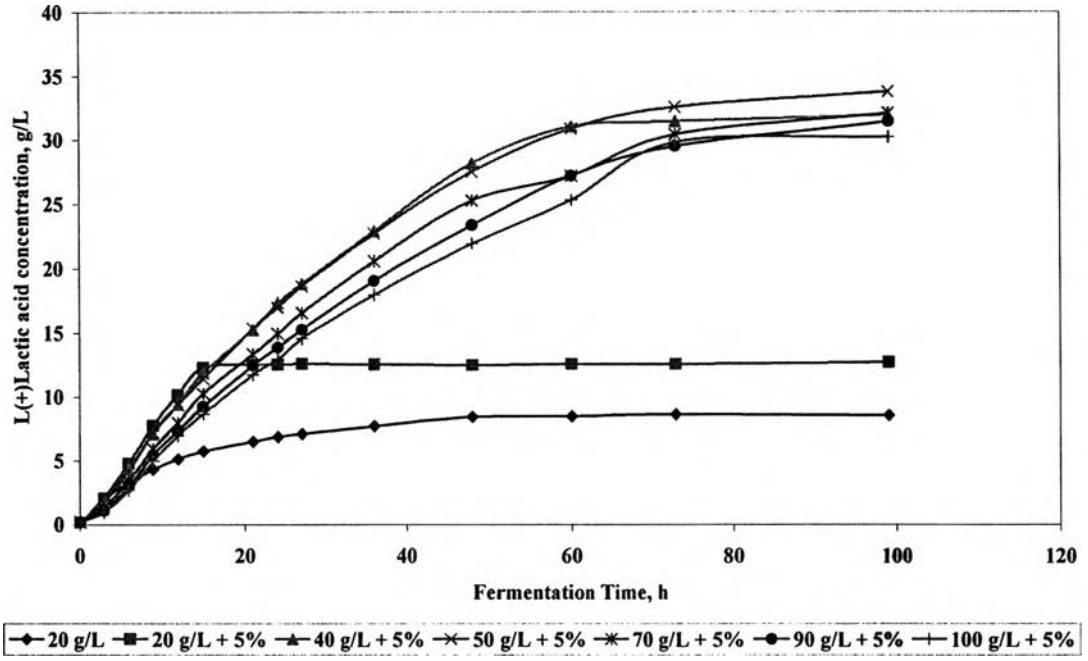



Figure 4.3 Effect of initial glucose concentration on L(+)lactic acid production

## 4.2 Immobilization materials

According that agricultural material has many environmental benefits, several materials have been selected to evaluate the possibility to use as an immobilization material in lactic acid fermentation. In this research, loofa sponge, sugarcane bagasse, tamarind fruit fibre, and coconut fibre were examined.

Loofa (*Luffa cylindrical*) grows well and is abundant in Thailand. It can provide loofa sponge which is natural, biodegradable and has fibrous network. The sponge has high porosity, high specific pore volume and high stability for long term use. Its interconnecting void and open network allow rapid contact of immobilized cells with medium. Loofa sponge was used for immobilization of different kinds of cells; bacteria producing clavulanic acid [31], sorbitol [26], yeasts producing ethanol [24, 25, 33 – 35], fungus producing giberellic acid [36], lactic acid [48] and plant cells

[60]. Loofa sponge can be used to immobilize flocculating cells very well [24, 25, 33 – 35]. However, non-flocculating cells can not well adhere to loofa sponge.

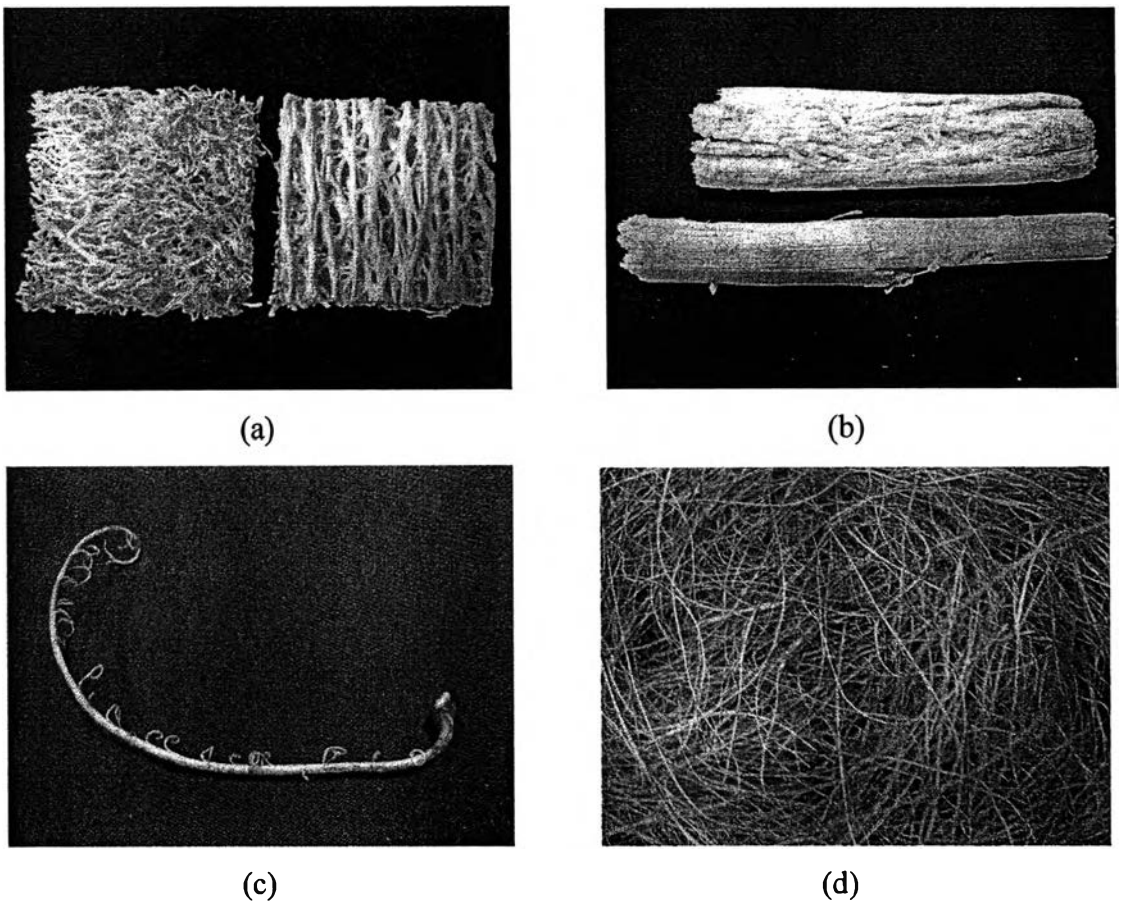
Sugarcane bagasse is an abundant agroindustrial byproduct. It is a fibrous lignocellulosic material generated by the sugar industry from cane stalks after juice extraction. A major part of this byproduct is used as an energy source in the sugarcane industry. However, there is an excess of bagasse and some alternatives for its use have been researched, including the production of alcohol and alkaloids, mushrooms, protein-enriched animal feed, and enzymes [37, 51, 52].

Tamarind (*Tamarindus indica L.*) grows in more than 50 countries of the world. The major areas of production are in Asian countries like India, Bangladesh, Sri Lanka, Thailand, and Indonesia, and in the African and the American continents. The pulp is thick and blackish-brown in colour. Tamarind pulp is surrounding the seed and lined with a tough parched like membrane, and joined to each other with tough fibers [61].

The coconut palm (*Cocos nucifera*) is a large palm which is grown throughout the tropical world, for decoration as well as for its many culinary and non-culinary uses; virtually every part of the coconut palm has some human uses. In Thailand the coconut husk is used as a potting medium because of its cost-effectiveness to produce healthy forest tree saplings [62]. The process of husk extraction from the coir bypasses the retting process [63].

### 4.2.1 Physical appearance of the materials

After thoroughly cleaned, boiled in deionized water and dried in hot air oven, fibres of the materials were demonstrated in the following figures to understand the structure of them.



(a) (b)

(c) (d)

Figure 4.4 (a) Loofa sponge: outer fibre (left) and inner fibre (right), (b) Sugarcane bagasse: inner fibre (top) and outer fibre (bottom), (c) Tamarind fruit fibre, and (d) Coconut fibre

The zeta potential of agricultural residues was determined and the data were shown in Table 4.2.

Table 4.2 Zeta potential of agricultural residues in medium

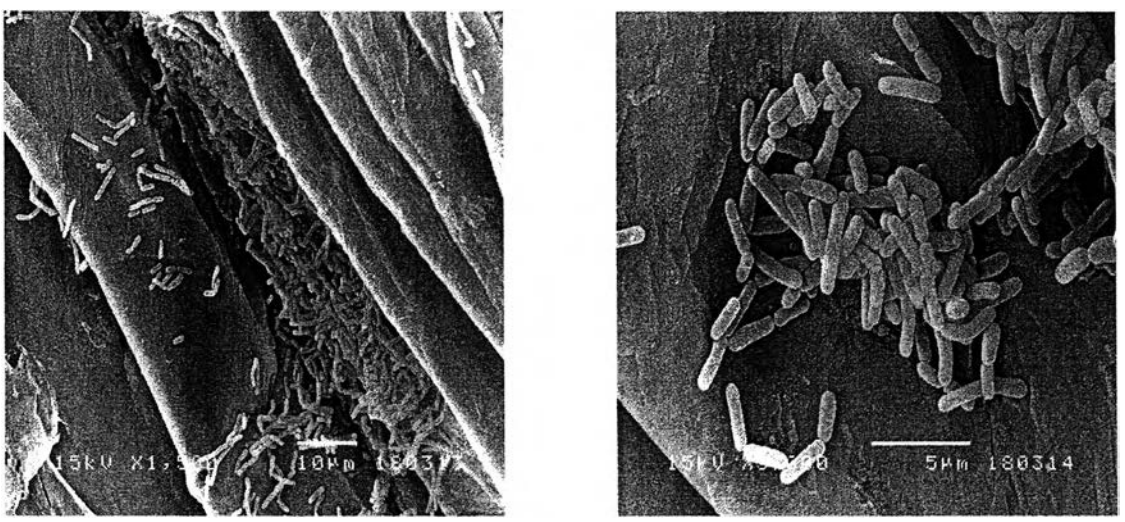
| Agricutural Residues | Zeta Potential, mV |
|---|---|
| Loofa Sponge | -8.57 |
| Sugarcane Bagasse | -3.85 |
| Tamarind fruit fibre | -6.36 |
| Coconut fibre | -8.46 |

For the preparation of the tamarind fibre, a large amount of the water and labour work were required in order to clean the fibre thoroughly. The overall process was time consuming and was not suitable for mass production. As a result, tamarind fruit fiber was not suitable to apply to industrial process. Therefore, it was not further studied in cell immobilization.

4.2.2 Preliminary study of cell immobilization on untreated fibre

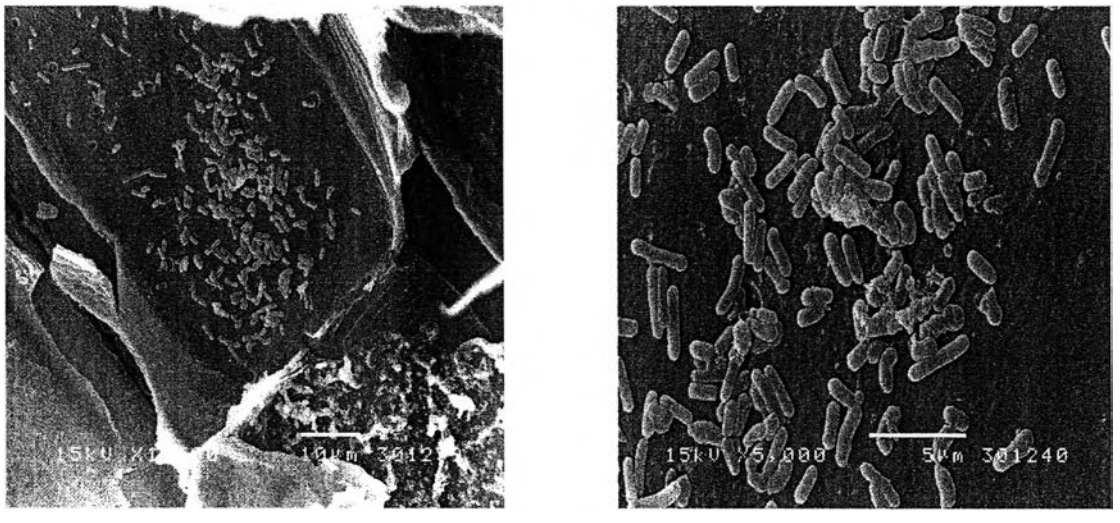
The experiments were conducted in shaking flask with initial glucose concentration at 50 g $L^{-1}$, 5% $CaCO_3$, 100 rpm, and 37°C.

SEM micrographs of the immobilized *L. salivarius* on loofa sponge, sugarcane bagasse, and coconut fibre for 24 hours were shown below. It can be observed that loofa sponge and sugarcane bagasse have potential to develop as immobilization carriers due to cell adhesion ability on fibre surface. This result also corresponded to the work of Santos et al. [29] which immobilized *Candida guilliermondii* in sugarcane bagasse in order to produce xylitol for xylose. On the contrary, there are only few cells attached on the coconut fibre. As a result, coconut fibre was considered ineffective to apply as cell immobilization carrier and no longer evaluated in further study.



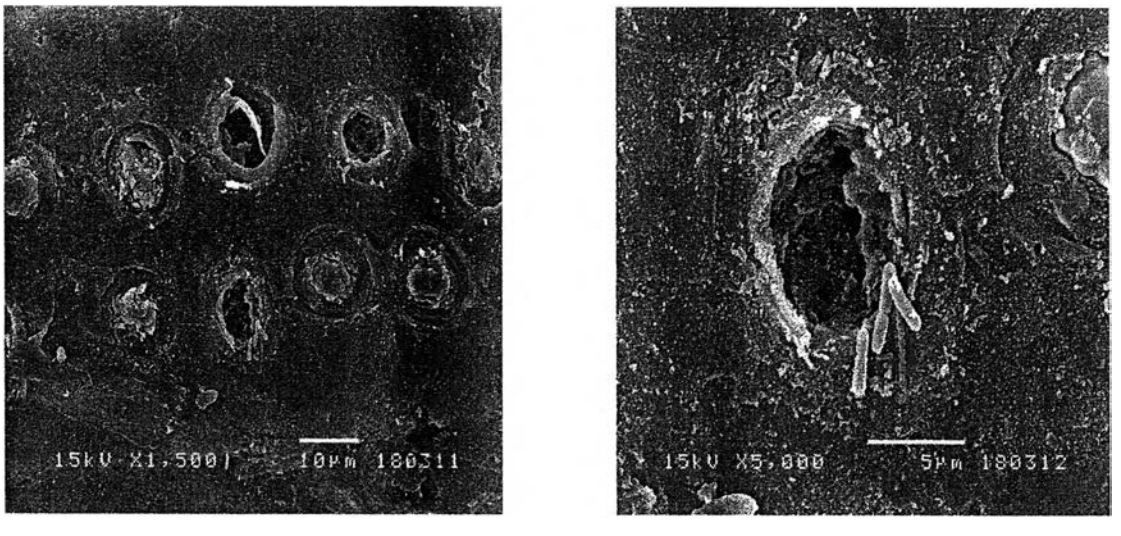
(a) (b)

Figure 4.5 Cell immobilization on untreated loofa sponge (a) x1,500 and (b) x5,000



(c) (d)

Figure 4.6 Cell immobilization on untreated sugarcane bagasse (a) x1,500 and (b) x5,000



(e) (f)

Figure 4.7 Cell immobilization on untreated coconut fibre (a) x1,500 and (b) x5,000

Consequently, the materials of interest will be loofa sponge and sugarcane bagasse for further study. Loofa sponge was first selected to evaluate the potential of being immobilization carrier.

4.2.3 $H_2O_2$ treatment of loofa sponge

From SEM images of cell immobilization on bare loofa sponge and sugarcane bagasse, it can be seen that there are cells adhere on the surface of the fibre. If the surface is rougher, in another word, higher surface area, it may improve the adhesion between cell and fibre surface when it is used as immobilization material. Therefore, treatment the bare fibre with $H_2O_2$ was introduced to the research. The duration of treatment, and percentage of hydrogen peroxide were studied parameters and the temperature of these operations was fixed at 60°C in order to maintain the loofa fibre in mat structure [27].

To determine the effect of hydrogen peroxide concentration, the treatment duration was fixed at 60 minutes [27]. From SEM micrograph in Figure 4.8 and 4.9, it was found that the surface of the treated loofa sponge was rougher and had more scratches than the untreated loofa sponge. This might be caused by the removal of waxy and gummy substance that laminated on the surface of the fibre by $H_2O_2$ [27]. Among the different percentages of hydrogen peroxide, the surface roughness was slightly different. For that reason, hydrogen peroxide at 0.25% was chosen for further studied on the duration of treatment, 20, 40, and 60 minutes.

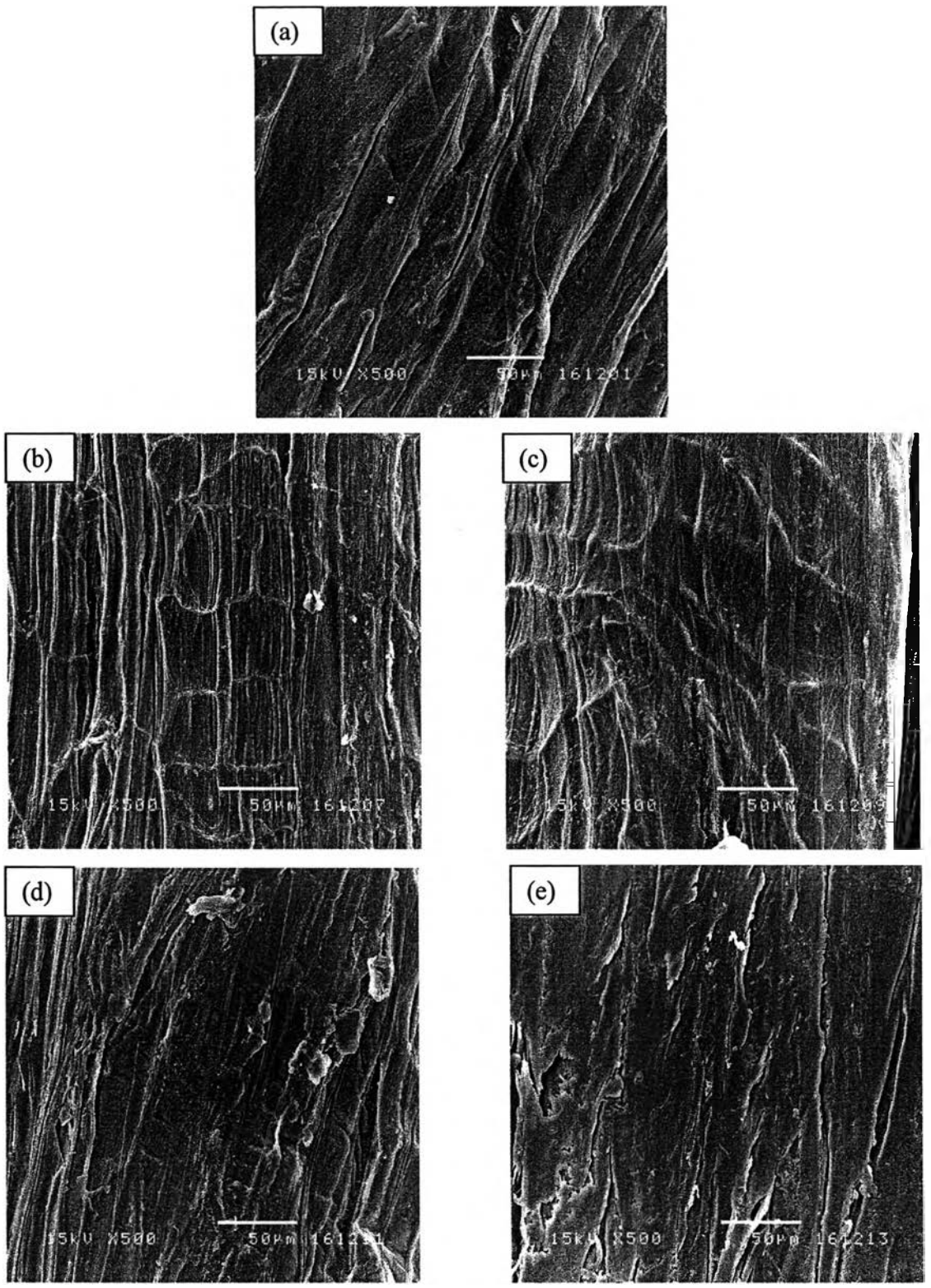



Figure 4.8 Inner loofa sponge fibre (a) untreated, (b) 0.25% $H_2O_2$, (c) 0.5% $H_2O_2$, (d) 1.0% $H_2O_2$, and (e) 2.5% $H_2O_2$

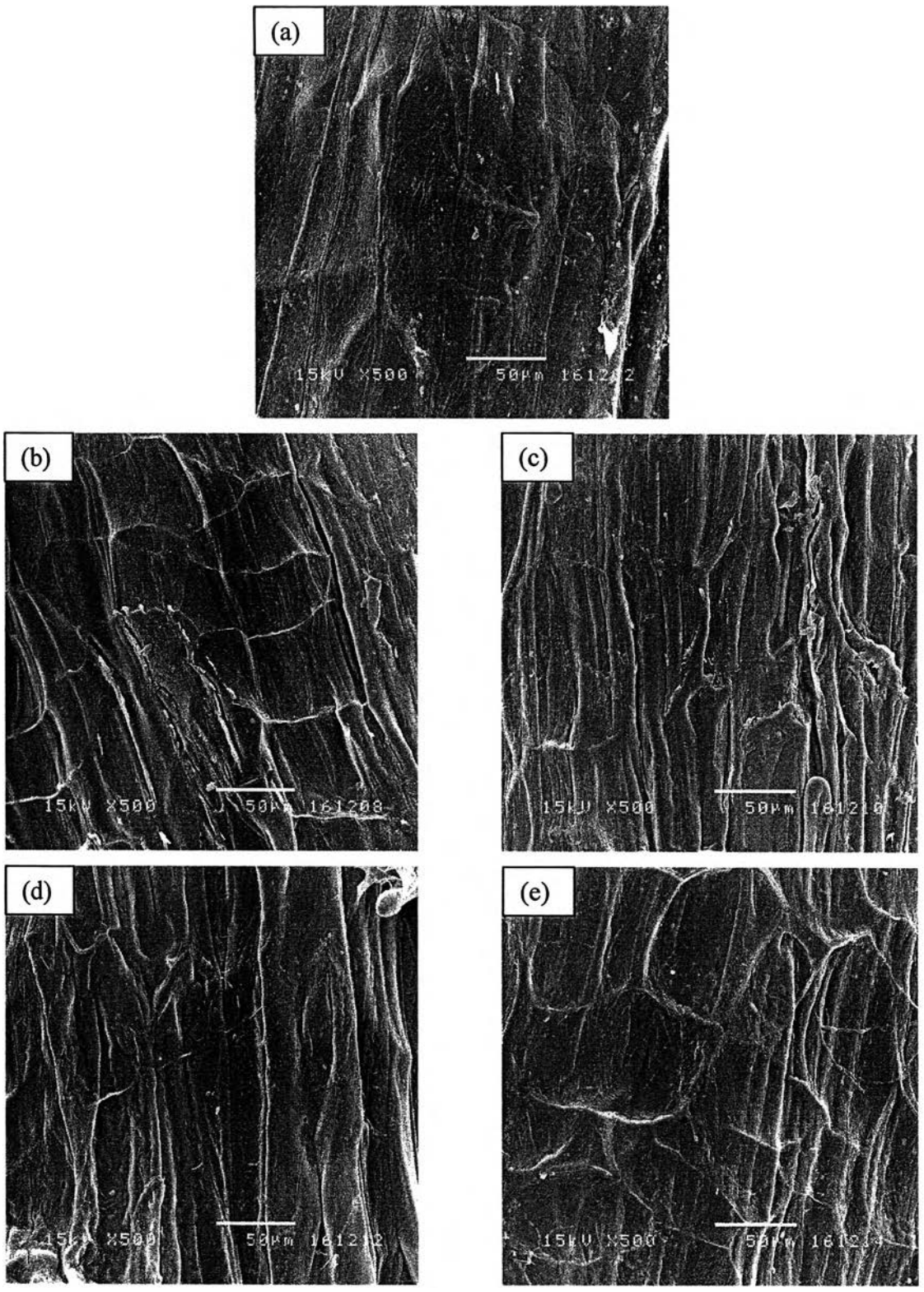



Figure 4.9 Outer loofa sponge fibre (a) untreated, (b) 0.25% $H_2O_2$, (c) 0.5% $H_2O_2$, (d) 1.0% $H_2O_2$, and (e) 2.5% $H_2O_2$

Closed look of the inner and outer surface of the untreated and loofa sponge treated by 0.25% $H_2O_2$ solution at 60°C for 20, 40, and 60 minutes were demonstrated in Figure 4.10 and Figure 4.11. It was found that surface of $H_2O_2$-treated loofa fibre at 60 minutes was the roughest. Consequently, treatment by hydrogen peroxide at 0.25% for 60 minutes at 60°C was applied as the $H_2O_2$-treated condition.

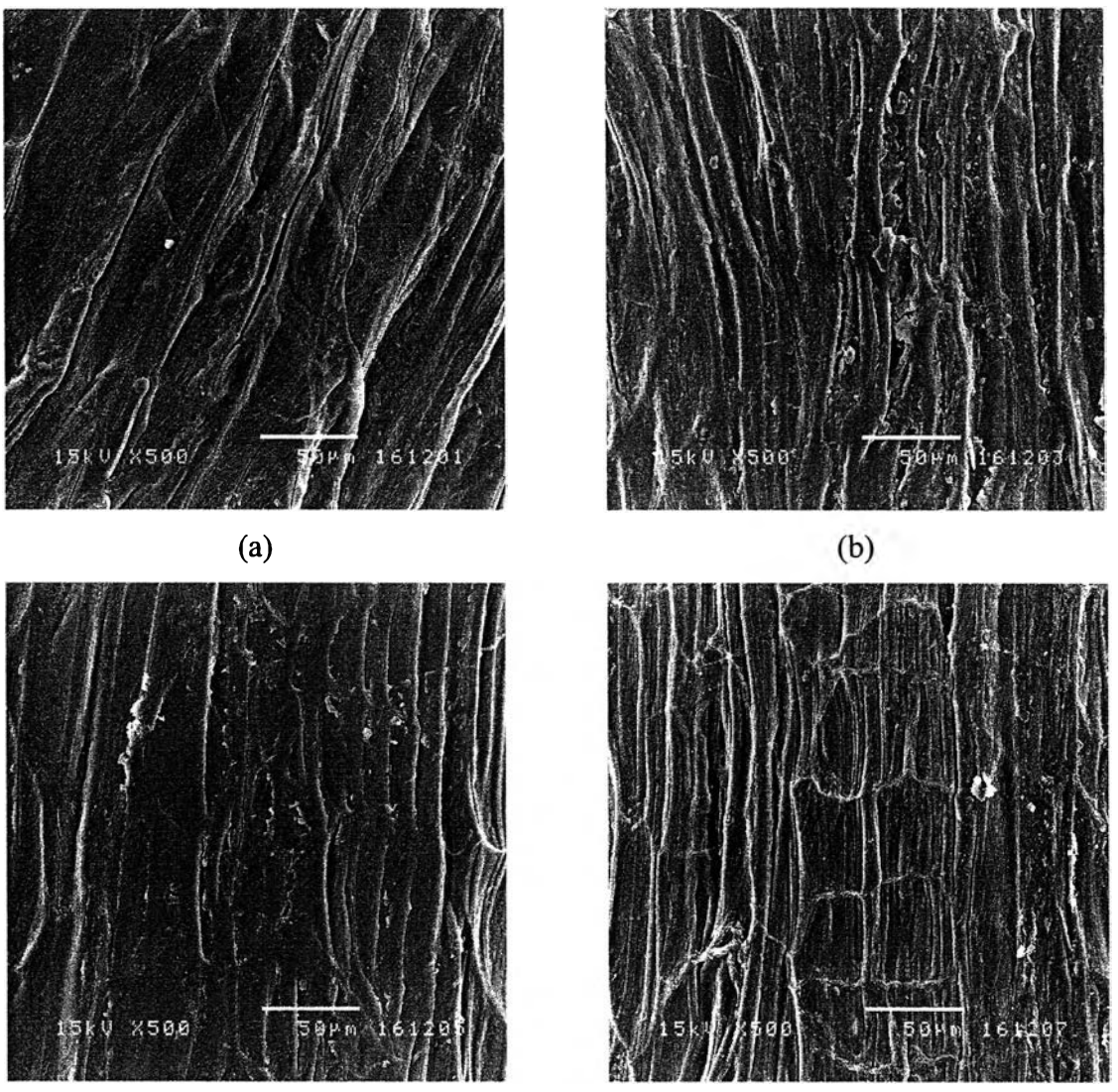
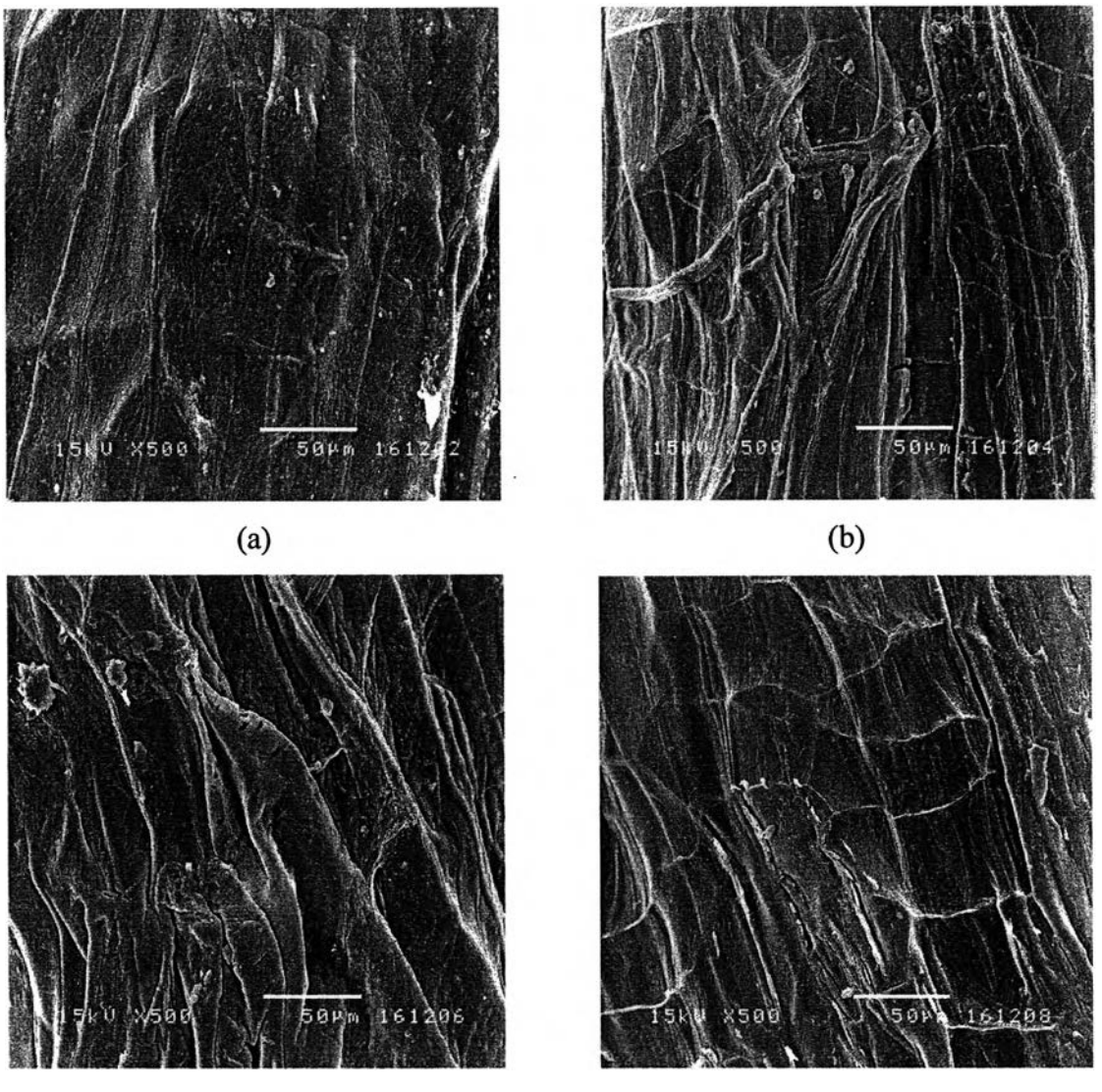



Figure 4.10 Inner loofa sponge fibre (a) untreated, (b) 0.25% $H_2O_2$-treated at 20 minutes, (c) 0.25% $H_2O_2$-treated at 40 minutes, and (d) 0.25% $H_2O_2$-treated at 60 minutes



(a) (b)

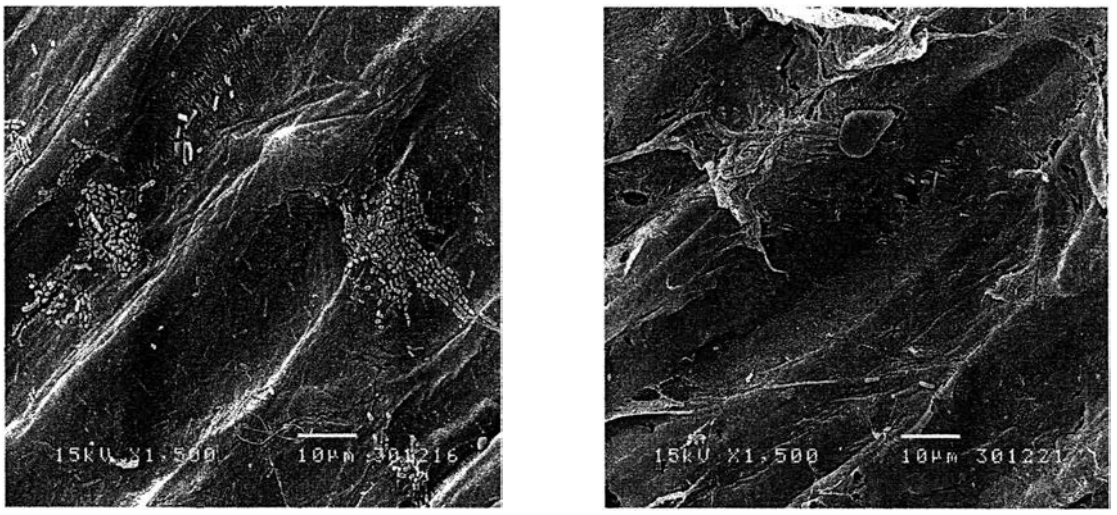
(c) (d)

Figure 4.11 Outer loofa sponge fibre (a) untreated, (b) 0.25% $H_2O_2$-treated at 20 minutes, (c) 0.25% $H_2O_2$-treated at 40 minutes, and (d) 0.25% $H_2O_2$-treated at 60 minutes

4.2.4 Cell immobilization on $H_2O_2$-treated loofa sponge

After treatment with hydrogen peroxide, the $H_2O_2$-treated loofa sponge was used as the immobilization carrier in lactic acid production. It can be reported that $H_2O_2$-treated loofa sponge was not successfully used in cell immobilization because there were only few cells adhered on the $H_2O_2$-treated loofa sponge surface as shown in Figure 4.12 and lactic acid production of the $H_2O_2$-treated loofa sponge fermentation was lower than the untreated loofa sponge about 27.45%. This may be

caused by chemical change of the treated surface. As a consequence, $H_2O_2$-treated fibre was not further used as an immobilization carrier.




(a) (b)

Figure 4.12 Immobilized cells on (a) untreated loofa sponge and (b) $H_2O_2$-treated loofa sponge

From the unsuccessful results of using $H_2O_2$-treated loofa sponge as an immobilization carrier, chitosan was introduced to modify the surface of loofa sponge and sugarcane bagasse to increase the potential of cell adsorption on the material surfaces.

## 4.3 Cell Immobilization

In order to improve cell immobilization on agricultural residues, chitosan was applied as a surface modifier of natural fibre. Because of its structure that composes of an amine group in every repeating unit resulting in the cation on the surface that can enhance the binding of cell on the fibre. Therefore, chitosan with high molecular weight should contain high level of amine groups that can be protonated with hydrogen ion in water into cationic surface [64]. Consequently, the chitosan molecule should have high power of static attraction and adsorption to anionic molecules, including cells. Characteristics of chitosan used in this experiment were tabulated in Table 4.3. The immobilized materials used in this experiment were untreated loofa

sponge, untreated sugarcane bagasse, $H_2O_2$-treated loofa sponge coated with various molecular weight chitosan and $H_2O_2$-treated sugarcane bagasse coated with various molecular weight chitosan.

In this experiment, lactic acid production after adsorption of cell on the fibre was investigated. Initial glucose concentration was about 50 g $L^{-1}$, a level which is considered ideal for maximized lactic acid production. The reusability of the biocatalyst in lactic acid fermentation was also determined in repeated batch mode after the main batch.

Table 4.3 Characteristics of chitosan used in this research.

| Molecular Weight, Da | DD, % | Viscosity*, cp | Zeta Potential, mV |
|---|---|---|---|
| 83,000 | 90 | 10.2 | 45.9 |
| 185,000 | 90 | 18.6 | 55.5 |
| 380,000 | 91 | 36.6 | 55.8 |
| 800,000 | 92 | 50.4 | 55.0 |

* Viscosity of 1% w/v chitosan in 2% v/v acetic acid.

4.3.1 Cell immobilization on loofa sponge

Five cultures were evaluated and labels of each culture investigated are tabulated in Table 4.4

Table 4.4 List of samples and labels for loofa sponge fermentation

| Immobilized carrier | Label |
|---|---|
| Untreated loofa sponge | L1 |
| $H_2O_2$-treated loofa sponge coated with chitosan MW 83,000 Da[a] | L2 |
| $H_2O_2$-treated loofa sponge coated with chitosan MW 185,000 Da[a] | L3 |
| $H_2O_2$-treated loofa sponge coated with chitosan MW 380,000 Da[a] | L4 |
| $H_2O_2$-treated loofa sponge coated with chitosan MW 800,000 Da[a] | L5 |

[a] 1% w/v chitosan in 2% v/v acetic acid

As shown in Figure 4.13, the shape of the glucose concentration profile was similar for all systems, indicating that the cell was behaving similarly in terms of

glucose consumption. It is suggested that there might be the same limiting factors in all systems so that by the end of every batch, the glucose concentration reached a similar level and the final glucose concentration of each cycle were not higher than 10 g $L^{-1}$.

Corresponding to glucose concentration curve, lactic acid concentration profile followed the opposite trend of the glucose consumption and produced L(+)lactic acid at the similar level between approximately 25 – 30 g $L^{-1}$ for every treatment from the main batch to repeated batch 5, as can be seen in Figure 4.14. At repeated batch 6 – 8, the lactic acid production was rapidly decreased until the lowest value of approximately 10 g $L^{-1}$.

From Figure 4.13 and 4.14, it can be noticed that lag phase occurred every other cycle beginning at the main batch.

Glucose consumption and lactic acid production of cell-immobilized loofa sponge in each treatment were insignificantly different. That meant the molecular weight of chitosan was not significantly affect on cell behaviours including substrate consumption and product formation.

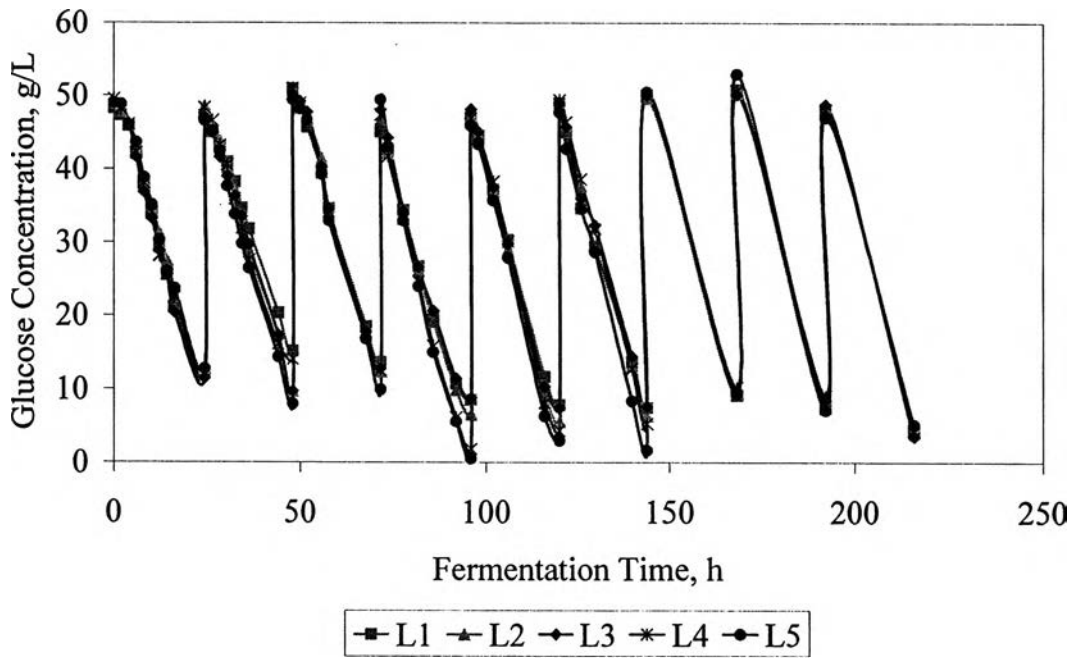



Figure 4.13 Glucose concentration profile of cell-immobilized loofa sponge for all cycles

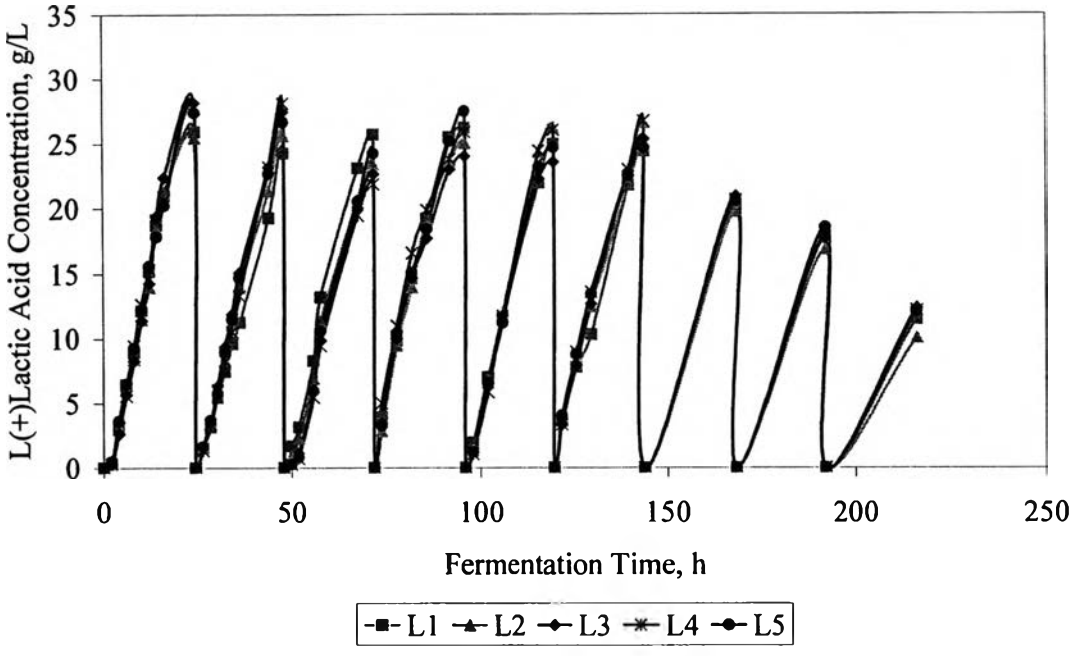



Figure 4.14 L(+)Lactic acid concentration profile of cell-immobilized loofa sponge for all cycles

From Figure 4.15, we can see that Yp/s from the main batch to the repeated batch 5 reduced gradually from approximately 0.85 to 0.55 g lactic acid/g glucose. After that in the repeated batch 6 to 8, the Yp/s dropped more rapidly from approximately 0.50 to 0.25 g lactic acid/g glucose. It might be due to the decreased efficiency of the immobilized cells during every passing cycle.

As shown in Figure 4.16, the productivity of the main batch to the repeated batch 5 was rather constant and had the values between approximately 0.90 to 1.20 g $L^{-1}$ $h^{-1}$. After that, it reduced more rapidly from repeated batch 6 to 8 at the lowest value of approximately 0.40 g $L^{-1}$ $h^{-1}$. The reason was the same as in product yield case.

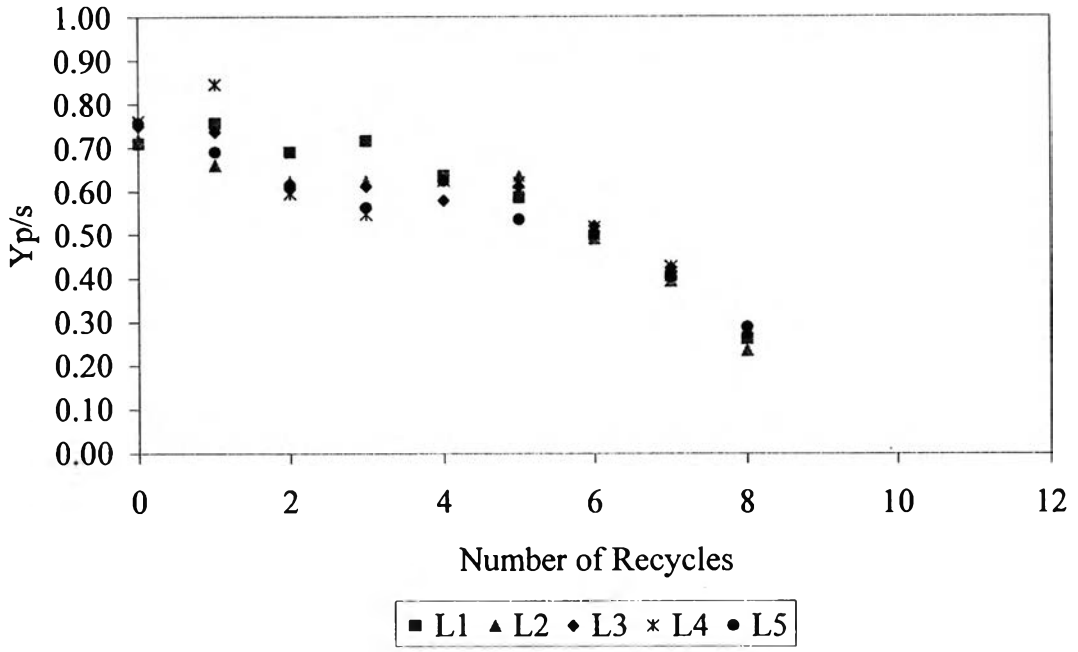



Figure 4.15 Total Yp/s of cell-immobilized loofa sponge for all cycles (1 cycle = 24 hours)

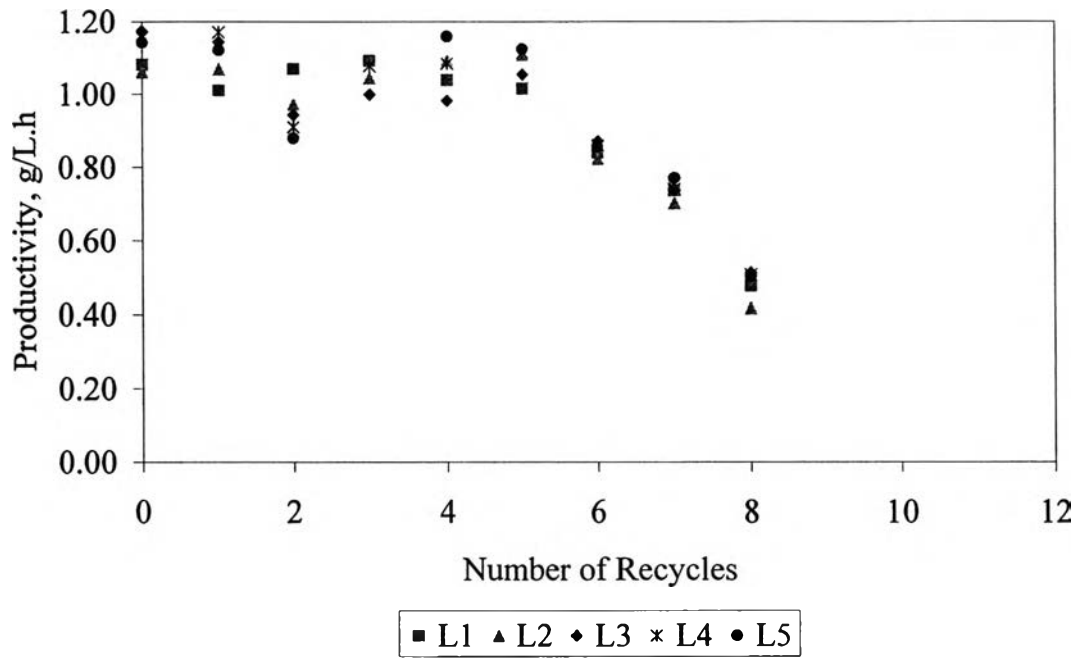



Figure 4.16 Productivity of cell-immobilized loofa sponge for all cycles (1 cycle = 24 hours)

SEM micrographs of cell-immobilized loofa sponge were shown below.

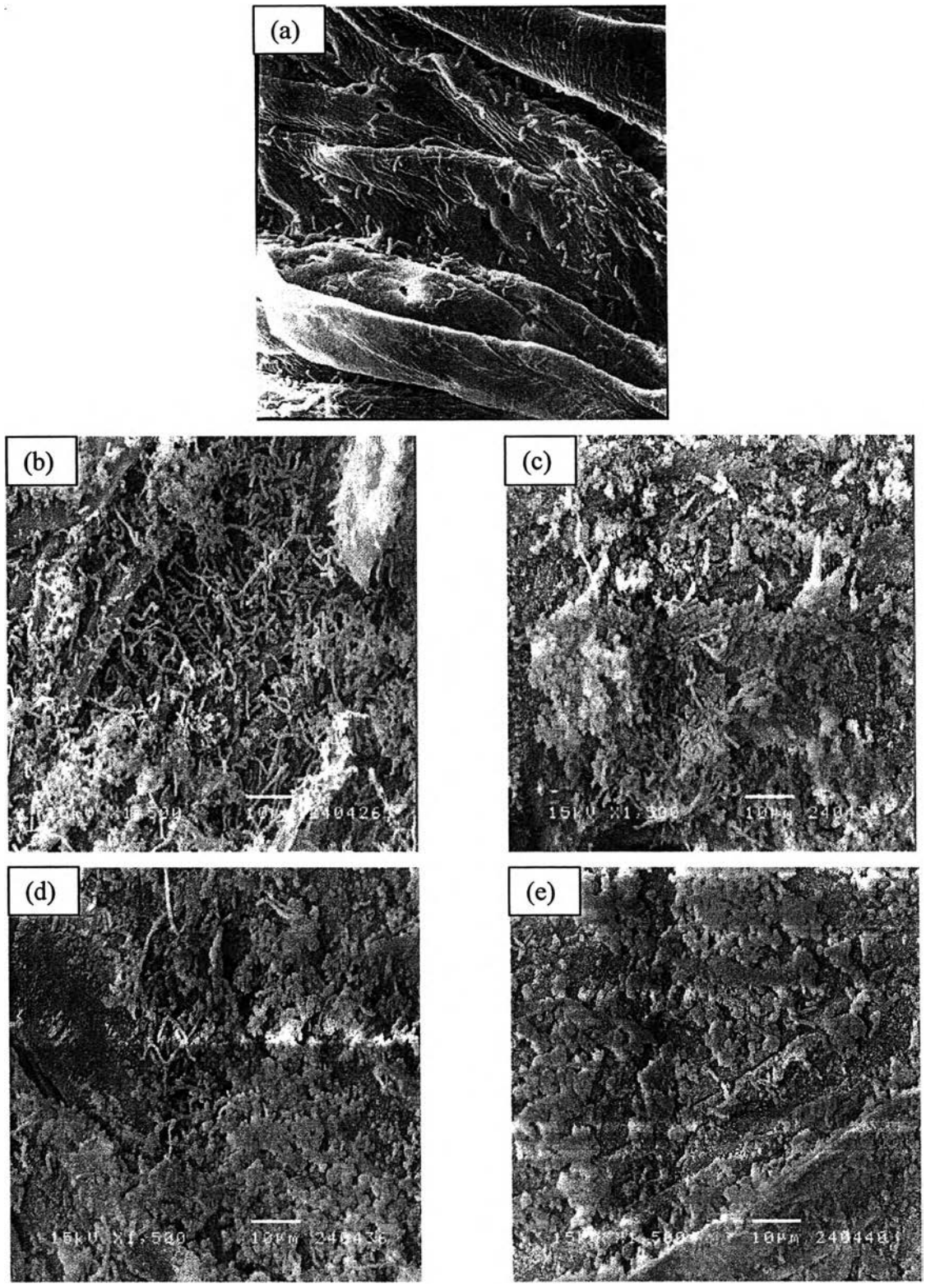



Figure 4.17 Cell-immobilized loofa sponge (a) L1 after main batch, (b) L2, (c) L3, (d) L4, and (e) L5 after repeated batch 5

Cell adsorption on the support surface, like in this research, was considered being external mass transfer resistance because the substrate had to diffuse through the liquid around the cells.

By noticing from the experiments, no mucous substance occurred on the surface of cell-immobilized loofa sponge. It can also be confirmed by the SEM images in Figure 4.17 that the cells attached to the support did not produce any mucous substance or polysaccharide.

Focusing on the various molecular weights of chitosan, there were no marked differences in glucose consumption and lactic acid formation. This might be due to slightly difference values of zeta potential of chitosan. This can also be seen from that the productivities and the product yields of different molecular weights of chitosan were not significantly different.

### 4.3.2 Cell immobilization on sugarcane bagasse

Five cultures were examined and labels of each culture investigated are listed in Table 4.5.

Table 4.5 List of samples and labels for sugarcane bagasse fermentation

| Immobilized carrier | Label |
|---|---|
| Untreated sugarcane bagasse | B1 |
| $H_2O_2$-treated sugarcane bagasse coated with chitosan MW 83,000 Da[a] | B2 |
| $H_2O_2$-treated sugarcane bagasse coated with chitosan MW 185,000 Da[a] | B3 |
| $H_2O_2$-treated sugarcane bagasse coated with chitosan MW 380,000 Da[a] | B4 |
| $H_2O_2$-treated sugarcane bagasse coated with chitosan MW 800,000 Da[a] | B5 |

[a] 1% w/v chitosan in 2% v/v acetic acid

As shown in Figure 4.18, the shape of the glucose concentration profile was similar for all systems, indicating that the cell was behaving similarly in terms of glucose consumption, like in the case of loofa sponge. It is suggested that there might be the same limiting factors in all systems so that by the end of the every batch, the glucose concentration reached a similar level and the final glucose concentration of each cycle were a little higher than 10 g $L^{-1}$.

Analogous to glucose concentration curve, lactic acid concentration profile followed the opposite trend of the glucose consumption and produced L(+)-lactic acid at the similar level between approximately 22 – 25 g $L^{-1}$ for every treatment from the main batch to repeated batch 3, as can be seen in Figure 4.19. At repeated batch 4 – 7, the lactic acid production was rapidly decreased from approximately 20 g $L^{-1}$ to the lowest value of approximately 15 g $L^{-1}$.

From Figure 4.18 and 4.19, no lag phase was noticed in every cycle which was different from the case of loofa sponge. This meant that cells adhered on sugarcane bagasse grew and produced lactic acid without adaptation to the fresh medium.

Analogous to the cell-immobilized loofa sponge case, glucose consumption and lactic acid production of cell-immobilized sugarcane bagasse in each treatment were insignificantly different. That meant the molecular weight of chitosan was not significantly affect on cell behaviours including substrate consumption and product formation.

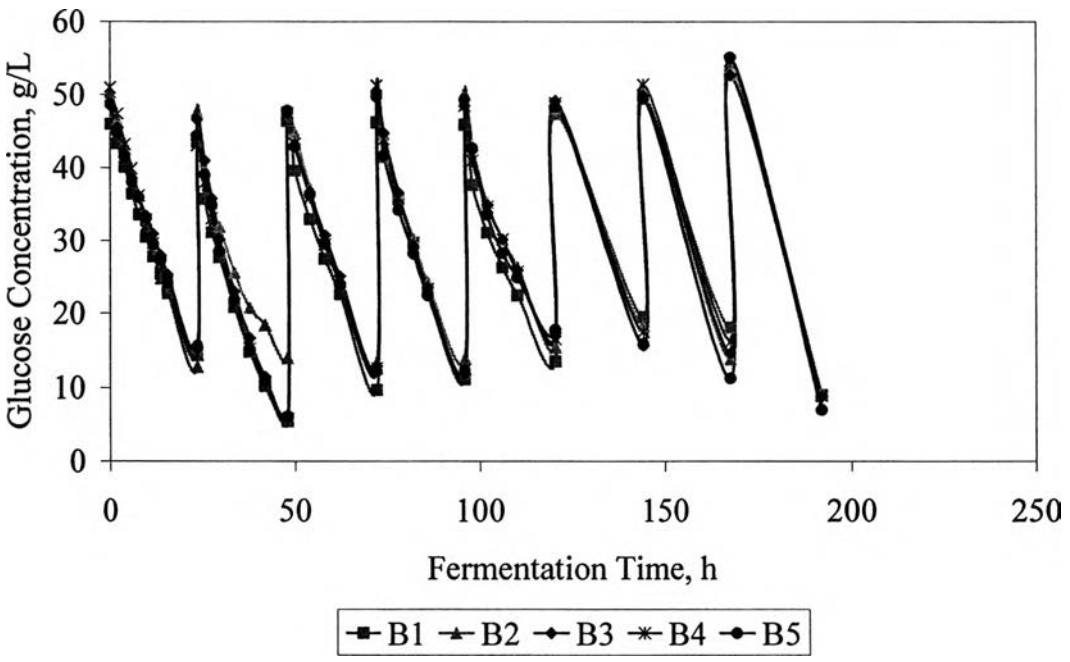



Figure 4.18 Glucose concentration profile of cell-immobilized on sugarcane bagasse for all cycles

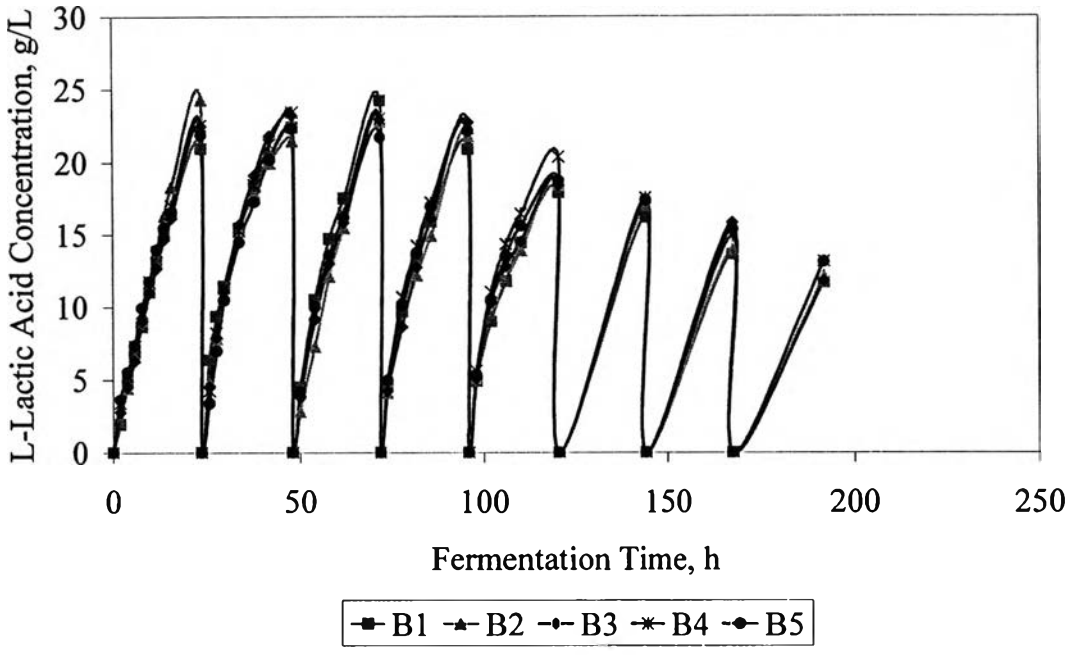



Figure 4.19 L(+)Lactic acid concentration profile of cell-immobilized on sugarcane bagasse for all cycles

From Figure 4.20, it can be seen that Yp/s from the main batch to the repeated batch 4 reduced slightly from approximately 0.70 to 0.60 g lactic acid/g glucose. After that in the repeated batch 5 to 7, the Yp/s dropped more rapidly from approximately 0.55 to 0.3 g lactic acid/g glucose. It might be due to the decreased efficiency of the immobilized cells during every passing cycle.

As shown in Figure 4.21, the productivity of the main batch to the repeated batch 3 was rather constant and had the values between approximately 0.90 to 1.00 g $L^{-1}$ $h^{-1}$. After that, it reduced more rapidly from repeated batch 4 to 7 from approximately 0.75 g $L^{-1}$ $h^{-1}$ to the lowest value of approximately 0.50 g $L^{-1}$ $h^{-1}$. The reason was the same as in product yield case.

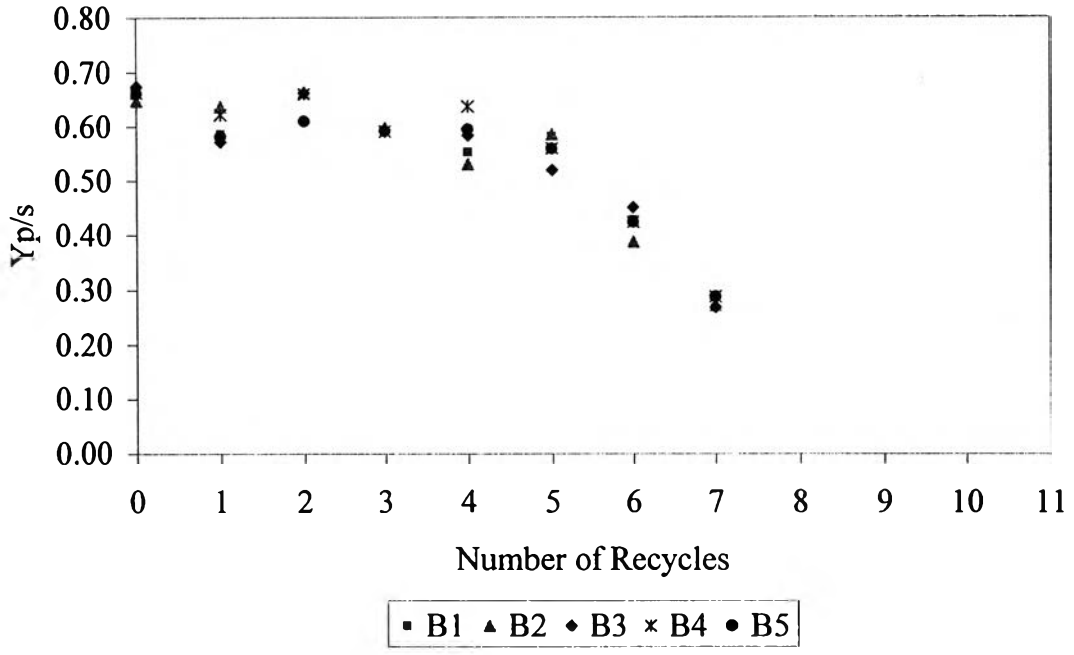



Figure 4.20 Total Yp/s of cell-immobilized on sugarcane bagasse for all cycles (1 cycle = 24 hours)

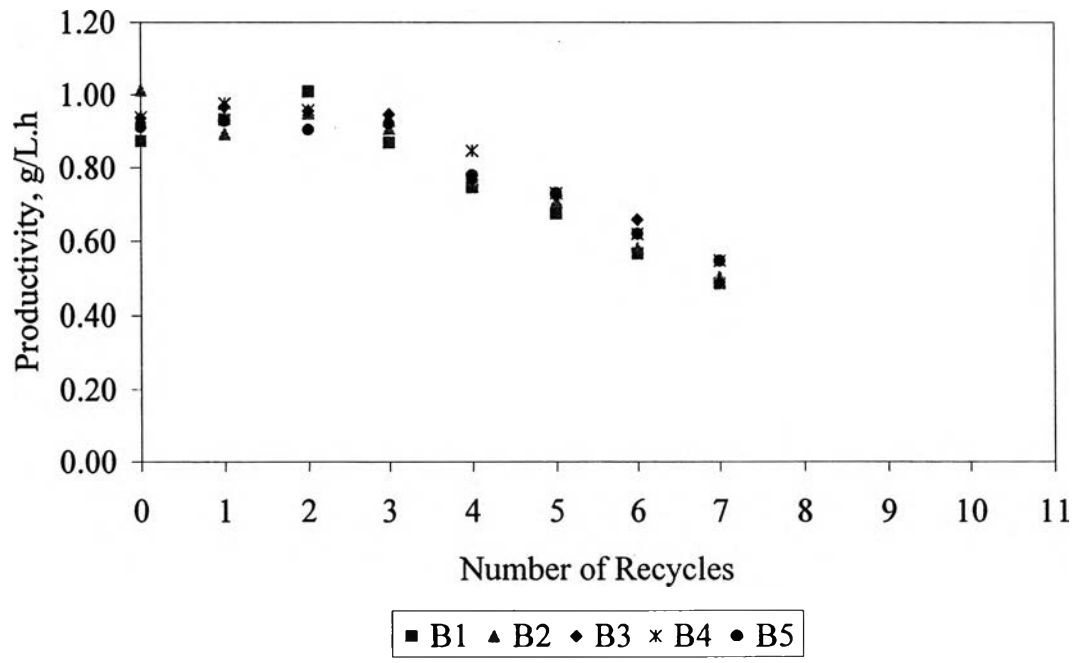



Figure 4.21 Productivity of cell-immobilized on sugarcane bagasse for all cycles (1 cycle = 24 hours)

SEM micrographs of cell-immobilized sugarcane bagasse were shown below.

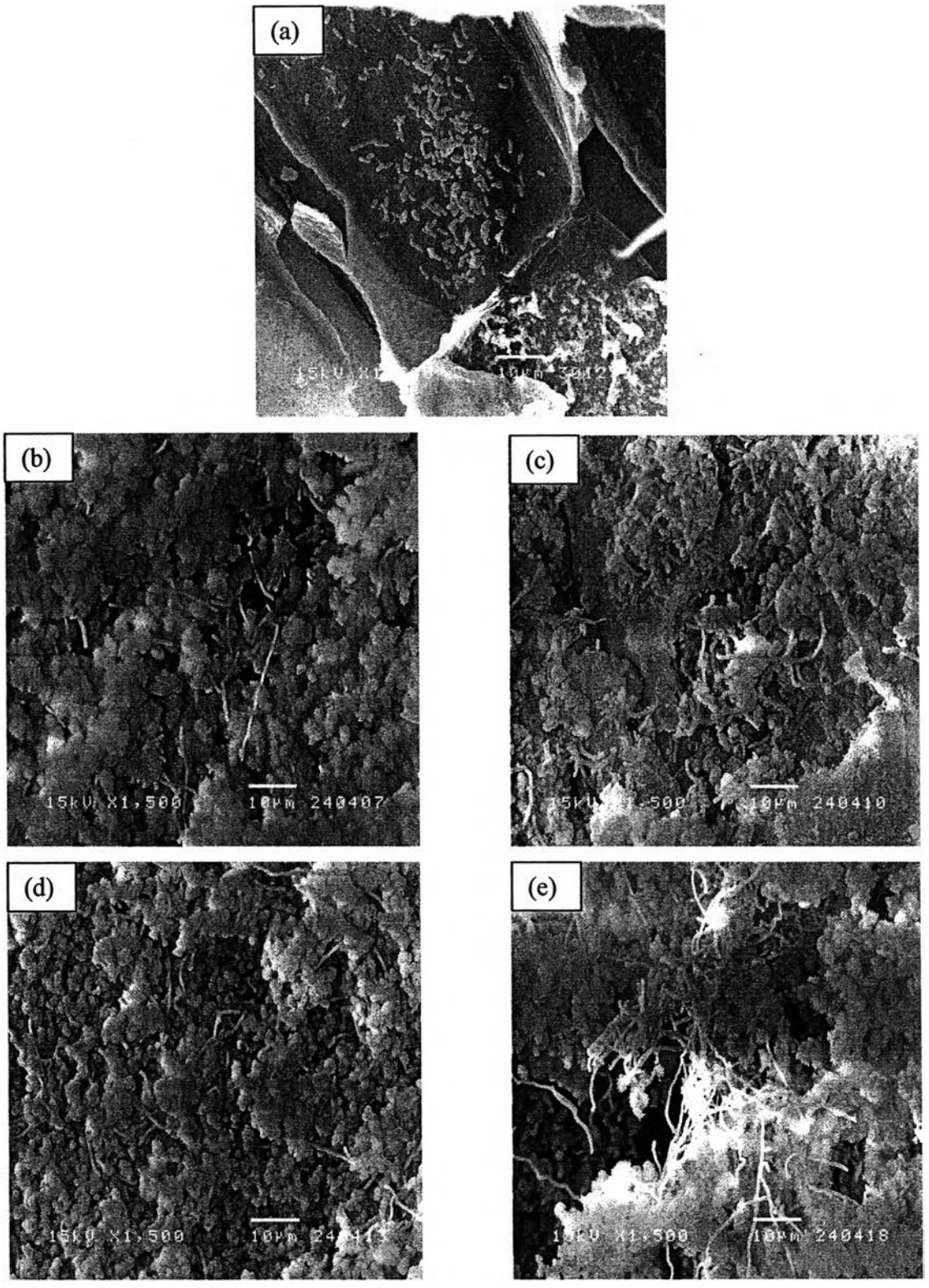



Figure 4.22 Cell-immobilized sugarcane bagasse (a) B1 after main batch, (b) B2, (c) B3, (d) B4, and (e) B5 after repeated batch 4

Similar to the cell-immobilized loofa sponge case, cell adsorption on the support surface was considered being external mass transfer resistance.

No mucous substance was noticed on the surface of cell-immobilized sugarcane bagasse. It can also be verified by the SEM images in Figure 4.22 that the cells attached to the support did not produce any mucous substance or polysaccharide.

Like in loofa sponge case, considering different molecular weights of chitosan, there were no marked differences in glucose consumption and lactic acid formation. This might be due to slightly difference values of zeta potential of chitosan. This can also be seen from that the productivities and the product yields of different molecular weights of chitosan were not significantly different.

### 4.3.3 Comparison of lactic acid production via various fermentation systems

In order to compare the lactic acid production between different conditions, lactic acid concentration data were plotted with fermentation time of the suspended cell controlled pH at 5.5 with NaOH (SC+NaOH), suspended cell controlled pH with 5% $CaCO_3$ (SC+5% $CaCO_3$), suspended cell controlled pH with 5% $CaCO_3$ in repeated batch mode (SC+5% $CaCO_3$ in repeated batch), loofa sponge coated with chitosan at MW 800,000 Da (L5), and sugarcane bagasse coated with chitosan at MW 800,000 Da (B5).

From Figure 4.23, SC+NaOH system could produce the lactic acid the most rapidly, as confirmed with the highest productivity value among all the systems from Table 4.6. This may be due to the ability of NaOH in liquid form to neutralize the produced lactic acid immediately. The pH was controlled at 5.5 constantly thus made cell not necessary to adapt themselves to microenvironment. Consequently, they could produce lactic acid continuously until the depletion of substrate occurred and gained the highest lactic acid concentration among these systems. When pH of the system was controlled by $CaCO_3$, the lactic acid production rate was less than the system that controlled pH by NaOH. This can be explained that $CaCO_3$ can dissolve in acidic solution. At the beginning of the fermentation, the pH was about 6.5 so $CaCO_3$ could not dissolve well. When lactic acid was more produced then the pH dropped, this made $CaCO_3$ more dissolved. $CaCO_3$ can neutralized lactic acid and pH of the system that controlled by $CaCO_3$ was stabilized at 4.7, which was not the optimum condition

for this microbial. So the amount of lactic acid produced was less than that of the NaOH system.

The advantage of immobilized cell culture over suspended cell culture was that it can prolong the fermentation because cells could be reused in repeated batch as demonstrated in Figure 4.23. Moreover, the productivities of repeated batch cultures were higher than the suspended cell system. On the contrary, the product yields of repeated batch system were lower than that of suspended cell system, as can be seen in Table 4.6.

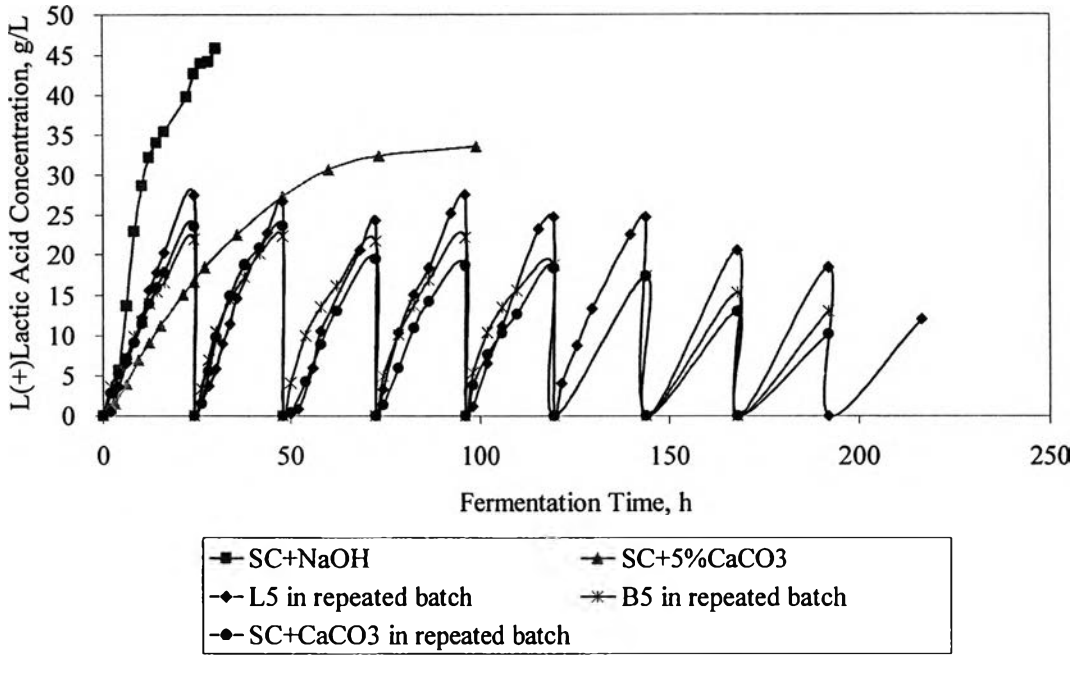



Figure 4.23 L(+)lactic acid production of various fermentation conditions with initial glucose concentration at 50 g $L^{-1}$.

Table 4.6 Yield and productivity of various fermentation conditions with initial glucose concentration at 50 g $L^{-1}$

| System | Operating Mode | Yp/s | Productivity, g $L^{-1}$ $h^{-1}$ | Fermentation Time, h |
|---|---|---|---|---|
| SC + NaOH | Batch | 1.12 | 2.87 | 30 |
| SC + 5% $CaCO_3$ | Batch | 1.19 | 0.69 | 99 |
| SC + 5% $CaCO_3$ in repeated batch | Repeated Batch | 0.55 | 0.75 | 192 |
| L5 in repeated batch | Repeated Batch | 0.47 | 0.95 | 216 |
| B5 in repeated batch | Repeated Batch | 0.51 | 0.79 | 192 |

Among the repeated batch cultures, cell immobilized on loofa sponge system provided the lactic acid concentration better than the cell immobilized on sugarcane bagasse system. The suspended cell system with $CaCO_3$ in repeated batch provided the lowest lactic acid concentration. The reason that suspended cell with $CaCO_3$ could be reused was that cells could attach on $CaCO_3$ as shown in SEM image below. Thus when the fermentation broth was decanted, some cells still remained in the system.

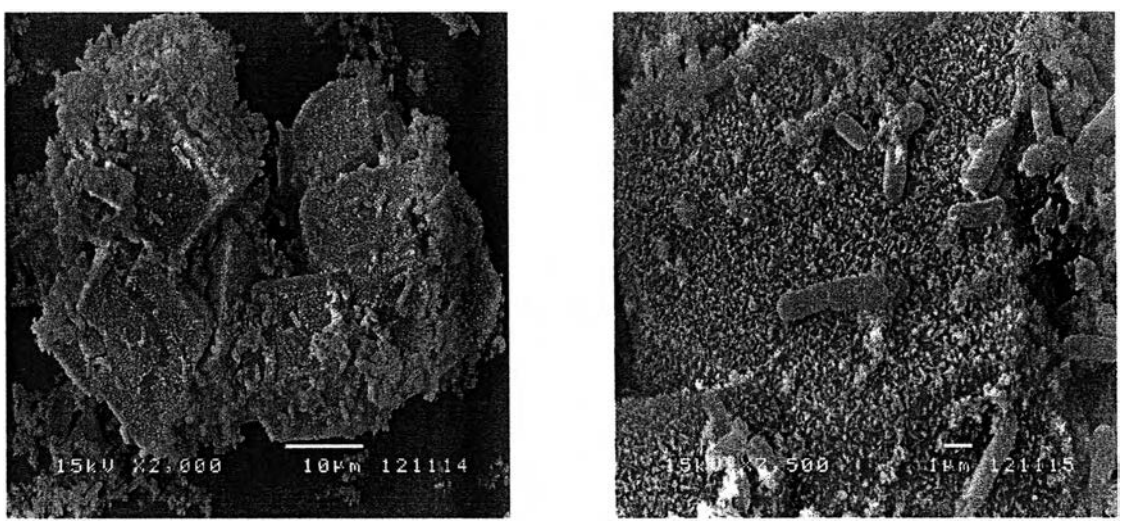



Figure 4.24 Cells adhered on $CaCO_3$

4.3.4 Comparison of loofa sponge and sugarcane bagassse as immobilization materials

In order to compare the effectiveness of loofa sponge and sugarcane bagasse as immobilization materials, the average product yield, average productivity, and the number of effective cycle of both materials were calculated and tabulated in Table 4.7. It can be seen that cell immobilized on loofa sponge could produced lactic acid slightly faster than cell immobilized on sugarcane bagasse, as in the terms of average productivity through repeated batch mode of operation. Although the average product yield of both loofa sponge case and sugarcane bagasse case were comparable, the number of effective cycle of loofa sponge case was a little higher than that of sugarcane bagasse case. All data were shown in Table 4.7. Therefore, it can be indicated that loofa sponge and sugarcane bagasse performed indifferently as immobilization material. For further discussion, cell-immobilized loofa sponge was selected as a case for study.

Table 4.7 Comparison of average product yield, average productivity, and number of repeated batches using loofa sponge and sugarcane bagasse as immobilization materials

| Immobilization Materials | Yp/s | Productivity, g $L^{-1}$ $h^{-1}$ | No. of Repeated Batches |
|---|---|---|---|
| Loofa sponge | 0.56 | 0.94 | 5 |
| Sugarcane bagassse | 0.53 | 0.80 | 3 |

From the past decade, many attempts in lactic acid fermentation using immobilized cell in various operation modes have been reported. Immobilization materials used in these studies were diversifying, including organic and inorganic materials. The experimental data were categorized and reported in Table 4.8. Comparing productivity of this work and other repeated batch studies, the productivity of this work, 1.2 g $L^{-1}$ $h^{-1}$, was close to other works, 0.5 – 2.0 g $L^{-1}$ $h^{-1}$, There may be some factors affecting productivities such as type of microorganisms, operating conditions especially pH regulation.

Comparing this work with repeated batch of Ho et al [63], the reason of considerable difference of the both productivities of Ho et al. [63], 4.26 g $L^{-1}$ $h^{-1}$, and this research, 1.2 g $L^{-1}$ $h^{-1}$, might be because Ho et al. [63] performed the fermentation with biofilm of microorganism, while the biofilm of *L. salivarius* from this research was quite thin and not evenly distributed on the support surface. Less microorganism density might cause less product yield and less production rate. However, the productivities of continuous cultivation of Cotton et al. [46], 9.0 g $L^{-1}$ $h^{-1}$, was significantly higher than this research, 1.2 g $L^{-1}$ $h^{-1}$, because continuous culture contained cells that were in logarithmic phase all the time. New cells were continuously produced and the cells were totally mixed in the fermentation broth when overflowed from the fermentor and some highly active cells remained in the fermentor.

Table 4.8 Studies on lactic acid production of immobilized cells.

| Researchers | Operating Mode | Yp/s | Productivity, g $L^{-1}$ $h^{-1}$ | Immobilization Materials | Remarks |
|---|---|---|---|---|---|
| Rangaswamy and Ramakrishna [49] | Batch | 0.90 – 0.95 | 2 – 2.5 | Polyurethane foam | 4 times higher productivity than free cell |
| This research | Batch (Main batch) | 0.76 | 1.14 | Chitosan-treated loofa sponge | 24 hours fermentation time |
| Krishna et al. [23] | Repeated batch in shake flask mode | 0.90 – 0.97 | 0.5 – 2.0 | Chitosan-treated polypropylene matrix | 12 Effective cycles |
| Elezi et al. [38] | Repeated batch in shake flask | 0.71 | 1.21 | Delignified cellulosic material | 10 Effective cycles |
| This research | Repeated batch in shake flask | 0.61 | 1.0 – 1.2 | Chitosan-treated loofa sponge | 6 Effective cycles |
| Ho et al. [63] | Repeated batch biofilm fermentation | 0.95 | 4.26 | Plastic composite support | Shorten the lag time and fermentation time |
| Shahbazi et al. [47] | Repeated batch of spiral-sheet bioreactor | 0.84 | 0.61 | Polymeric matrix | Studied only 3 repeated batch |

Table 4.8 Studies on lactic acid production of immobilized cells. (Cont.)

| Researchers | Operating Mode | Yp/s | Productivity, g $L^{-1}$ $h^{-1}$ | Immobilization Materials | Remarks |
|---|---|---|---|---|---|
| Roble et al. [32] | Repeated fed-batch of circulating loop bioreactor | 0.76 | 1.6 | Loofa sponge with alginate as the polymer support | More than 400 h fermentation time |
| Cotton et al. [46] | Continuous stirred tank bioreactor | 0.70 | 9.0 | Plastic composite support | At dilution rate of 0.4 $h^{-1}$ |
| Krishna et al. [23] | Packed bed column bioreactor | 0.97 | 7.66 | Chitosan-treated polypropylene matrix | Average residence time of 30 h |
| Rangaswamy and Ramakrishna [49] | Packed bed biofilm reactor | - | 5 | Polyurethane foam | 1000 hours of fermentation time |