CHAPTER IV

RESULTS AND DISCUSSION

4.1 The effect of pH on lactic acid production in batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

The purpose of this experiment is to determine the suitable pH for growth and lactic acid production. The experiments were set up in batch fermentation processes by using 10 g/l Meat extract, 5 g/l Yeast extract, 10 g/l Peptone, 20 g/l D-glucose, 1 g/l Tween 80, 2 g/l K_2HPO_4, 0.2 g/l $MgSO_4 \cdot 7H_2O$ and 0.2 g/l $MnSO_4 \cdot H_2O$ as fermentation medium (Appendix B). During batch process, pH (5.0, 5.5, 6.0 and uncontrolled pH), temperature (37^0C) and stirrer speed (100 rpm) were controlled with no aeration condition.

From Figure 4-1, the effect of pH on cell dry weight has been compared. It has observed that the uncontrolled pH has more lag time than controlled pH (4 h and 2 h, respectively). Ghaly et al. [49] reported that the length of the lag phase depended on the extent to which the medium and the environmental factors such as pH, temperature and dissolved oxygen, were different from those under which the inoculum was prepared.

In addition, the maximum specific growth rate obtained from uncontrolled pH was higher than the maximum specific growth rate obtained from controlled pH (0.41 h^{-1} for uncontrolled pH, 0.29 h^{-1} for pH 5.0, 0.34 h^{-1} for pH 5.5 and 0.33 h^{-1} for pH 6.0)(Figure 4-4). However, the lactic acid concentration (Figure 4-3) and the productivity (Figure 4-4) have been obtained from uncontrolled pH (10.11 g/l and 0.82 g/l h, respectively) show lower value than controlled pH at 5.5 (10.21 g/l and 1.64 g/l h, respectively). It was indicated that the lactic acid production under controlled pH at 5.5 was better than uncontrolled pH. Moreover, at the maximum lactic acid concentration, the fermentation time of controlled pH obtained is faster than uncontrolled pH (6 h and 12 h, respectively) that exhibit the consistency with the shorter lag time. In fermentation without pH control, the pH of fermentation broth was dropped and the productivity was slowed by inhibitory effect of lactic acid and the lactic acid yield in uncontrolled pH of fermentation broth decreased significantly [5]. According to some authors [35,12], weak acids, e.g., lactic acid inhibit bacterial growth because as the external pH declines, the acid is protonized as soon as it is exported out of the bacteria. Uncharged, it diffuses back

into the cell and dissociates due to the higher intracellular pH. The cell then has to use ATP to pump out protons and energy eventually is depleted causing growth stop and bacteria die. From this experiment, the highest productivity (1.64 g/l h) and lactic acid yield (1.15) were obtained from the controlled pH at 5.5 (Figure 4-4).

Furthermore, this experiment was shown that at the controlled pH, it has substrate limitation that means the initial glucose concentration using in this experiment is not enough for cell growth. From Figure 4-1, 4-2 and 4-3, we observed that the cell dry weight and the lactic acid concentration of controlled pH were dropped when complete utilization of glucose. Therefore, the higher initial glucose concentration could be employed for solving this problem.

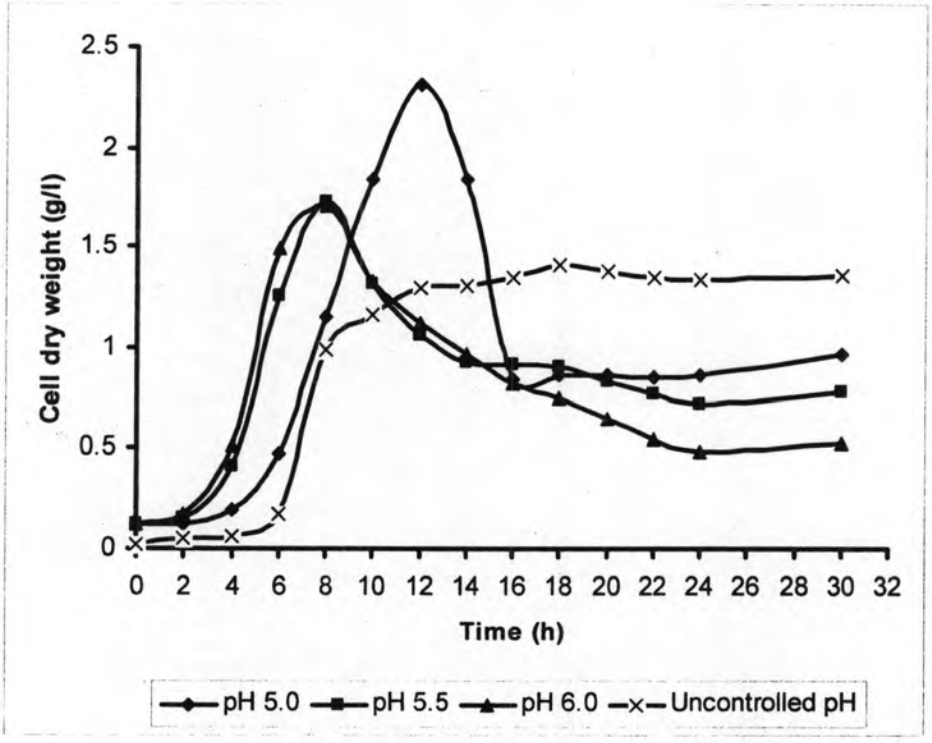

Figure 4-1 Effect of pH on cell growth of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

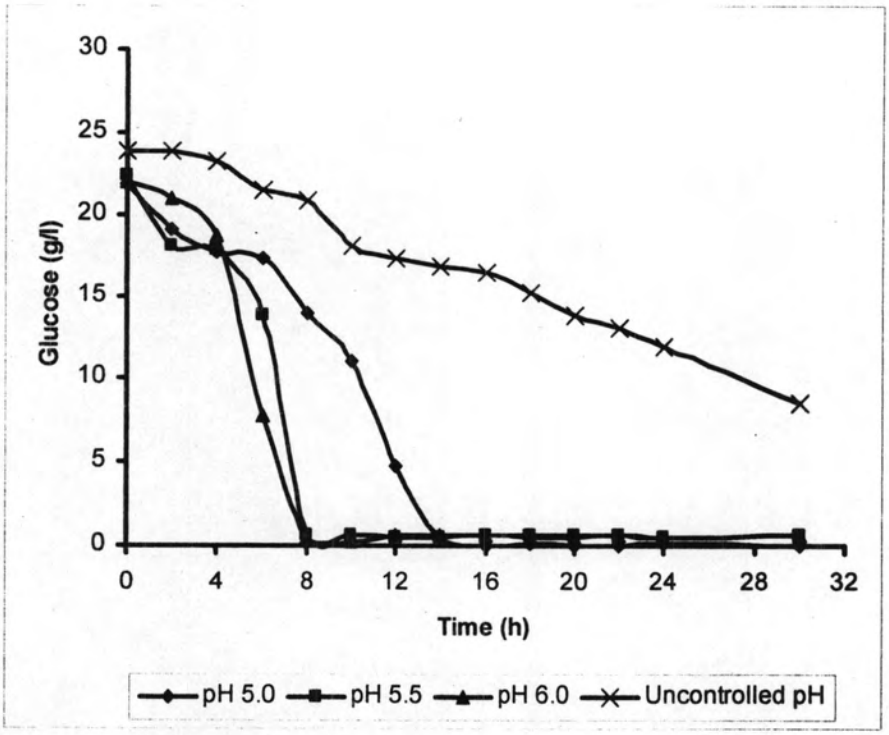

Figure 4-2 Effect of pH on glucose consumption of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

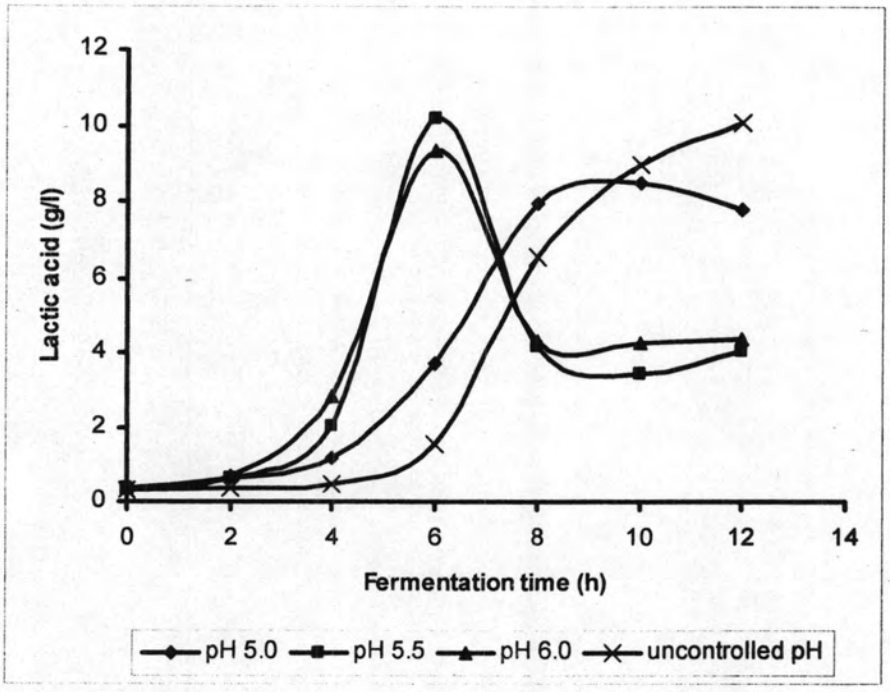

Figure 4-3 Effect of pH on Lactic acid production of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

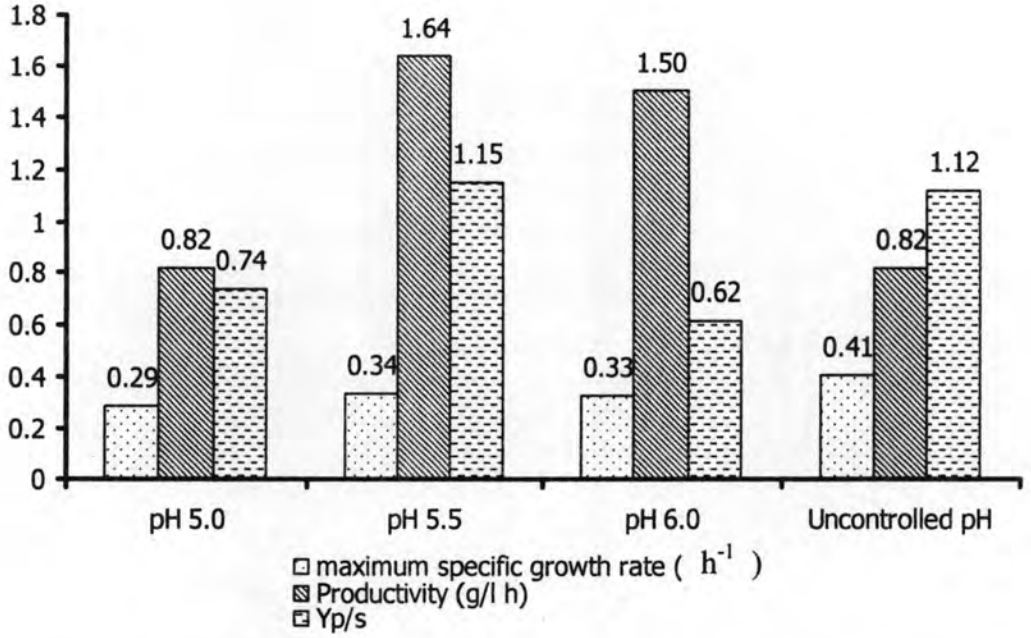

Figure 4-4 Effect of pH on kinetic parameters of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

4.2 The effect of the initial glucose concentration (Commercial grade, CG) on lactic acid production in batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

For solving the substrate limitation problem and to increase the lactic acid concentration, this experiments were set up in batch fermentation and varied the initial glucose concentration (30, 40, 50, 70, 80 and 100 g/l) to find the suitable concentration under the controlled pH at 5.5, temperature 37^{0}C, stirrer speed 100 rpm and using the other components of fermentation medium as same as previous experiment (4.1).

Figure 4-5 to Figure 4-7 indicated that the cell concentration and lactic acid concentration increased with increases in the initial substrate concentration up to 70 g/l and then decreased with further increases in the initial substrate concentration (at 80 and 100 g/l). This decrease in the cell concentration and lactic acid concentration were 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 [50]. Moreover, the initial glucose concentration from 30 g/l to 50 g/l have been remained the substrate limitation.

Figure 4-8 shows the specific growth rate, productivity and lactic acid yield at different initial glucose concentration. At different initial glucose, the maximum specific growth rate and productivity of each other were resembled (0.34-0.39 h^{-1} and 3.43-3.72 g/l h, respectively), that mean, the initial glucose concentration not effect the maximum specific growth rate and productivity. The maximum specific growth rate depended on the strains of the microorganism, temperature and pH [51]. In addition, the highest lactic acid yield (1.73 g product/ g substrate) was obtained from the initial glucose concentration 70 g/l. It indicated that the optimum initial glucose concentration for lactic acid production using *L.salivarius subsp. salivarius* at pH 5.5 was 70 g/l.

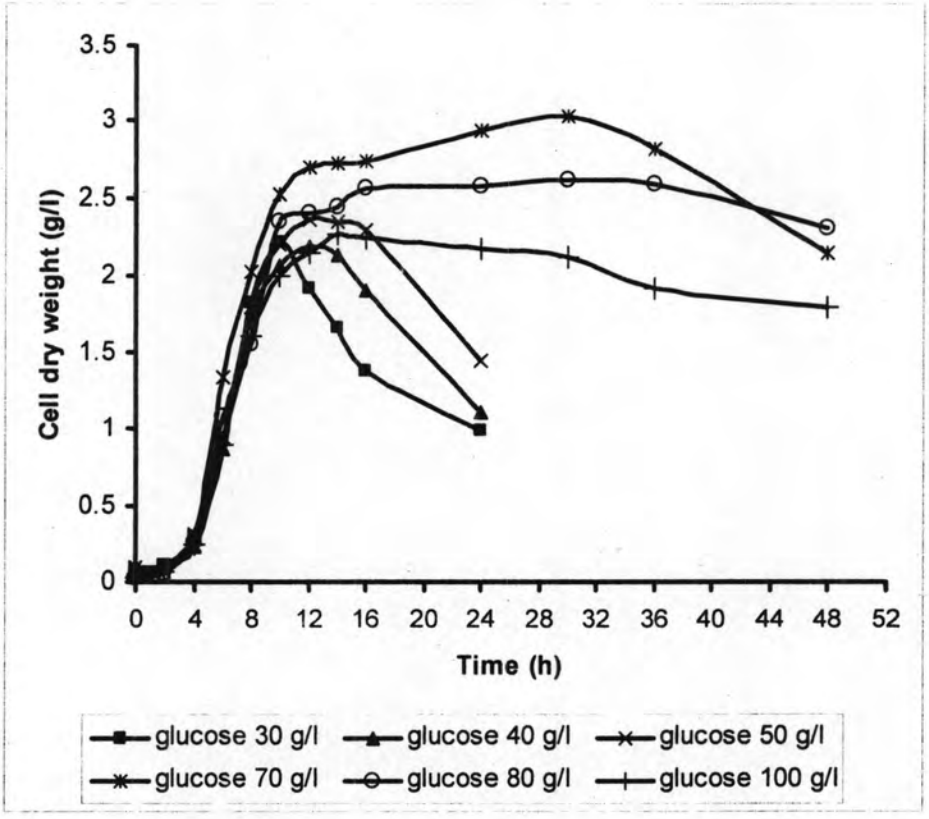

Figure 4-5 Effect of the initial glucose concentration on cell growth of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

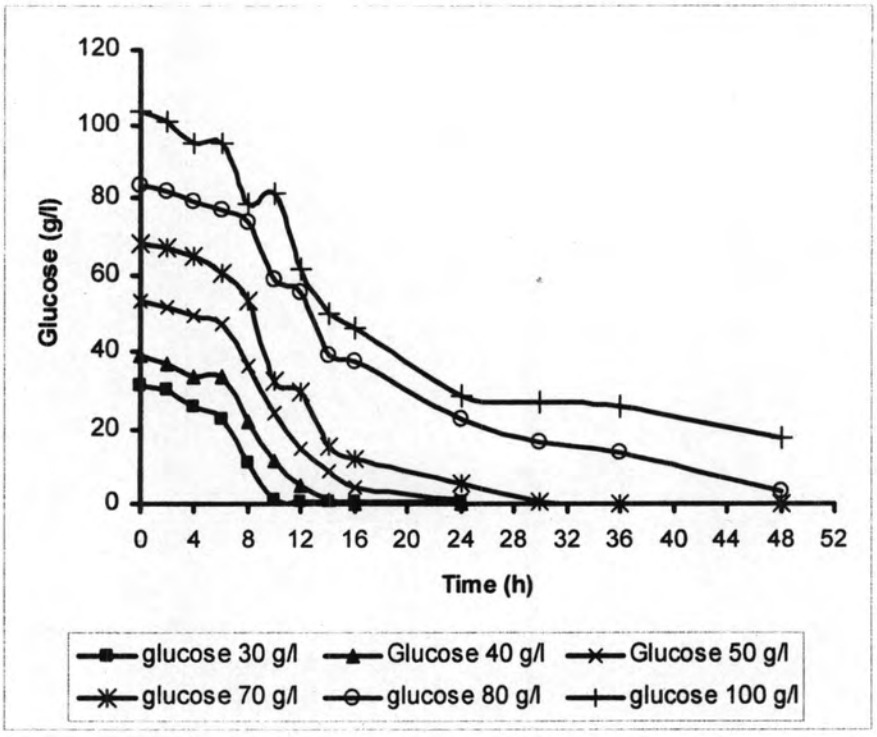

Figure 4-6 Effect of the initial glucose concentration on glucose consumption of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

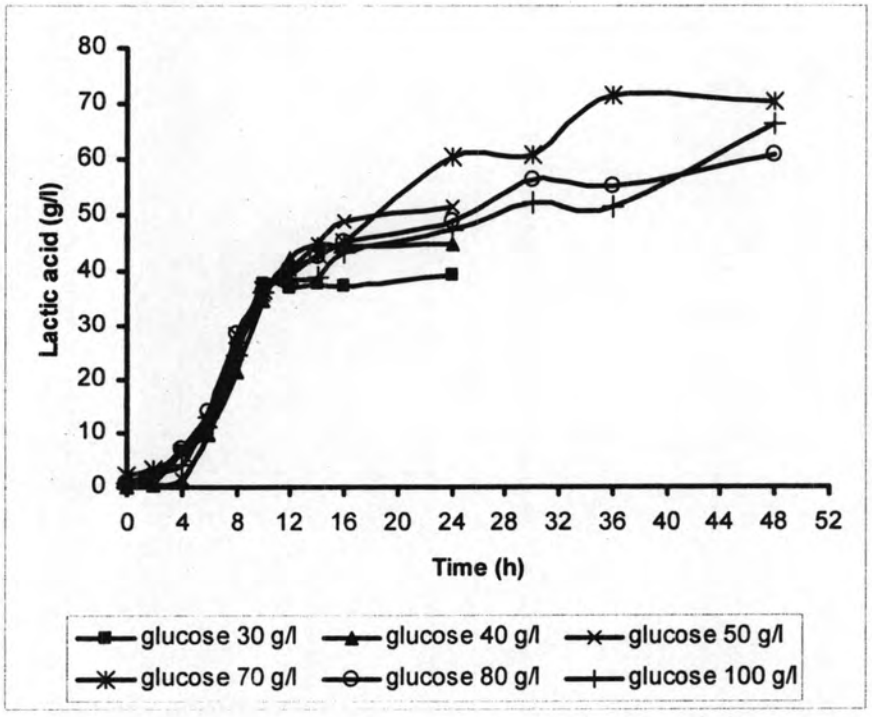

Figure 4-7 Effect of the initial glucose glucose concentration on lactic acid production of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

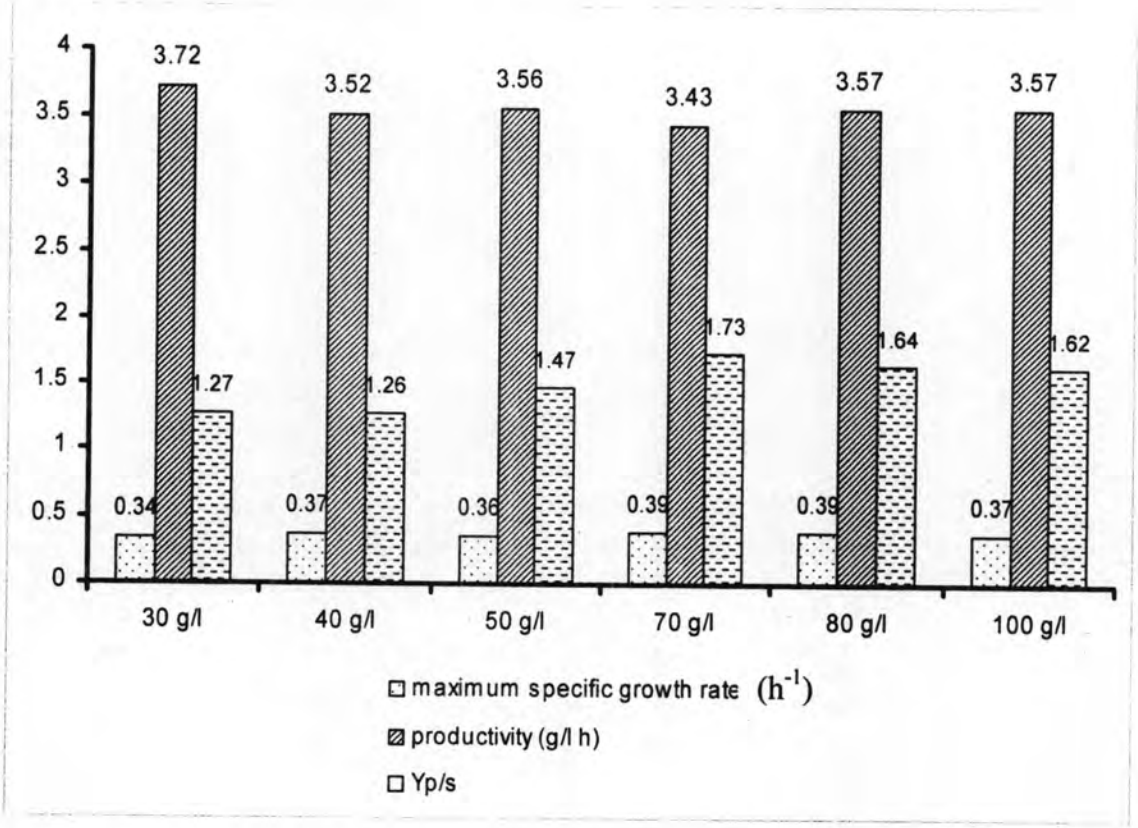

Figure 4-8 Effect of the initial glucose concentration on kinetic parameters of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

4.3 The effect of glucose concentration (from Cassava starch hydrolysate, CSH) on lactic acid production in batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

From previous experiment, it was found that the optimum initial glucose concentration for lactic acid production using *L.salivarius subsp. salivarius* at pH 5.5 was 70 g/l, so in this experiment, the glucose from cassava starch hydrolysate was used and varied about the initial glucose (CSH) concentration at 70 g/l, 80 g/l and 100 g/l to find the suitable initial glucose (CSH) concentration. This experiment were set up in batch fermentation under the controlled temperature at 37^0C, stirrer speed 100 rpm and using the other components of fermentation medium as same as previous experiment (4.1).

Figure.4-9 shows that the substrate inhibition occurred at the initial glucose concentrations (CSH) above 70 g/l. Moreover, Figure.4-10 to 4-11 indicated that lactic acid concentration and maximum specific growth rate at the initial glucose concentration (CSH) 70 g/l were resembled to another but it had a highest lactic acid yield (1.00 g product/ g substrate.

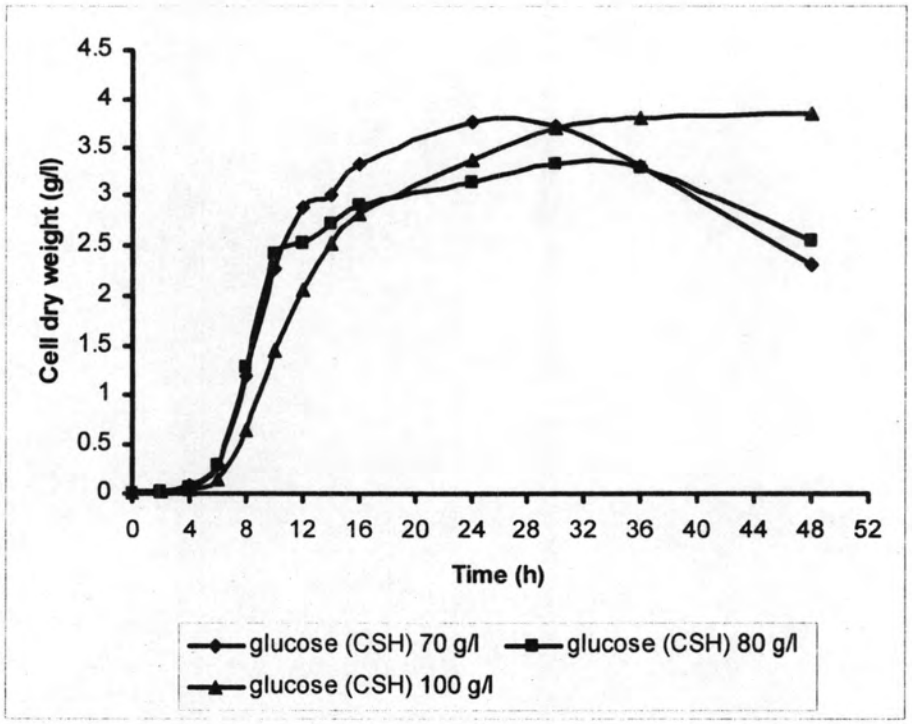

Figure 4-9 Effect of glucose concentration from CSH on cell growth of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

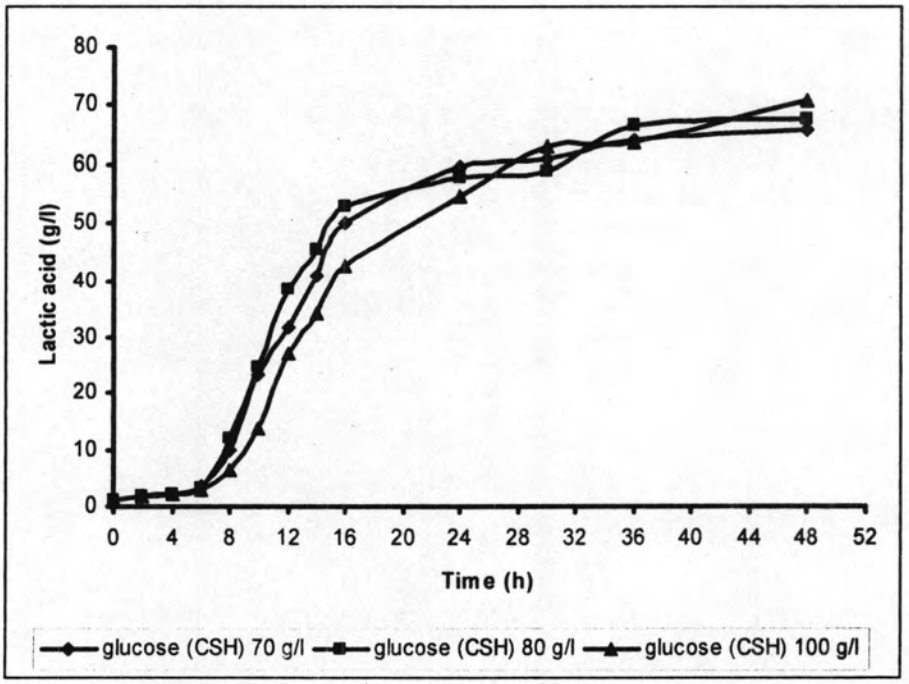

Figure 4-10 Effect of glucose concentration from CSH on lactic acid production of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

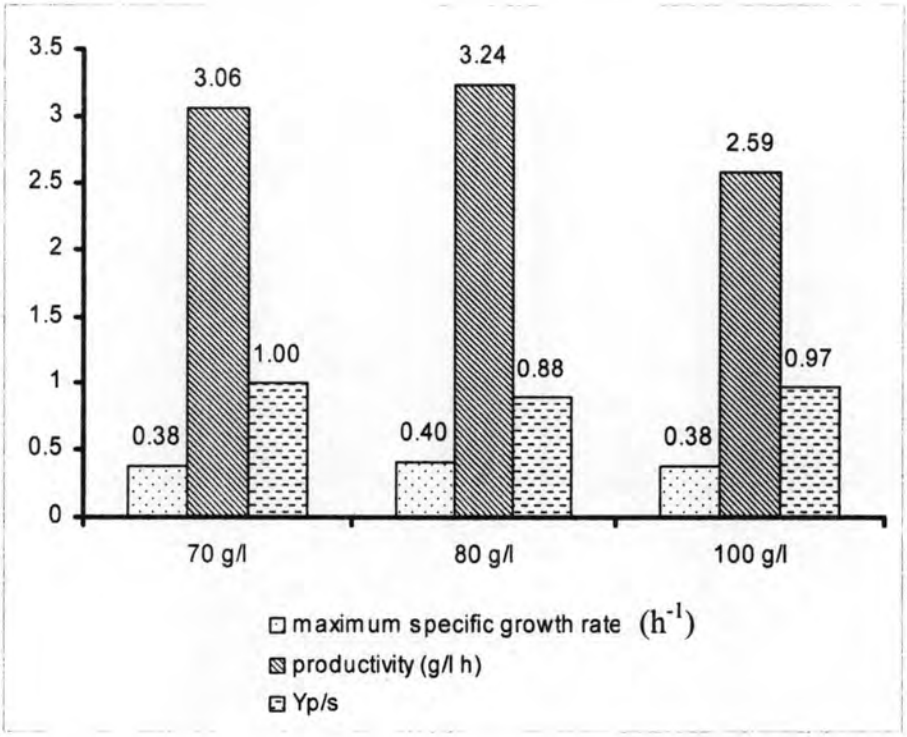

Figure 4-11 Effect of the initial glucose concentration from CSH on kinetic parameters of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

4.4 The comparison of the commercial grade glucose (CG) and glucose from cassava starch hydrolysate (CSH) on lactic acid production of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741.

The effect of the commercial grade glucose (CG) and glucose from cassava starch hydrolysate (CSH) on lactic acid production of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741 were compared. This experiment was studied under the suitable condition that received from previous experiments (initial CG concentration and initial CSH concentration were 70 g/l)

The comparison between the commercial grade glucose and glucose from cassava starch hydrolysate (CSH) at initial glucose concentration 70 g/l was shown in Figure.4-12 to 4-14. It can be observed that commercial grade glucose had shorter lag time and lower cell dry weight than glucose from cassava starch hydrolysate (2 h, 3.76 g/l for CG and 4 h, 3.02 g/l for CSH). It indicated that when we used glucose from cassava starch hydrolysate, the cells spent more time to use new nutrients to regenerate pools of essential nutrients and for adjustment to new environment [36]. However, it can be noticed that the glucose from cassava starch hydrolysate had more appropriate than commercial grade glucose for cell growth although it had mor///e lag time.

In addition, Figure 4-13 and 4-14 indicated that the trend of glucose and lactic acid concentration of commercial grade glucose and glucose from cassava starch hydrolysate were being similar. Figure 4-15 shows the effect of both types of carbon source on the kinetic parameters of the lactic acid production using *L.salivarius subsp. salivarius*. It indicated that the productivity and lactic acid yield of cassava starch hydrolysate were lower than commercial grade glucose (3.06 g/l h, 1.00 g product / g substrate and 3.43 g/l/h, 1.73 g product / g substrate, respectively). Although the lactic acid yield of CSH had lower than CG but it is still moderately high, moreover, the lactic acid concentration of both type of carbon source were resembled (60.79 g/l for CG, 59.57 g/l for CSH), so this experiment recommends that the blending of CG with CSH for lactic acid production is the one choice for reduce the cost of carbon source.

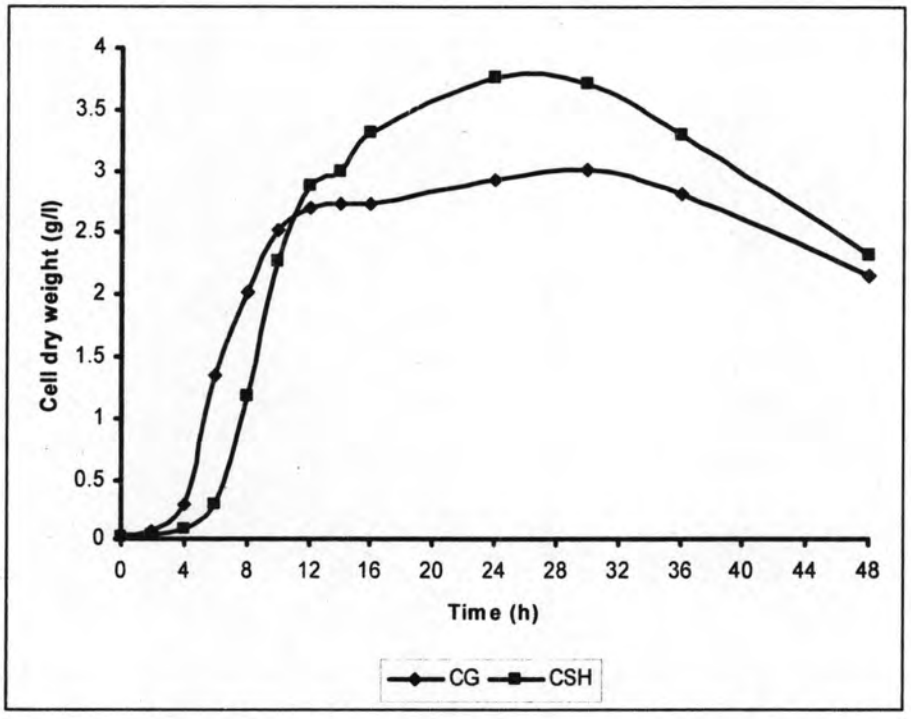

Figure.4-12 The effect of the commercial grade glucose (CG) and glucose from cassava starch hydrolysate (CSH) on cell growth of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741 (using YE = 5 g/l)

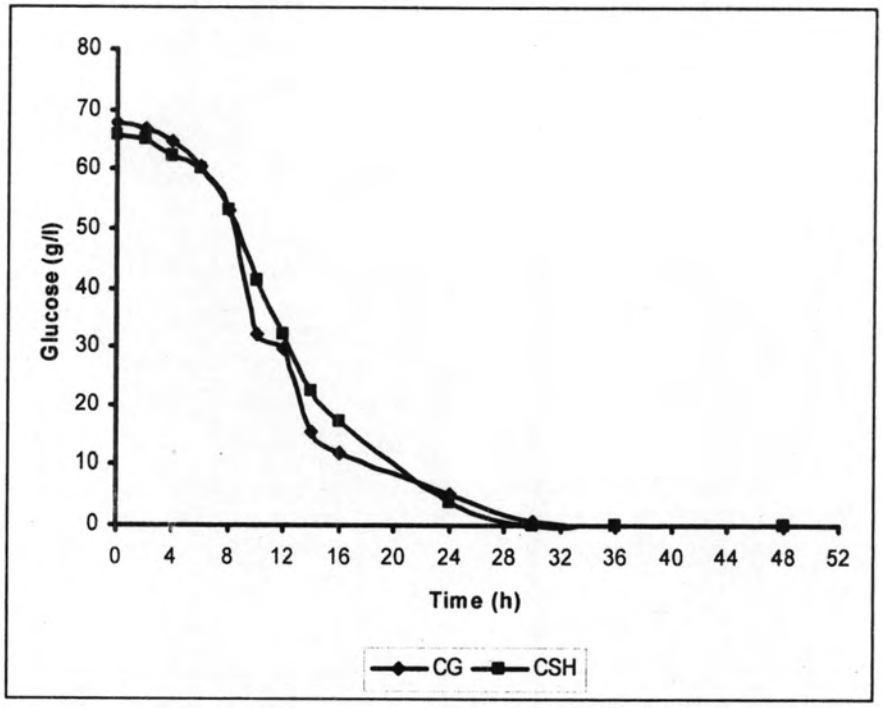

Figure.4-13 The effect of the commercial grade glucose (CG) and glucose from cassava starch hydrolysate (CSH) on glucose consumption of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741 (using YE = 5 g/l)

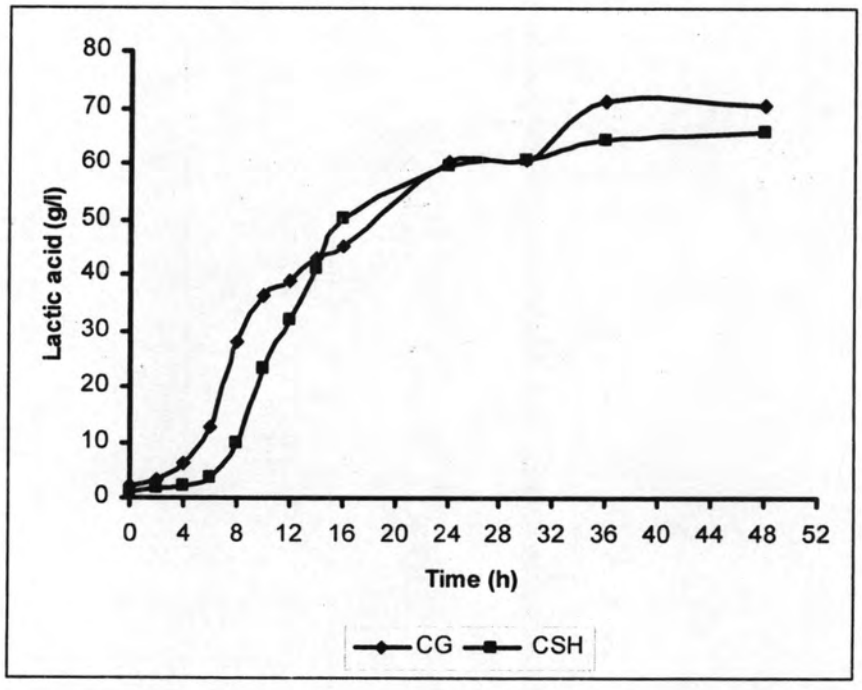

Figure.4-14 The effect of the commercial grade glucose (CG) and glucose from cassava starch hydrolysate (CSH) on lactic acid production of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741 (using YE = 5 g/l)

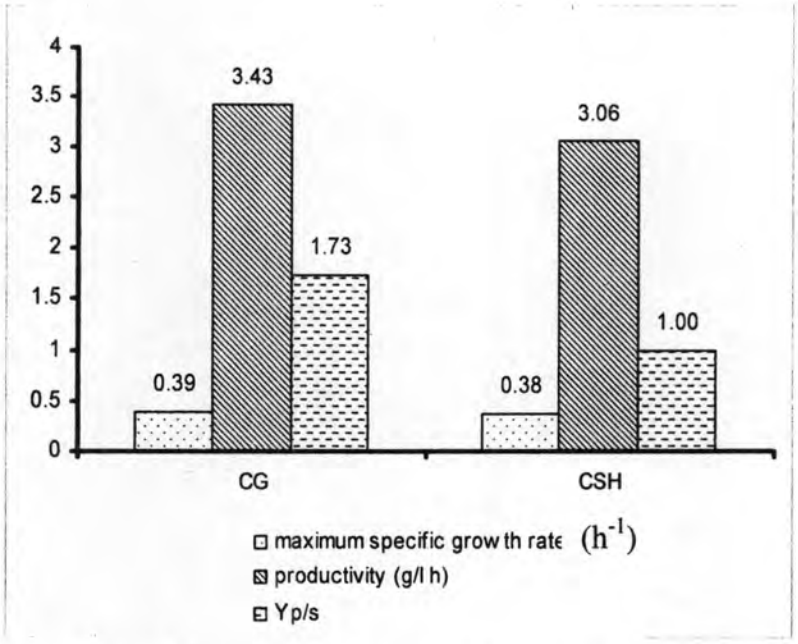

Figure.4-15 The effect of the commercial grade glucose (CG) and glucose from cassava starch hydrolysate (CSH) on kinetic parameters of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741 (using YE = 5 g/l)

4.5 The effect of brewer's yeast autolysate (BYA) on lactic acid production in batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

The propose of this experiment is to study the effect of brewer's yeast autolysate concentration and to find the suitable condition of brewer's yeast autolysate concentration on lactic acid production, so in this experiment, the brewer's yeast autolysate was used and varied about the initial concentration at 48 ml/l, 95 ml/l and 190 ml/l. This experiment were set up in batch fermentation under the controlled temperature at 37^0C, stirrer speed 100 rpm and using 70 g/l of glucose from cassava starch hydrolysate and the other components of fermentation medium as same as previous experiment (4.1). By Kjeldahl method [48], the brewer's yeast autolysate (BYA) concentration at 48 ml/l corresponding to yeast extracts concentration of 5 g/l.

From Figure 4-16, it indicated that the lag time was increased when the BYA concentration increased. The BYA at 190 ml/l had longest lag time (24 h). Moreover, the lactic acid concentration of BYA 190 ml/l also lowest (46.41 g/l), while the lactic acid concentration of BYA 48 and 95 ml/l were resembled (Figure 4-18). From these results, it can be noticed that the inhibitory action occurred at the BYA 190 ml/l.

From Figure 4-19, it indicated that the maximum specific growth rate of three concentration of BYA was resembled. The highest productivity and lactic acid yield were

obtained from BYA 48 ml/l (3.61 g/l h, 1.55 g product/g substrate, respectively). It indicated that the suitable brewer's yeast autolysate (BYA) concentration for lactic acid production using *L.salivarius subsp. salivarius* at pH 5.5 and initial glucose was 48 ml/l.

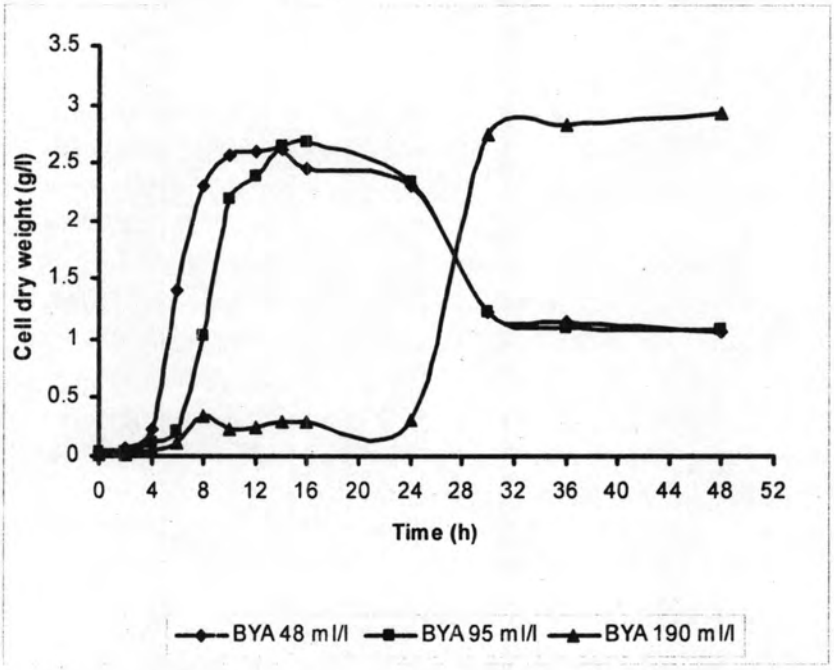

Figure 4-16 The effect of brewer's yeast autolysate (BYA) on cell growth of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741 (using 70 g/l of glucose from CSH)

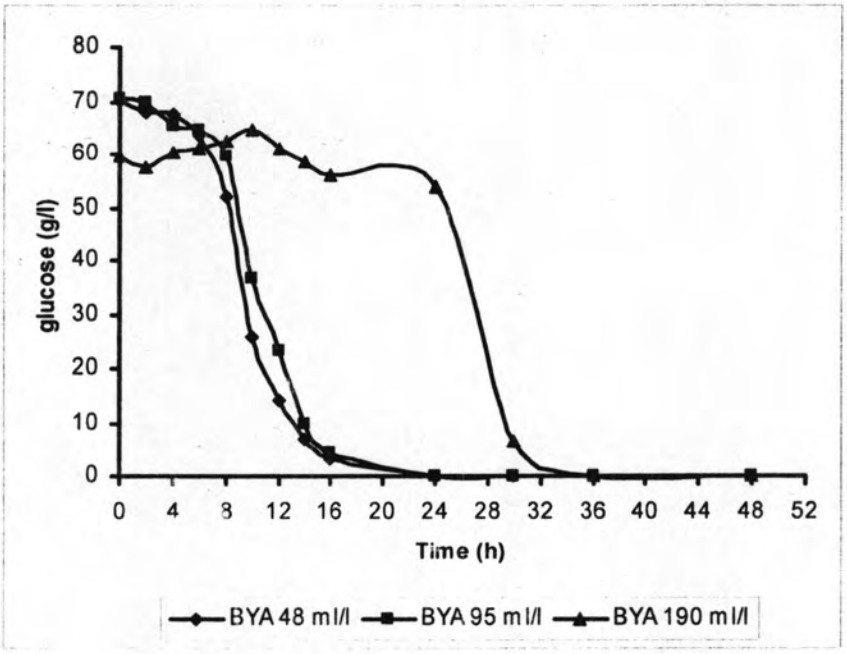

Figure 4-17 The effect of brewer's yeast autolysate (BYA) on glucose consumption of Batch fermentation of *L.salivarius subsp. salivarius* ATCC 11741(using 70 g/l of glucose from CSH)

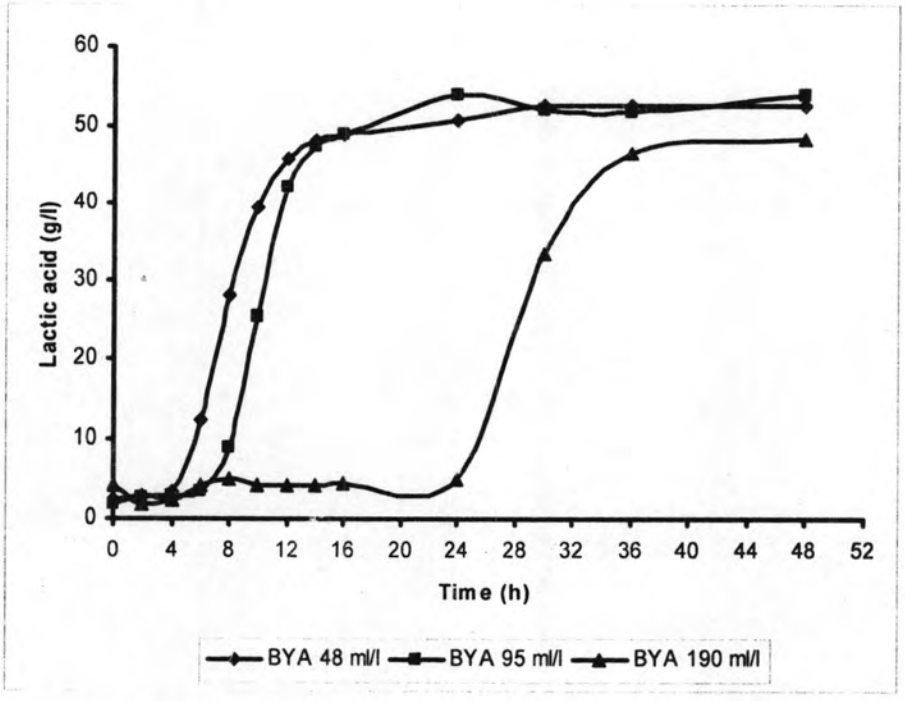

Figure 4-18 The effect of brewer's yeast autolysate (BYA) on lactic acid production of Batch fermentation of *L.salivarius subsp. salivarius* ATCC 11741(using 70 g/l of glucose from CSH)

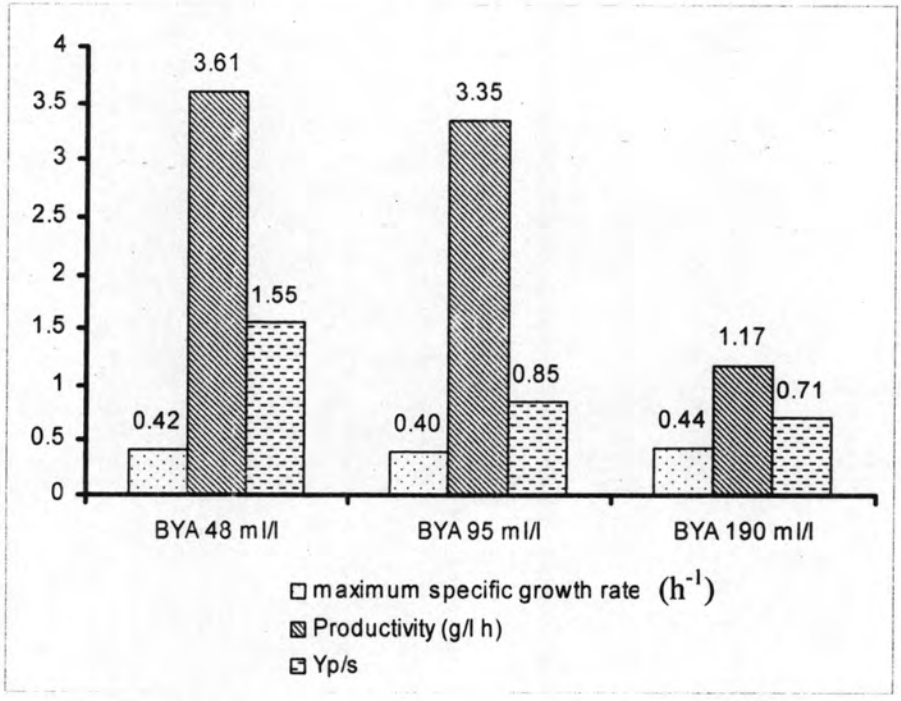

Figure 4-19 The effect of brewer's yeast autolysate (BYA) on kinetic parameters of Batch fermentation of *L.salivarius subsp. salivarius* ATCC 11741(using 70 g/l of glucose from CSH)

4.6 The comparison of yeast extract (YE) and brewer's yeast autolysate (BYA) on lactic acid production of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741.

The propose of this experiment is to compare yeast extract (YE) and brewer's yeast autolysate (BYA) on lactic acid production at the same condition (pH 5.5, temperature 37 ^{0}C, stirrer speed 100 rpm and CSH concentration 70 g/l). The YE and BYA were used at 5 g/l and 48 ml/l, respectively. The amount of total nitrogen of YE at 5 g/l and BYA at 48 ml/l were resembled (Analyzed by Kjeldahl method, [48]).

Figure 4-20 indicated that the cell dry weight of YE was higher than BYA (3.76 g/l and 2.61 g/l, respectively),whereas, lactic acid productivity and lactic acid yield (Figure 4-21) from BYA was higher than YE (3.61 g/l h, 1.55 g product/ g substrate for BYA and 3.06 g/l h, 1.00 g product/ g substrate for YE, respectively). This may be due to unavailability of vitamins, mineral and certain specific amino acids in yeast extract (YE) to the extent required by the organism [29]. The lactic acid bacteria generally have complex nutrient requirements to synthesis amino acids and vitamins for growth and fermentation [2]. From the above observed, the brewer's yeast autolysate may be used to produce lactic acid for reducing cost of nitrogen source.

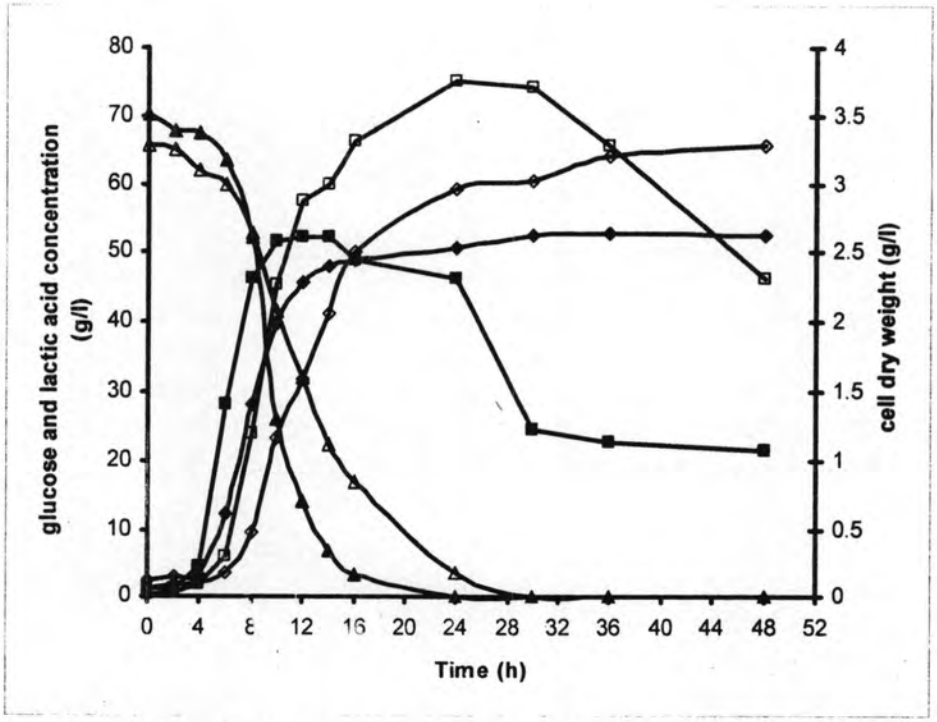

Figure 4-20 Batch fermentation of *L.salivarius subsp. salivarius* ATCC 11741 using glucose from CSH (70 g/l). (□) cell dry weight (g/l), (△) glucose concentration (g/l), (◇) lactic acid concentration (g/l) of yeast extract (YE); (■) cell dry weight (g/l), (▲) glucose concentration (g/l), (◆) lactic acid concentration (g/l) of Brewer's yeast autolysate (BYA)

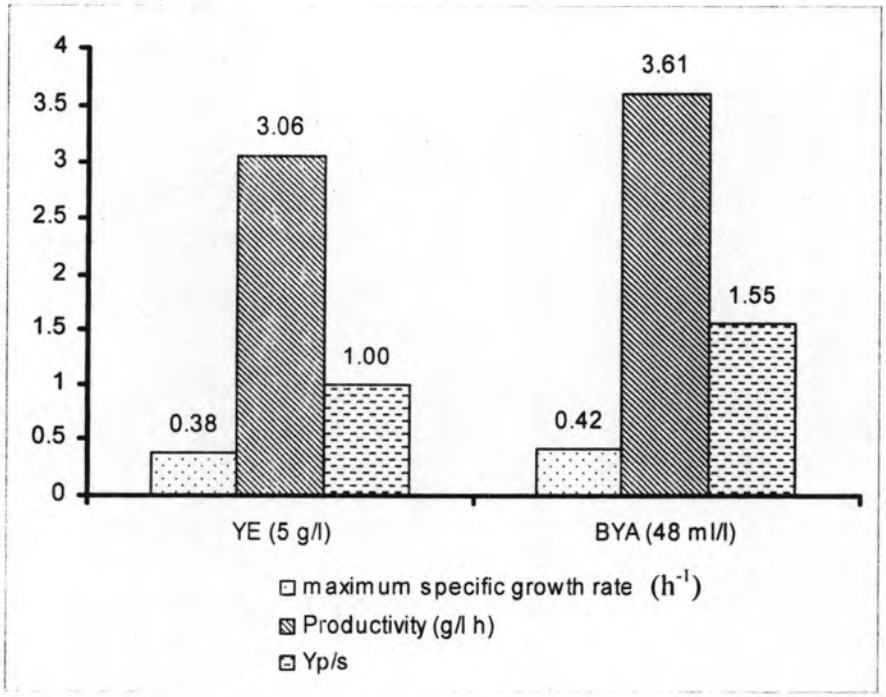

Figure 4-21 The effect of yeast extract (YE) and brewer's yeast autolysate (BYA) on kinetic parameter of Batch fermentation of *L.salivarius subsp. salivarius* ATCC 11741 using glucose from CSH (70 g/l)

4.7 The effect of the bitterness of brewer's yeast autolysate (BYA) on lactic acid production in batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

An autolysate is the total content after autolysis, or self-degradation, of the yeast. It is composed up of the particulate matter which is mainly the cell wall and debris suspended in the soluble fraction of the yeast. The brewer's yeast autolysate (BYA) contained the bitterness, iso-alpha acid (which is isomerized from the alpha acid that contained in hop), so, the propose of this experiment is to study the effect of the bitterness of brewer's yeast autolysate (BYA) on lactic acid production in batch fermentation using *L.salivarius subsp. salivarius* ATCC.

Figure 4-22 shows that glucose and lactic acid concentration of BYA were similar to the glucose and lactic acid concentration of BYA that debittering the bitterness, moreover, the specific growth rate and lactic acid yield were also resembled (0.48 h^{-1}, 1.21 g product/ g substrate, for BYA and 0.46 h^{-1}, 1.22 g product/ g substrate, for BYA (debitterness) (Figure.4-23). Whereas, the cell dry weight of BYA (1.91 g/l) was lower than BYA (debitterness)(2.39 g/l). The hop acids have pronounced bacteriostatic activity; they strongly inhibit the growth of Gram-positive bacteria. This action has been attributed to the interference of the prenyl group, characteristic of the side chains of the hop acids, with the function of the cell plasma membrane [24]. From above results, it shows that the bitterness of BYA didn't effect the lactic acid production but the debittering of the bitterness was good for cell growth.

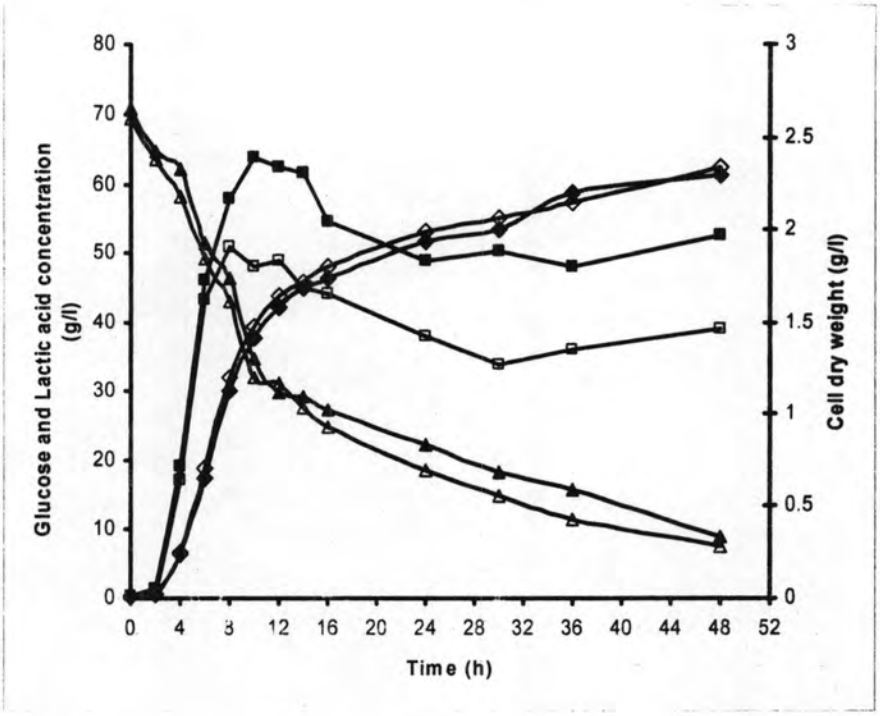

Figure 4-22 Batch fermentation of *L.salivarius subsp. salivarius* ATCC 11741 using glucose from CSH (70 g/l) and BYA (48 ml/l). (□) cell dry weight (g/l), (△) glucose concentration (g/l), (◇) lactic acid concentration (g/l) of Brewer's yeast autolysate (BYA); (■) cell dry weight (g/l), (▲) glucose concentration (g/l), (◆) lactic acid concentration (g/l) of Brewer's yeast autolysate (debitterness)

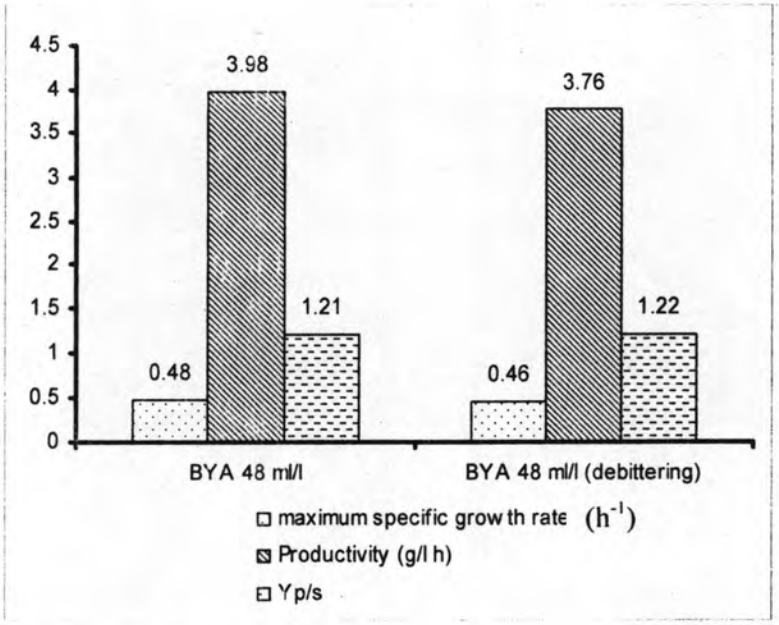

Figure 4-23 The effect of the bitterness of brewer's yeast autolysate (BYA) on lactic acid yield of Batch fermentation of *L.salivarius subsp. salivarius* ATCC 11741 using glucose from CSH (70 g/l) and BYA (48 ml/l).