# CHAPTER V

# RESULTS AND DISCUSSION

## 5.1 Morphology

Bacillus subtilis TISTR 25 that was cultivated in agar-based culture showed opaque white colonies with pretty rough rim. Its mature growth fully developed within 24 hours and turned a white colony into a light brown one as shown in Figure 5-1. In non-rotated nutrient broth, it formed a thin candle-drop-like over the clear broth. On the other hand, it grew well in a rotated condition, because oxygen was more transferred into microorganism. In complex medium [28], morphological growth of bacillus was as same as in nutrient broth. In microscopic observation, it had a single rod shape rather than in chain and endospore was found in stationary phase as shown in Figure 5-2.

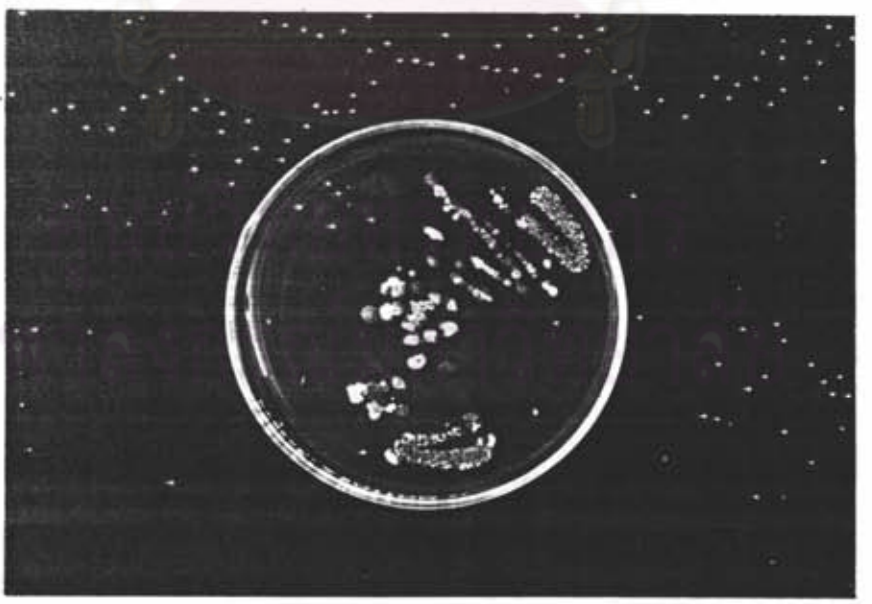
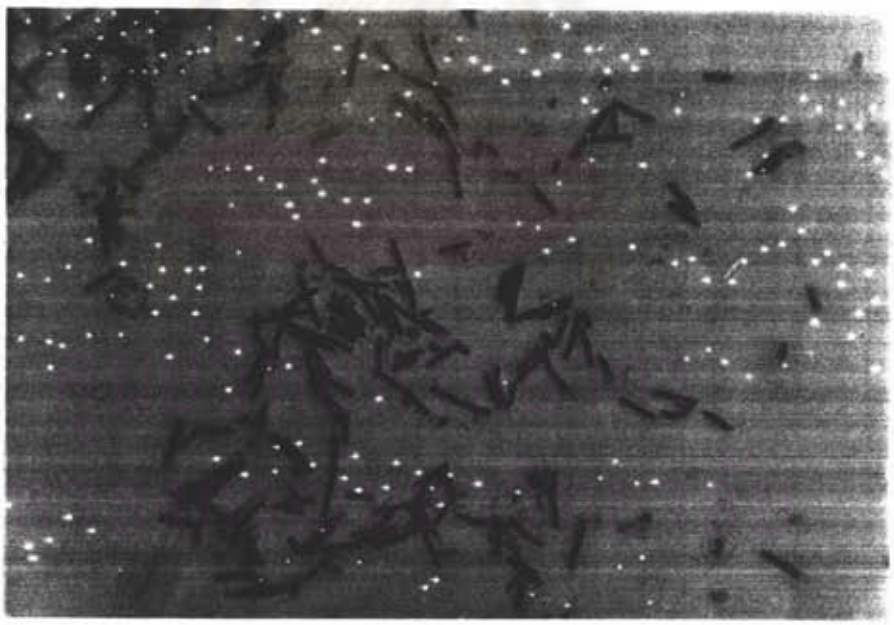


Figure 5-1 Colony forming of Bacillus subtilis TISTR 25

(A)



(B)

Figure 5-2 Microscopic observation of B. subtilis TISTR 25

(A) 400x - Enlargement (B) 1000x - Enlargement

## 5.2 The effect of controlled pH on growth and protease production in batch fermentation

The purpose of this experiment is to clarify the optimum pH for growth and protease production. The experiments were set up as batch fermentation processes by using 0.1 % $KH_2PO_4$, 0.05% $MgSO_4.7H_2O$ and 0.001% $CaCl_2.2H_2O$ as mineral substances together with 1.8 % starch hydrolysate and 2.0 % soy bean coupling with sunflower seed hydrolysate as a carbon and nitrogen source, respectively. During batch processes, pH (6.0, 6.5, 7.0, 7.5 and 8.0) and temperature (37.0°C) were controlled beyond maximum dissolved oxygen condition (Appendix B). Periodically, broth sample was withdrawn to analyze cell concentration, glucose concentration and protease.

Bacillus subtilis TISTR 25 has almost the same growth pattern in the experimental pH range (Figure 5-3 to 5-7). Approximately, its lag phase lasted 2 hours which there was no protease detected. Normally, active protease is always scientifically detected in unit activity form, so are other enzymes. In log phase, the bacillus consumed glucose and apparently rose up in cell concentration comparing to other phases. Considering on protease, the beginning of protease production was obviously found at the early log phase but was actually not associated with cell concentration. According to product and growth relationship, this protease production behavior is termed as mixed growth association. This is because the product is generated between log phase and stationary phase. Around the $18^{th}$ hour, the bacillus cell reached a stationary phase and proceeded constantly for almost all pH range except for pH 7.5 and 8.0 which their death rate took place around late stationary phase and were more than others as shown in Figure 5-6 and 5-7. That is why cell concentration relatively decreased in alkali condition.

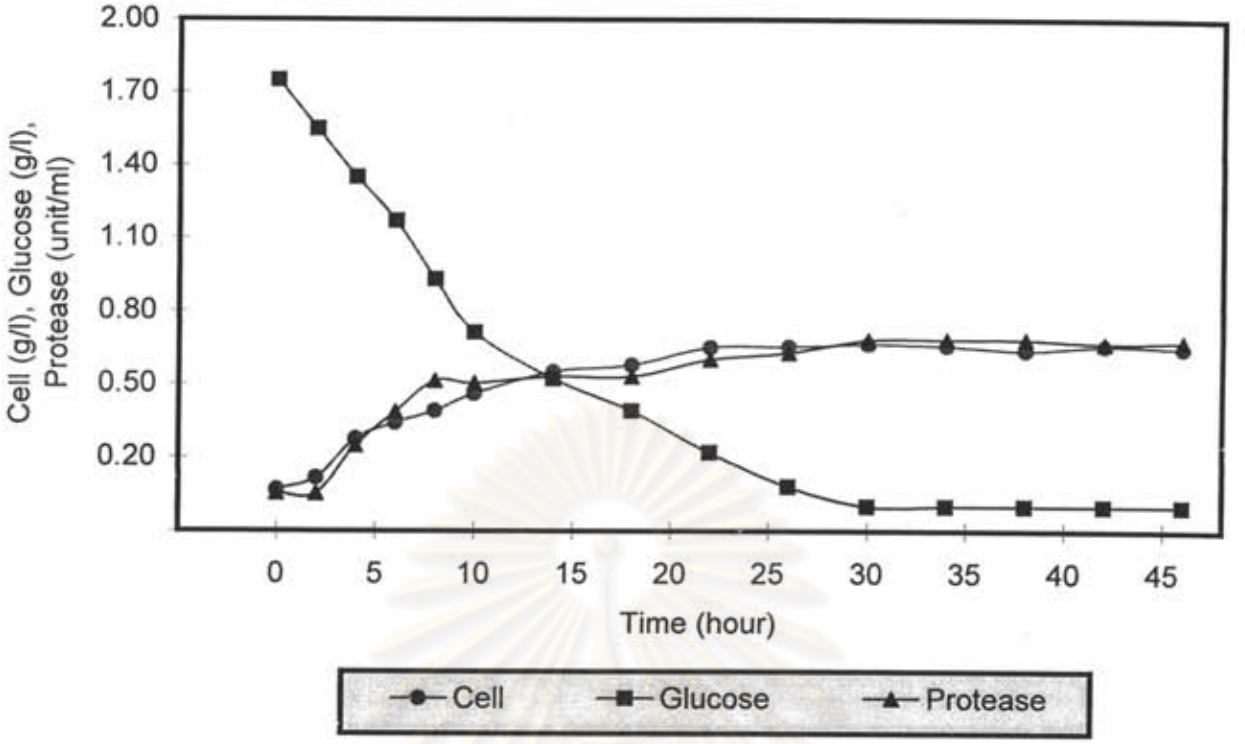



Figure 5-3 Batch Fermentation of B. subtilis TISTR 25 at controlled pH of 6.0

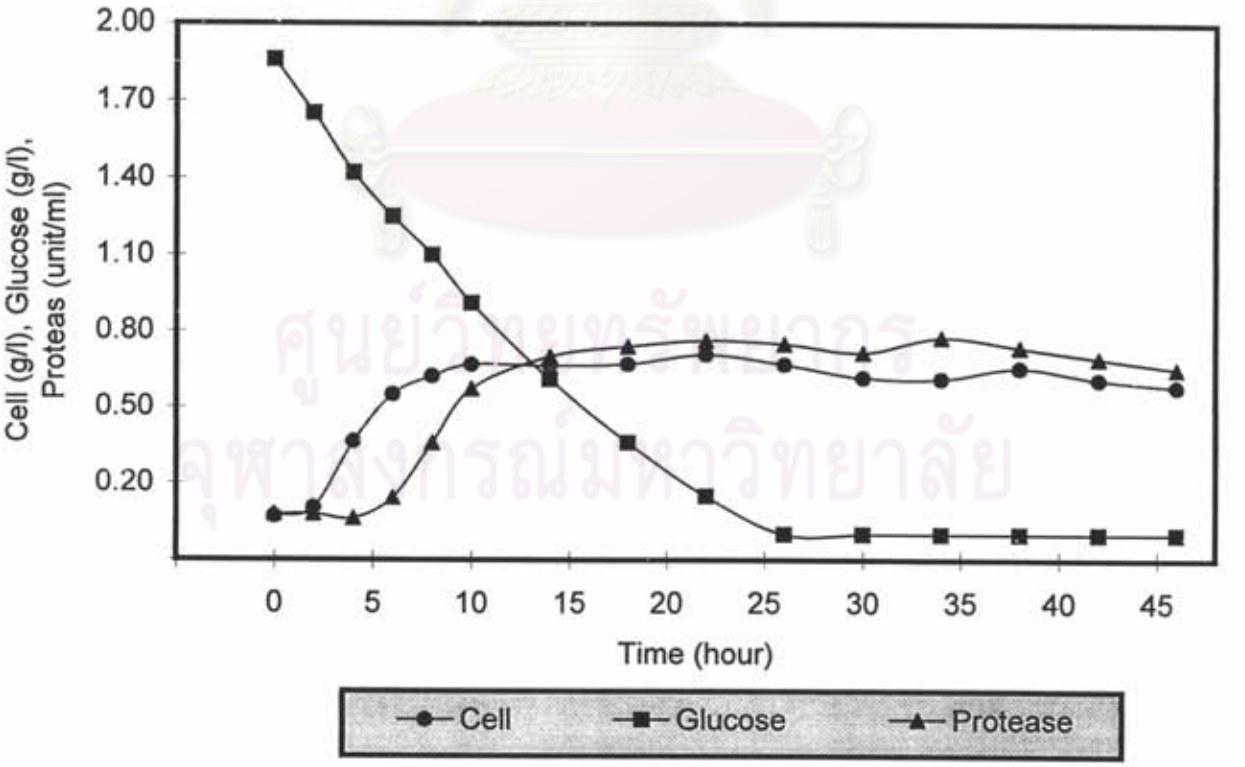



Figure 5-4 Batch Fermentation of B. subtilis TISTR 25 at controlled pH of 6.5

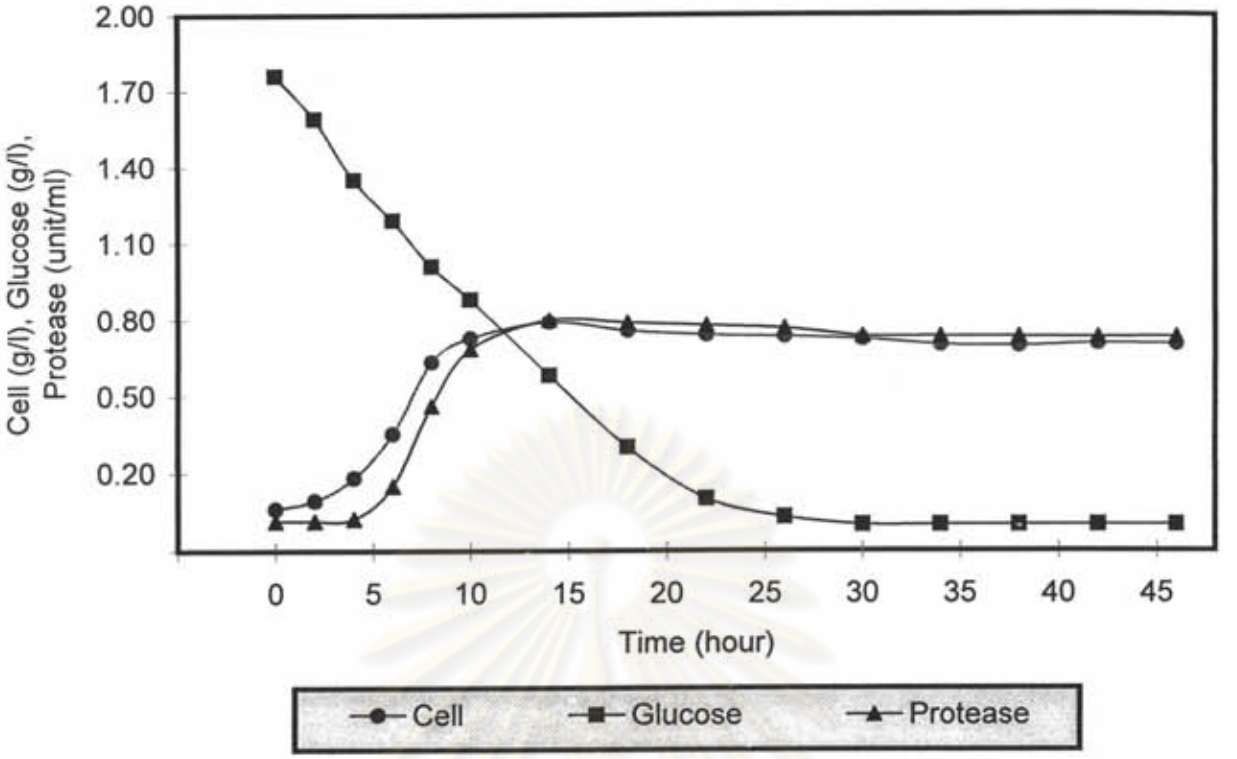



Figure 5-5 Batch Fermentation of B. subtilis TISTR 25 at controlled pH of 7.0

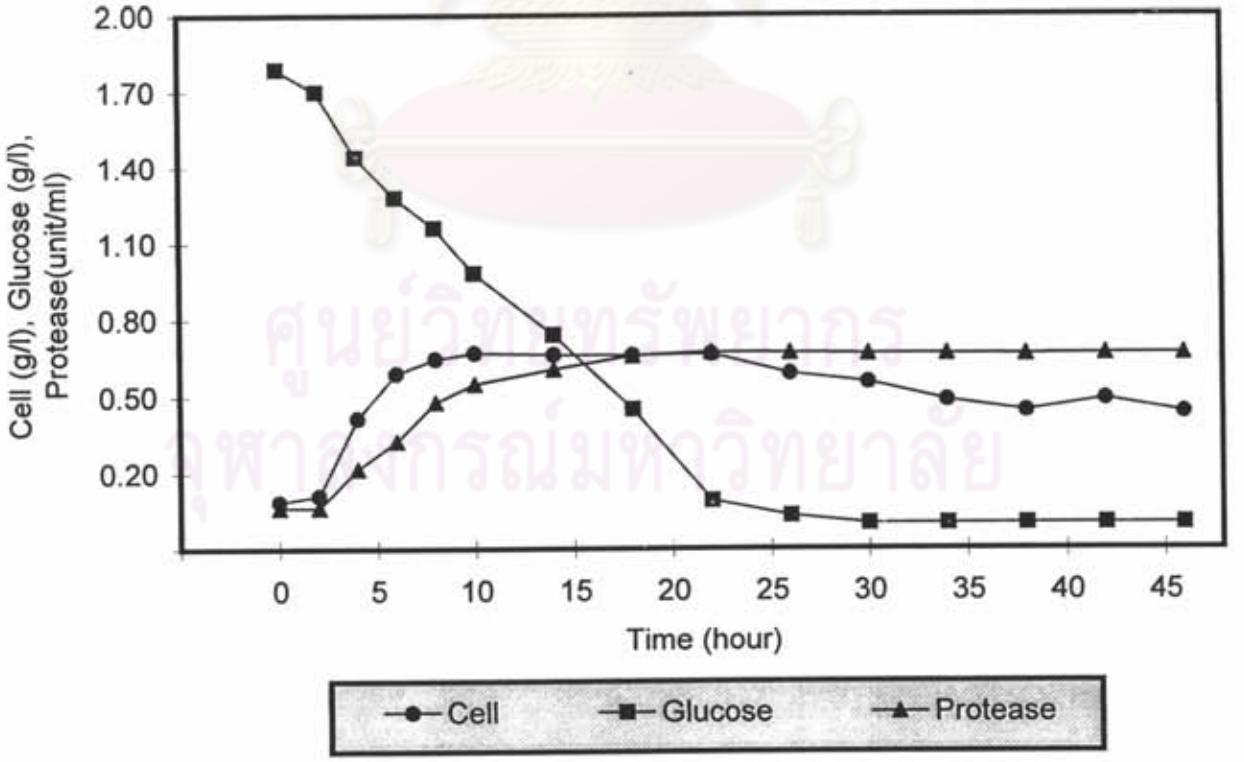



Figure 5-6 Batch Fermentation of B. subtilis TISTR 25 at controlled pH of 7.5

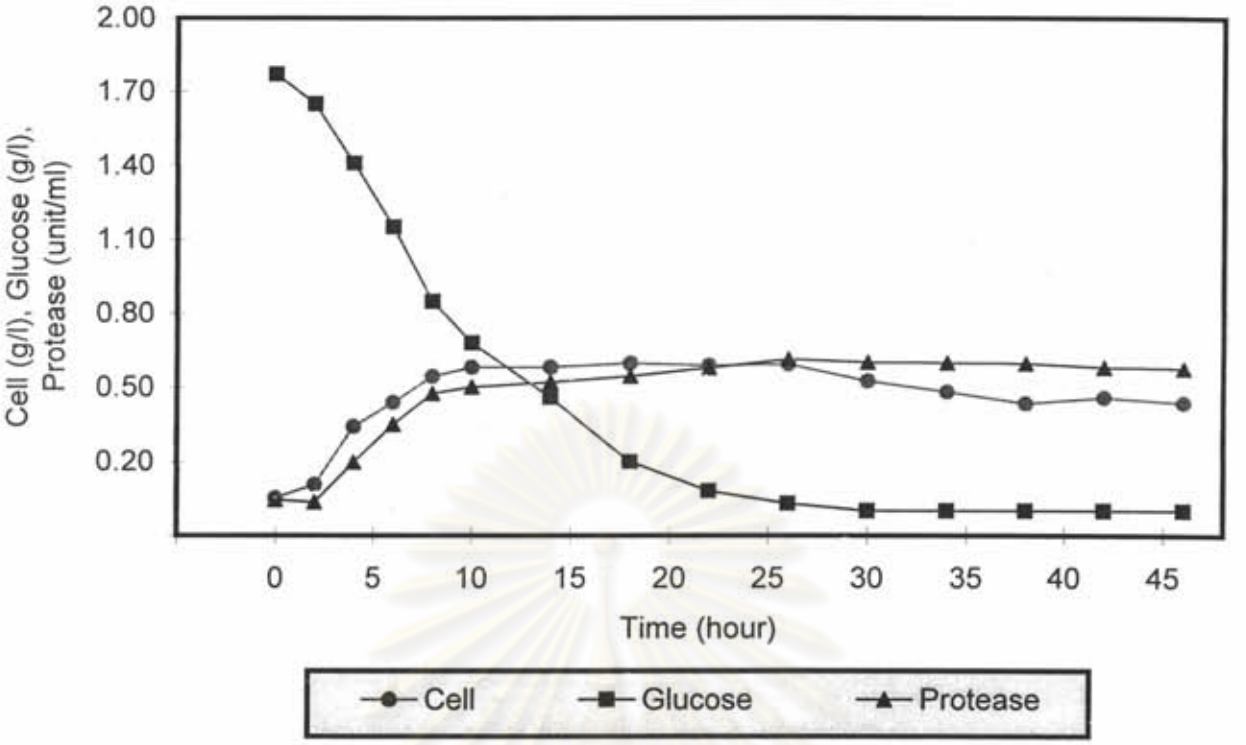



Figure 5-7 Batch Fermentation of B. subtilis TISTR 25 at controlled pH of 8.0

Main cause of the death is that cell lysis rate was faster than cell duplication rate. In spite of the fact that glucose was being consumed in the late stationary phase, the cell concentration was unstable, moreover, it decreased. It shows that glucose consumed in the stationary phase in alkali condition was employed to maintain the cell. It can be concluded that Bacillus subtilis TISTR 25 is not suitable to be cultivated in such alkali condition.

From Figure 5-3, at pH 6.0, it spent a long time to get cell concentration as much as others did, so the cultivation at controlled pH 6.0 was not an economically optimum condition for the growth. Investigating among various experimental pH ranges, the most effective growth was achieved in controlled pH 7.0 system. This system not only provided the maximum cell concentration (0.789 g/l) but also provided it the most quickly ($14^{th}$ hour). For cultivation in acid pH range (6.0 and 6.5), it took 30 and 22 hours to reach its maximum cell concentration, respectively. Furthermore, it took about 18 hours in the base pH range (8.0) for the maximum value. For neutral pH (7.0 and 7.5), it is faster than that of both acid and base pH

range and it was at the hour of 14 and 10, respectively as shown in Figure 5-8. The obtained graph resembled a statistically normal distribution histogram graph.

Being mixed growth association, protease production would follow the cell concentration, for instance, protease production decreased as well as the cell concentration did at pH 6.0, 6.5, and 7.0. As far as all batch experiment concerned, the best pH range for protease production is also at pH 7.0 which is the same as cell production. The combination effect of pH on cell and protease production is available at Figure A-3 in Appendix A. The maximum protease production was found in pH 7.0 batch experiment in the same way as that of the cell concentration that it is not only pH 7.0 system provided the maximum protease (0.798 unit/ml) but also provided it the most quickly ($14^{th}$ hour) as illustrated in Table 5-1 and Figure 5-8. The major advantage in choosing the condition which gives the highest product concentration is to decrease the cost of separation; however, the highest point of product concentration may spend a long time to achieve and that makes no economical reason to employ such a long time cultivation because of a lot of time-consuming and energy requirement. In order to show the effect of pH, the maximum product concentration is converted to a new kinetic parameter known as product productivity which is equal to the maximum product concentration divided by its cultivation time. From Figure 5-9, the protease productivity at different pH values also gave the same trend as shown in Figure 5-8.

Table 5-1 Maximum protease activities and their productivity

| pH | Protease activity (unit/ml) \| Cultivation time (hr) | Productivity (unit/ml-hr) |
|---|---|---|
| 6.0 | 0.681 \| 34 | 0.020 |
| 6.5 | 0.764 \| 22 | 0.035 |
| 7.0 | 0.798 \| 14 | 0.057 |
| 7.5 | 0.671 \| 22 | 0.031 |
| 8.0 | 0.614 \| 26 | 0.024 |

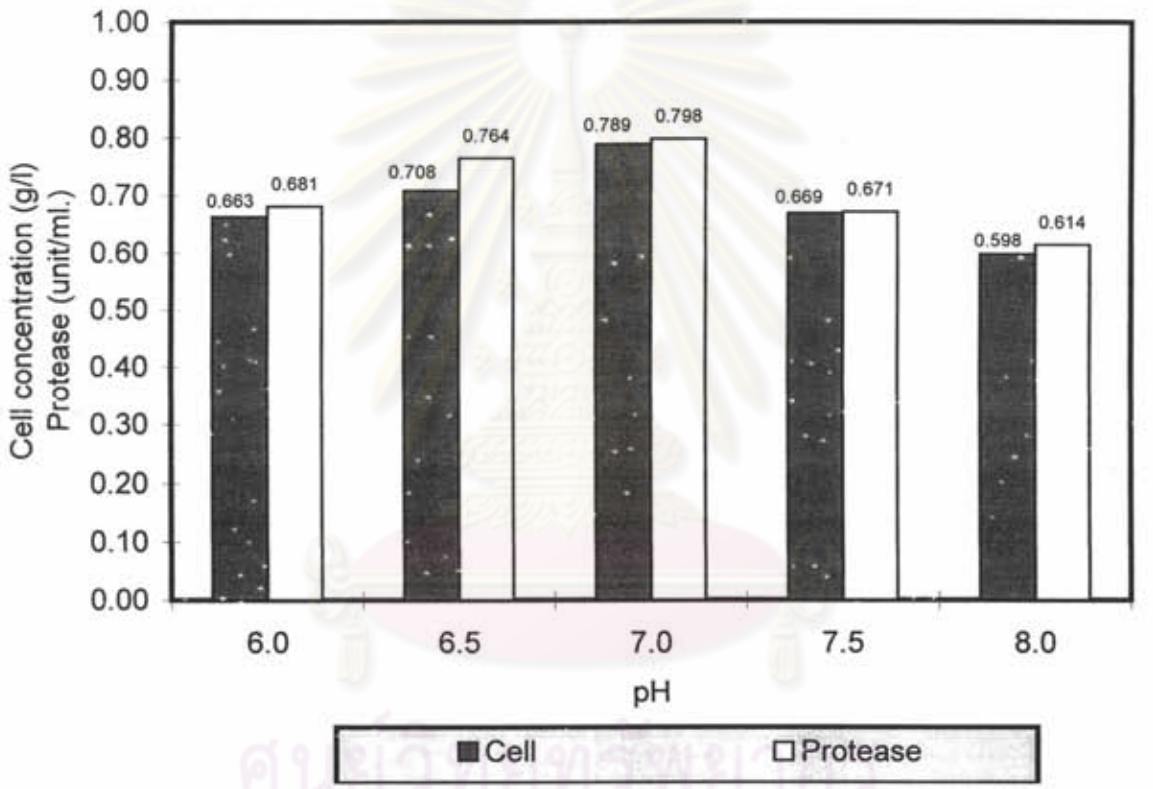



Figure 5-8 Maximum cell concentration and maximum activity of protease among experimental pH range.

The best condition which gave the highest protease productivity was also at controlled pH of 7.0 as shown in Figure 5-9.

Another kinetic parameters based on maximum protease production were also compared among experimental pH range. The cell concentration yield over substrate ($Y_{X/S}$) and protease yield over substrate ($Y_{P/S}$) had the same profile among experimental pH range that the highest peak was found at pH 7.0 (0.616 g-cell/g-glucose and 0.663 Act/mg-glucose, respectively). Moreover, not only cell concentration but also its quality which was defined as protease yield over cell concentration ($Y_{P/X}$) affected the protease production. The graph of protease yield over cell concentration also gave the similar profile which the highest peak was found at pH 7.0 (1.076 unit/mg-cell) as shown in Figure 5-10.

With the highest and fastest protease production, specific growth rate, specific product rate, and specific consumption rate at pH 7.0 was investigated to show kinetic behavior of Bacillus subtilis TISTR 25 in controlled pH 7.0 in batch fermentation. Their maximum values were spotted around mid-log phase as reported in Figure 5-11. Glucose was firstly consumed to produce cell and the highest specific consumption rate (1.101 g-glucose/g-cell-hr) was spotted at the 2 $^{nd}$ hour. After the cell was generated with the maximum specific growth rate (0.322 $hr^{-1}$) at the 4$^{th}$ hour, the glucose was lastly consumed to produce protease which the maximum specific product rate (0.316 unit/mg-cell-hr) was noticed at 8 $^{th}$ hour. The specific product rate curve versus cultivation time resembled the right hand shifted curve of the specific growth rate which the shift effect was not excess the stationary zone of specific growth rate curve. This behavior was termed as mixed growth association.

As far as all kinetic parameters confirm, the best condition for cell and product cultivation is at controlled pH at 7.0 in batch fermentation process.

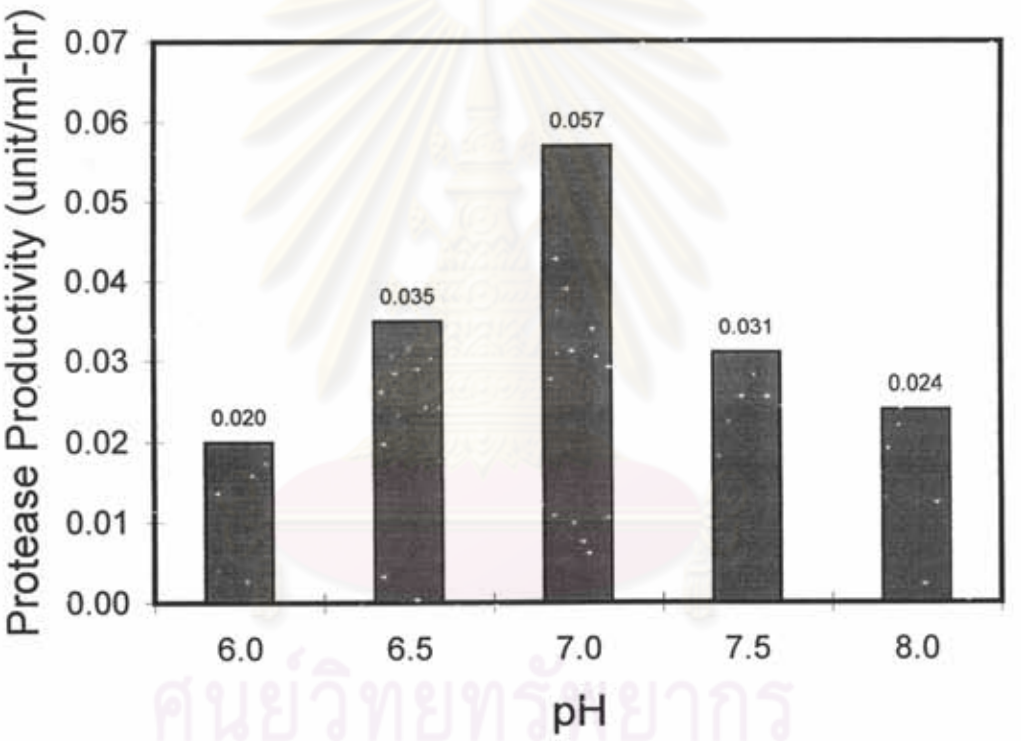





Figure 5-9 Protease productivity based on maximum protease comparison between various experimental pH range.

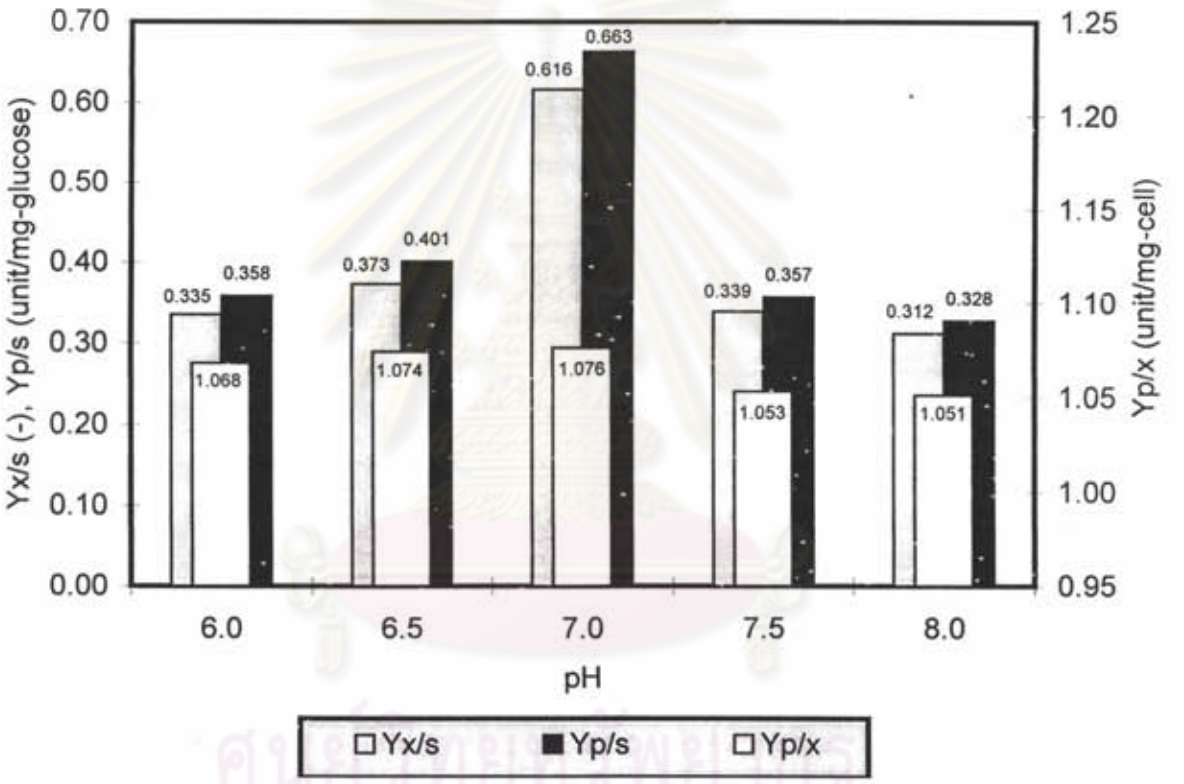



Figure 5-10 $Y_{x/s}$ , $Y_{p/s}$ and $Y_{p/x}$ based on maximum protease production among experimental pH range.

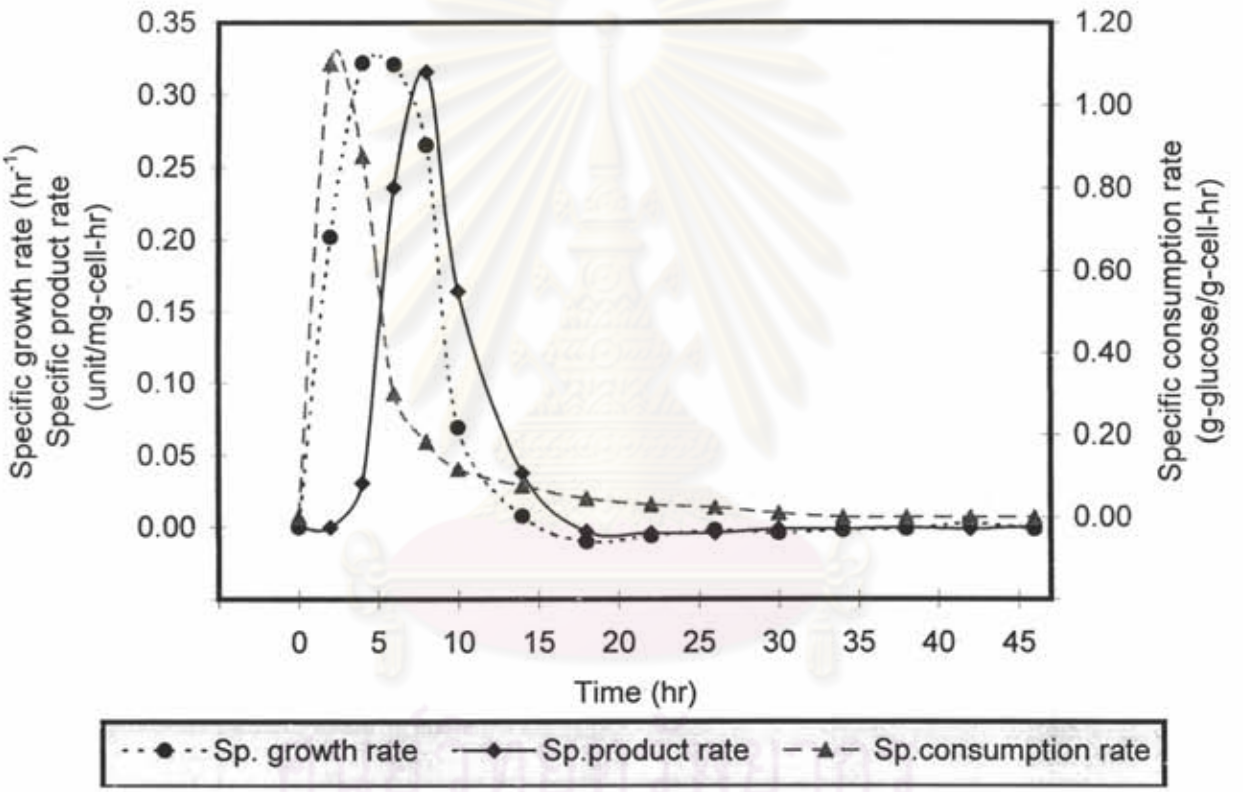



Figure 5-11 Specific growth rate, specific product rate, and specific consumption rate at pH 7.0

### 5.3 The effect of initial glucose concentrate on growth and protease production in batch fermentation.

The aim of this experiment is to clarify the optimum initial glucose concentration for protease production together with retrieving the growth curve of Bacillus subtilis TISTR 25 from varying starch hydrolysate concentrations (equivalent to 1.0, 1.8, 3.2, 4.5, and 7.5 g/l of glucose) in batch fermentation. During batch processes, pH 7.0 and 37°C were controlled beyond maximum dissolved oxygen condition, Periodically, the broth sample was withdrawn to analyze parameters as same as that of pH-varying experiment.

Obviously, an increase in the initial glucose concentration from 1.0 to 7.5 g/l affected the growth of Bacillus subtilis TISTR 25. As illustrated in Figure 5-12, the stationary phases of each batch were plotted to show the effect of initial glucose concentration. In log phase, the cell rose up while glucose depleted. After both of them approached to stationary phase, the maximum cell concentration of each batch was obtained around at the $14^{th}$ hour to the $18^{th}$ hour. The higher initial glucose was employed, the higher cell concentration was earned. After that, the cell concentration started to level off at the initial glucose concentration of 4.5 g/l. In Figure 5-13, the initial glucose concentration versus protease activity did not show consistent relationship between each other. The maximum protease activity (0.798 unit/ml) was obtained in 1.8 g/l initial glucose concentration experiment in which the maximum cell concentration (1.070 g/l) was not achieved. The same result has been stated in shaken flask fermentation experiment [28]

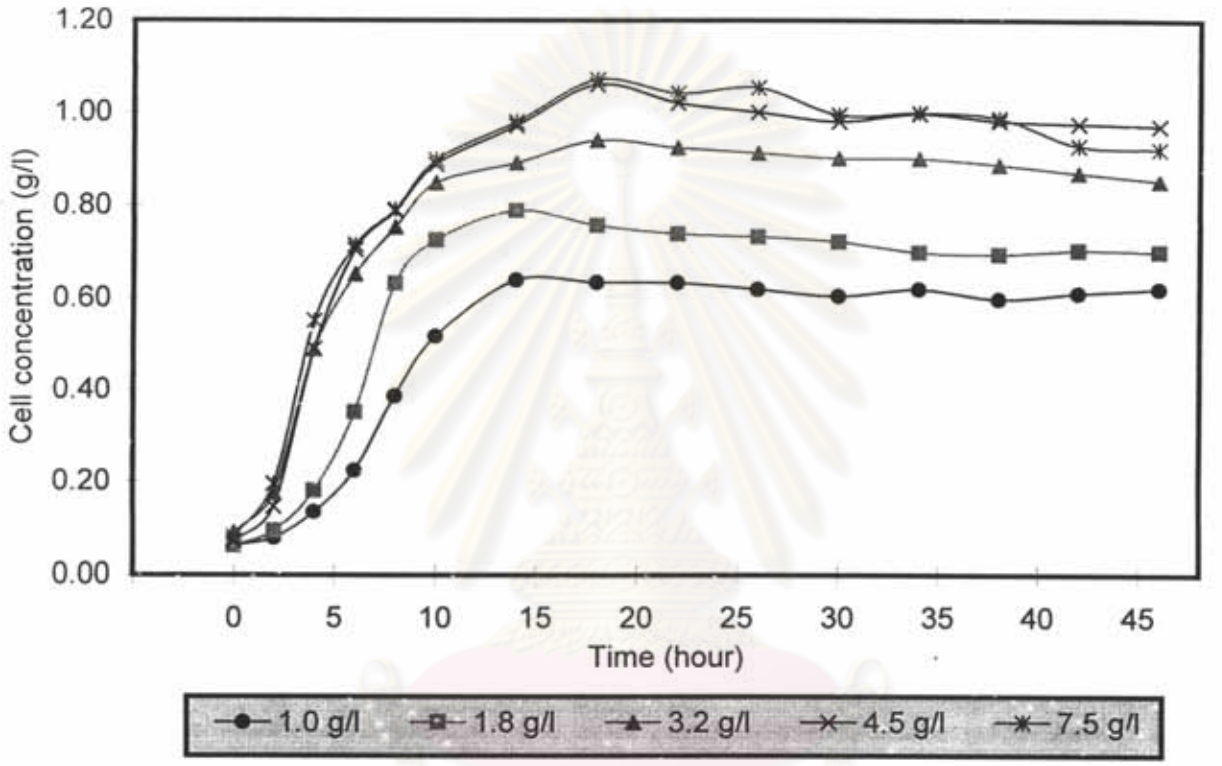



Figure 5-12 Cell concentration comparison among various initial glucose concentration

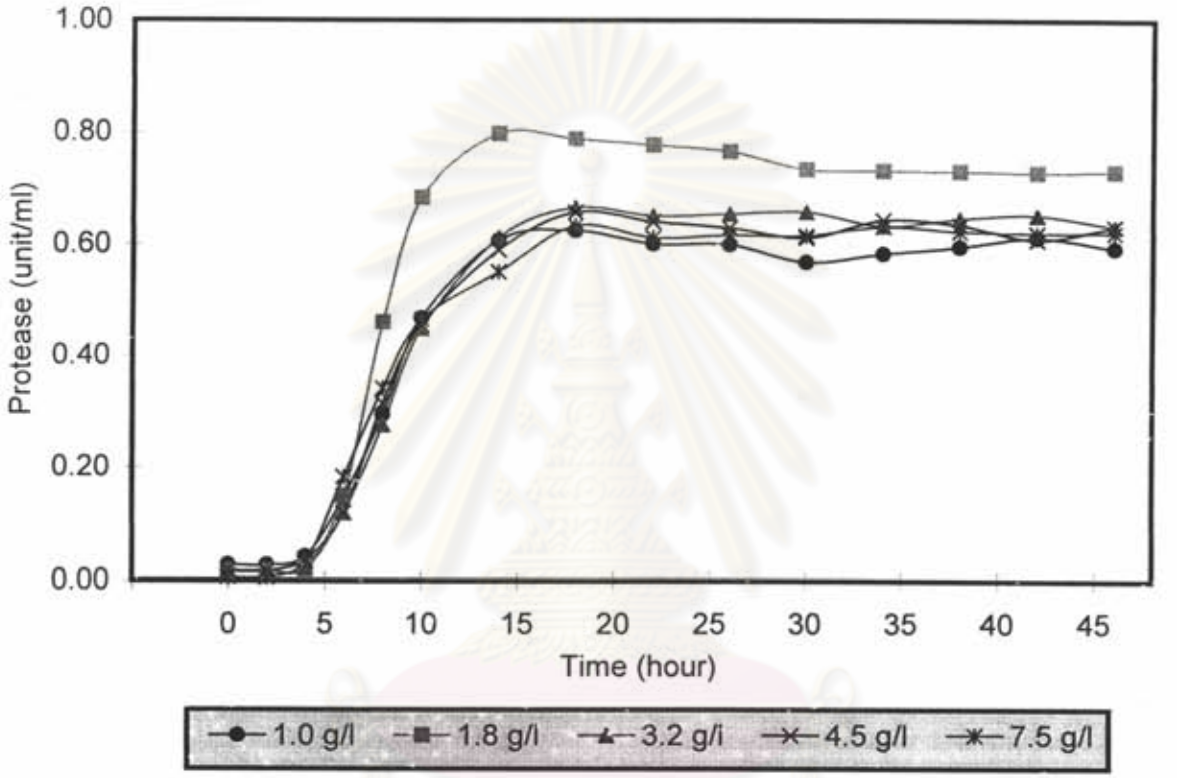



Figure 5-13 Protease comparison
among various initial glucose concentration.

In kinetic parameter investigation, both $Y_{P/S}$ and $Y_{P/X}$ had the same profile which both highest peak (0.663 unitt/mg-glucose of $Y_{P/S}$ and 1.076 unit/mg-cell of $Y_{P/X}$) were obtained at the 1.8 g/l initial glucose concentration. But $Y_{X/S}$ had a increasing profile with initial glucose concentration from 1.0 to 4.5 g/l, but there came a little decrease since the glucose concentration of 7.5 g/l as plotted in Figure 5-14. The cell productivity and protease productivity were also observed as shown in Figure 5-15. The relationship between cell productivity and initial glucose concentration showed the identical profile with $Y_{X/S}$. At the low initial glucose concentration, there came little bit difference that was a sharper slope for cell productivity but not exist in $Y_{X/S}$ curve. Furthermore, the protease productivity resembled both $Y_{P/S}$ and $Y_{P/X}$ when it was plotted against initial glucose concentration .The highest protease productivity was 0.057 unit/ml-hr. Like cell productivity, the relationship between specific growth rate and initial glucose concentration at the beginning showed the same profile. From Figure 5-16 the maximum value of both specific product rate and specific consumption rate were 0.316 unit/mg-cell-hr and 1.101 g-glucose/g-cell hr at the initial glucose concentration of 1.8 g/l, respectively.

In conclusion, as much as many kinetic parameters dealt with protease were confirmed by their maximum value, the best initial glucose concentration for protease production was 1.8 g/l although the cell concentration and cell production rate was not much.

The growth curve of Bacillus subtilis TISTR 25 was retrieved by plotting inverse value of both initial glucose concentration and initial specific growth rate (Lineweaver-Burk plot) as illustrated in Figure 5-17. The obtained slope and interception at inverse glucose concentration axis represented $Ks/\mu_{max}$ and $1/\mu_{max}$, respectively. The evaluated constants from Lineweaver-Burk plot (Ks = 0.98 g/l and $\mu_{max} = 0.52\ hr^{-1}$) was closed to those from growth curve estimation (Ks = 1.0 g/l and $\mu_{max} = 0.52\ hr^{-1}$) as shown in Figure 5-18.

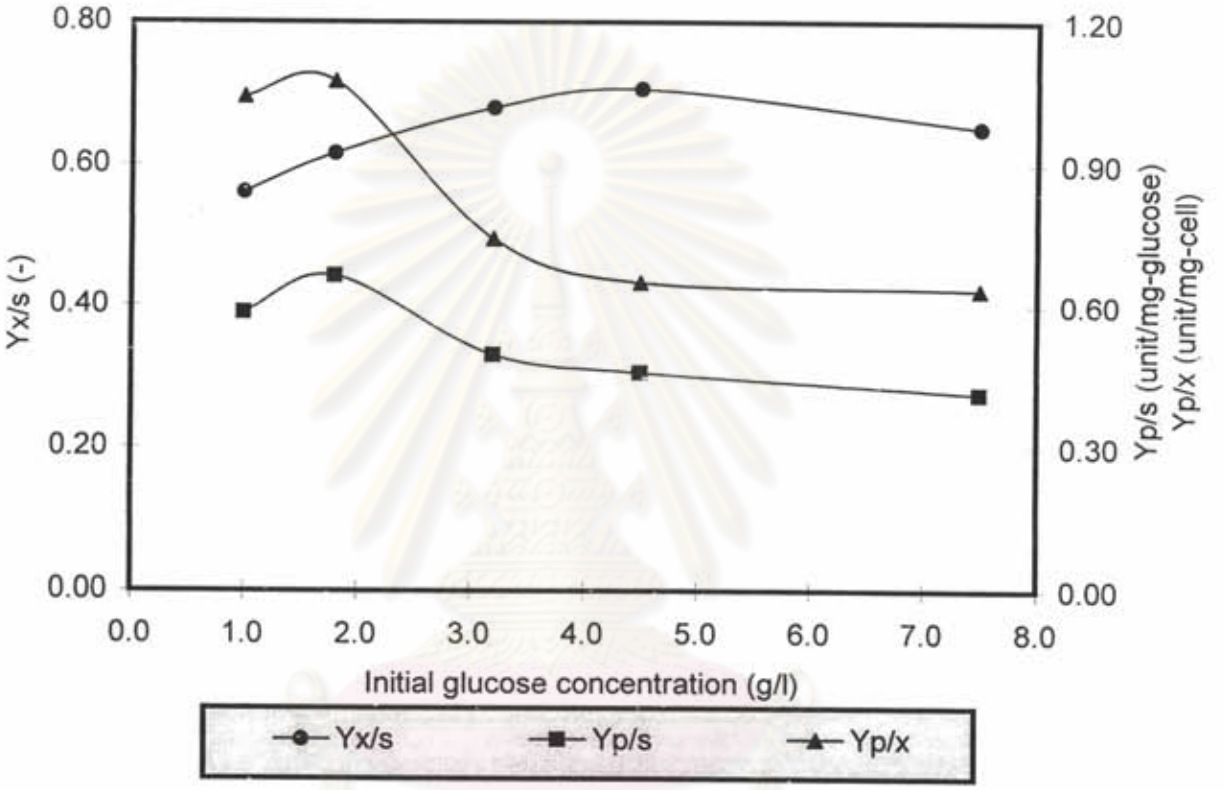





Figure 5-14 $Y_{x/s}$, $Y_{p/s}$, and $Y_{p/s}$ among various initial glucose concentration.

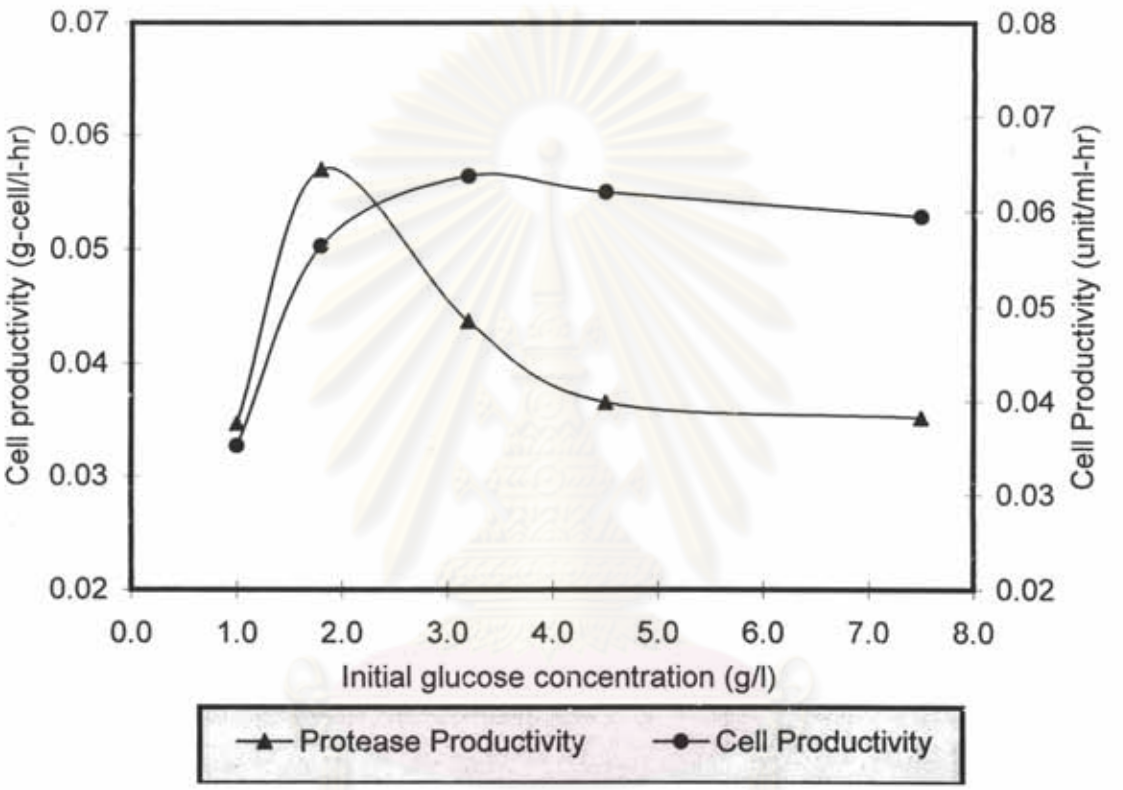





Figure 5-15 Cell productivity and Product productivity among various initial glucose concentration.

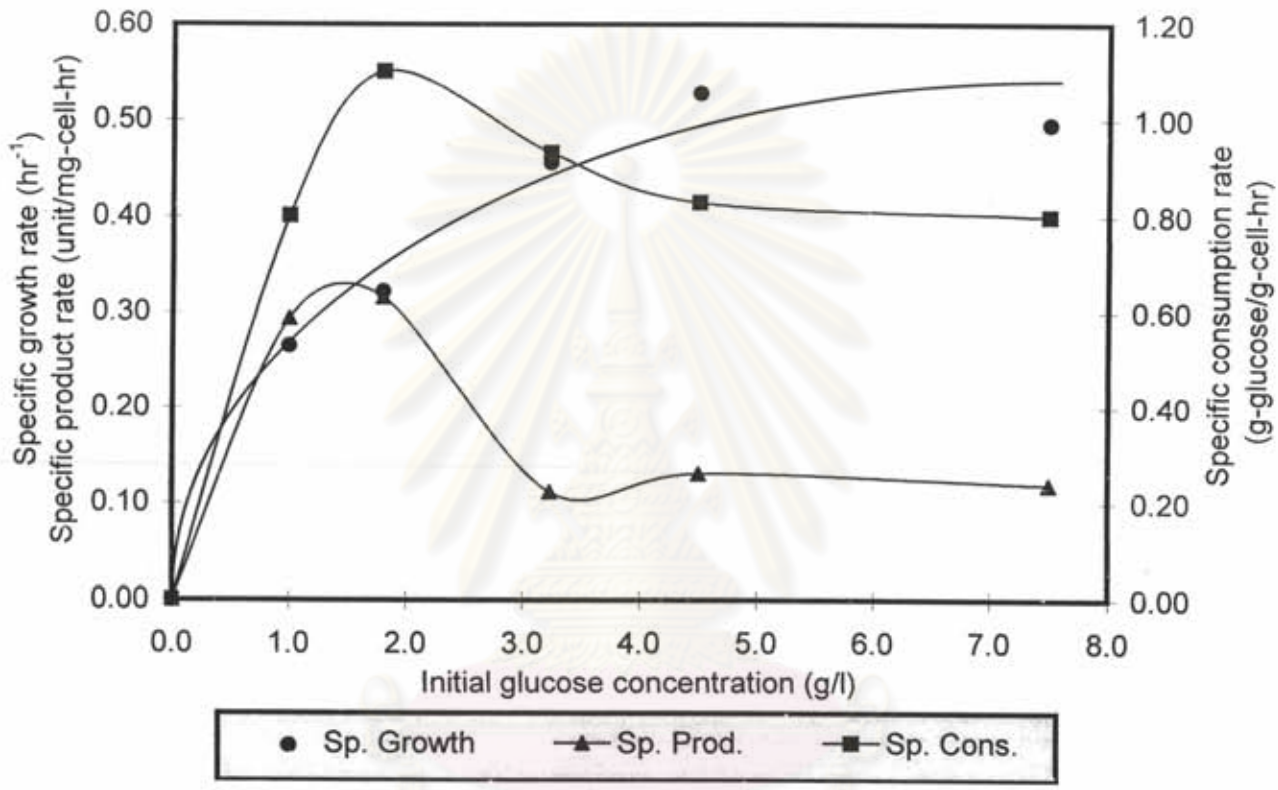



Figure 5-16 Specific product rate, specific growth, and specific consumption among various initial glucose concentration.

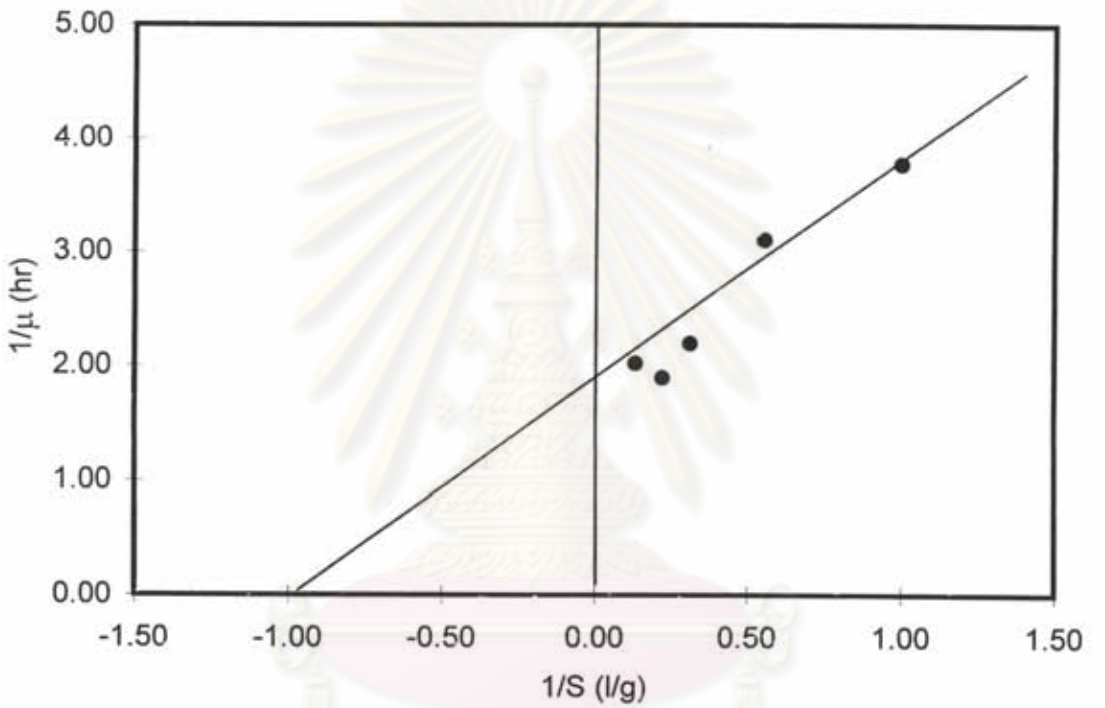





Figure 5-17 The Lineweaver-Burk plot of Bacillus subtilis TISTR 25 growth

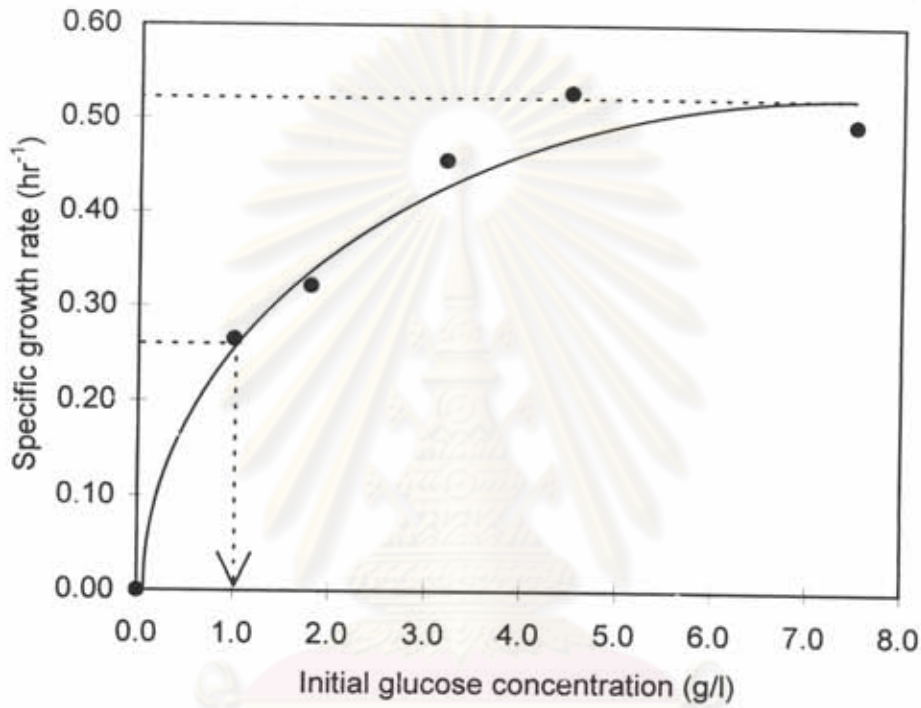





Figure 5-18 The growth curve of Bacillus subtilis TISTR 25

## 5.4 The study of microfiltration characteristics

Ceramic membrane filter type 1M-1, Japan was equipped with a continuous fermenter as a microfiltration unit. The study for characteristics took place over 4 cell concentrations whereas 5 pressure drop and 3 recirculation flow rate were varied. A calculated permeate flux was an effective criteria to show the performance of the microfiltration membrane which was calculated by dividing the permeate flow rate with filtration area (0.2030 $m^2$).

### 5.4.1 The effect of applied pressure on permeate flux

The permeate flux will linearly increase with transmembrane pressure while distilled water was filtered through a ceramic membrane.

At various microbial suspensions, the permeate flux was non-linearly increased with increasing applied pressure from $0.0^+$ to $0.8^+$ $kg_f/cm^2$ because the so-called concentration polarization occurred that made an increasing resistant which is resulted from increasing the applied pressure. The more pressure was introduced to a system, the less increasing permeate flux was detected as illustrated in a less slope at high applied pressure in Figure 5-19.

For higher concentration, the permeate flux was almost constant over a high applied pressure ($0.6^+$ to $0.8^+$ $kg_f/cm^2$) at the cell concentration of 8.70 and 14.42 g/l. The reason why the permeate flux is constant is that a gel layer was formed over filtration areas and acted as gel resistance which would lessen the permeate flux. Although pressure is one of the driving force, it can cause a resistive gel. The higher pressure was introduced, the thicker gel accumulated on membrane surface was found and this made permeate flux reach a certain maximum point, then became relatively constant with any further applied pressure; however, higher concentrations would lessen the permeate flux comparing to the lower ones as shown in Figure 5-20

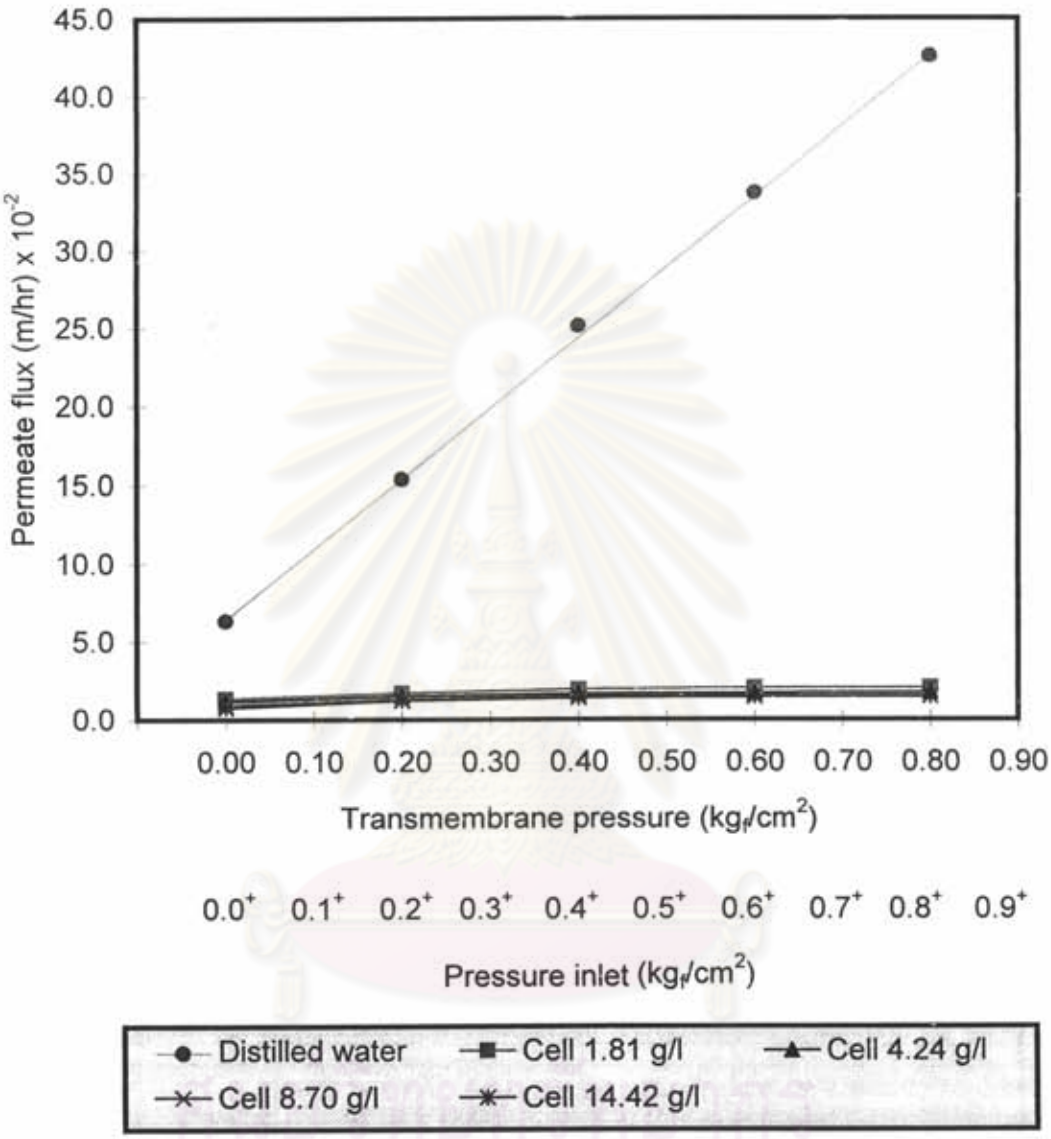



Figure 5-19 The effect of applied pressure on permeate flux of distilled water and fermentation broth
- Operating temperature of 37 °C
- Recirculation flow rate of 0.4 $m^3$/hr

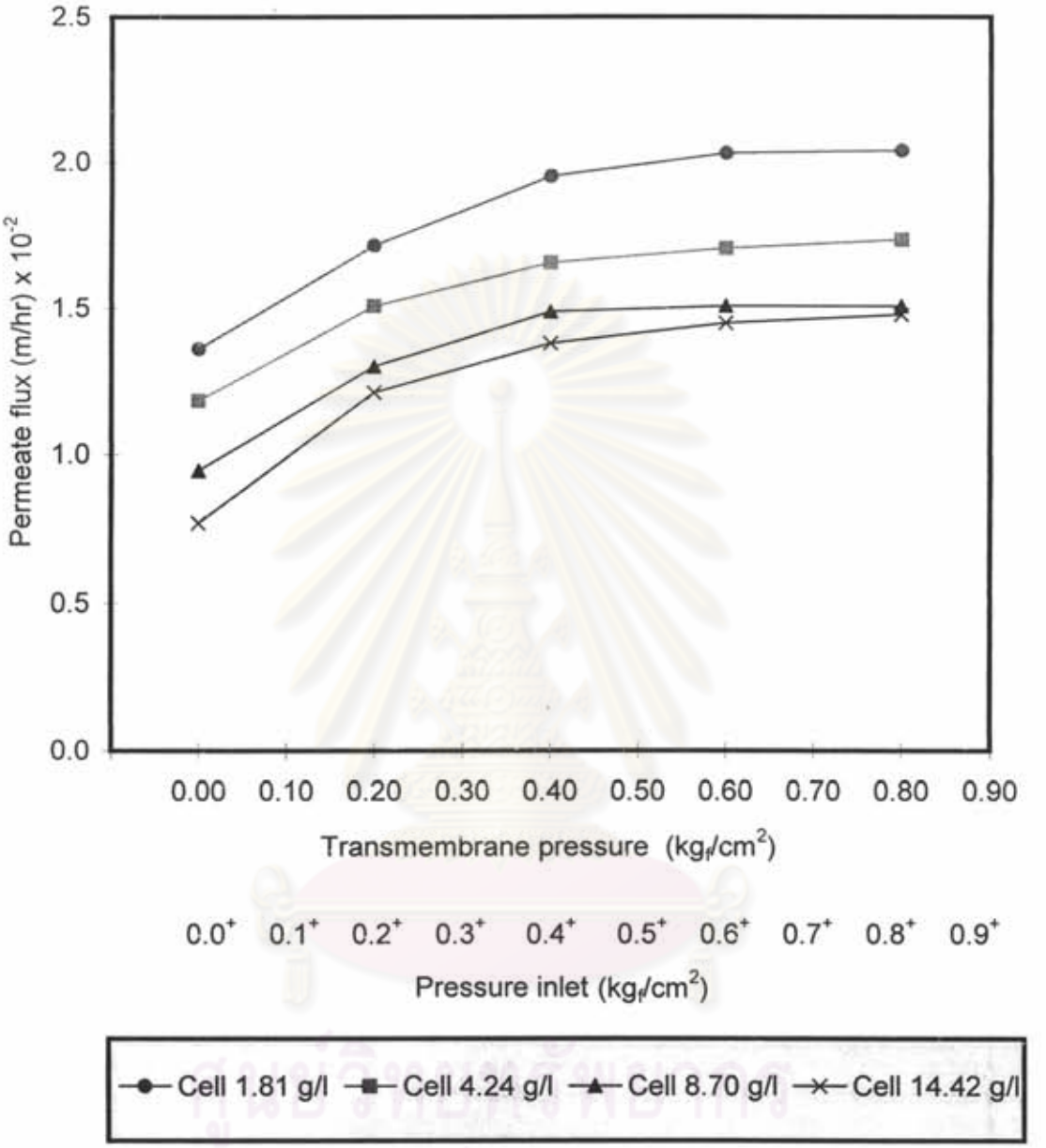



Figure 5-20 Permeate flux of fermentation broth
as a function of applied pressure
- Operating temperature of 37 °C
- Recirculation flow rate of 0.4 $m^3$/hr

It was indicated that the applied pressure of more than $0.0^{+}$ $kg_f/cm^2$ would not only cause rapid fouling of membrane but also cause an increase in $\Delta P$ which enhanced the fluid velocity and shear force, subsequently. The more shear force applied on microbial cell, the more breaking opportunity of cell appeared [30]. From these two reasons, the applied pressure of $0.0^{+}$ $kg_f/cm^2$ was selected for a further experiment of studying the influence of recirculation flow rate.

The plus superscript (+) over applied pressure was defined in order to correct the real applied pressure. The inlet pressure gauge should have indicated more than zero value while fluid flowed through the pipe and should be at least equal to the pressure drop in that pipe, which was affected by many parameters such as Reynold number, dimension, shape and smoothness of pipe, etc. Therefore, the plus superscript over the applied pressure was intended to correct the rough-scaled inlet pressure gauge by adding the pressure gauge readings with the pressure drop.

### 5.4.2 The effect of recirculation flow rate on permeate flux

The permeate flux increased with the recirculation flow rate (0.4, 0.5 and 0.6 $m^3/hr$) as illustrated in Figure 5-21

As far as the theory of microfiltration concerned, the mass transfer coefficient (k) increased with the square root of recirculation flow rate in laminar flow and almost be linear in turbulent. The increasing of recirculation flow rate enhanced the shear force at the membrane surface, hence the thickness and resistance of the gel layer decreased with recirculation flow rate. On the other hand, the recirculation flow rate would lessen the pressure drop across membrane (transmembrane pressure, $\Delta PTM$). But the previously mentioned effect was very low in comparison with the effect of reducing gel resistance and increasing the mass transfer coefficient. Therefore, the permeate flux increased with recirculation flow rate . The higher cell concentration was introduced, the less permeate flux was obtained. Such the high turbulent condition at employing 0.5 and 0.6 $m^3/hr$ of recirculation rate, there came

some problems that were difficulty in level control in fermenter, foaming of the broth and disintegrated microorganism. Although the foam problem could be diminished by introducing antifoam into the fermentor, the antifoam agent always lessened the performance of oxygen transferation by reducing its coefficient which caused the microorganism suffocated. So the selected recirculation flow rate for application in cell recycle system was set at 0.4 $m^3$/hr.

### 5.4.3 The effect of cell concentration on permeate flux

Under the applied pressure of $0.0^+$ $kg_f/cm^2$ and the recirculation flow rate of 0.4 $m^3$/hr, a set of experiment was done on studying the effect of four cell concentrations (1.81, 4.24 8.70 and 14.42 g/l). The permeate flux was reduced with increasing cell concentration as shown in Figure 5-22

According to gel polarization theory, flux is a function of the bulk cell concentration following $J = k \ln (C_g/C_b)$ as plotted in Figure 5-23. The slope (2.86 x $10^{-3}$ m/hr) obtained from the previous equation was the mass transfer coefficient and $C_g$ could be evaluated from extrapolated curve to the permeate free position which was approximately 230 g/l .

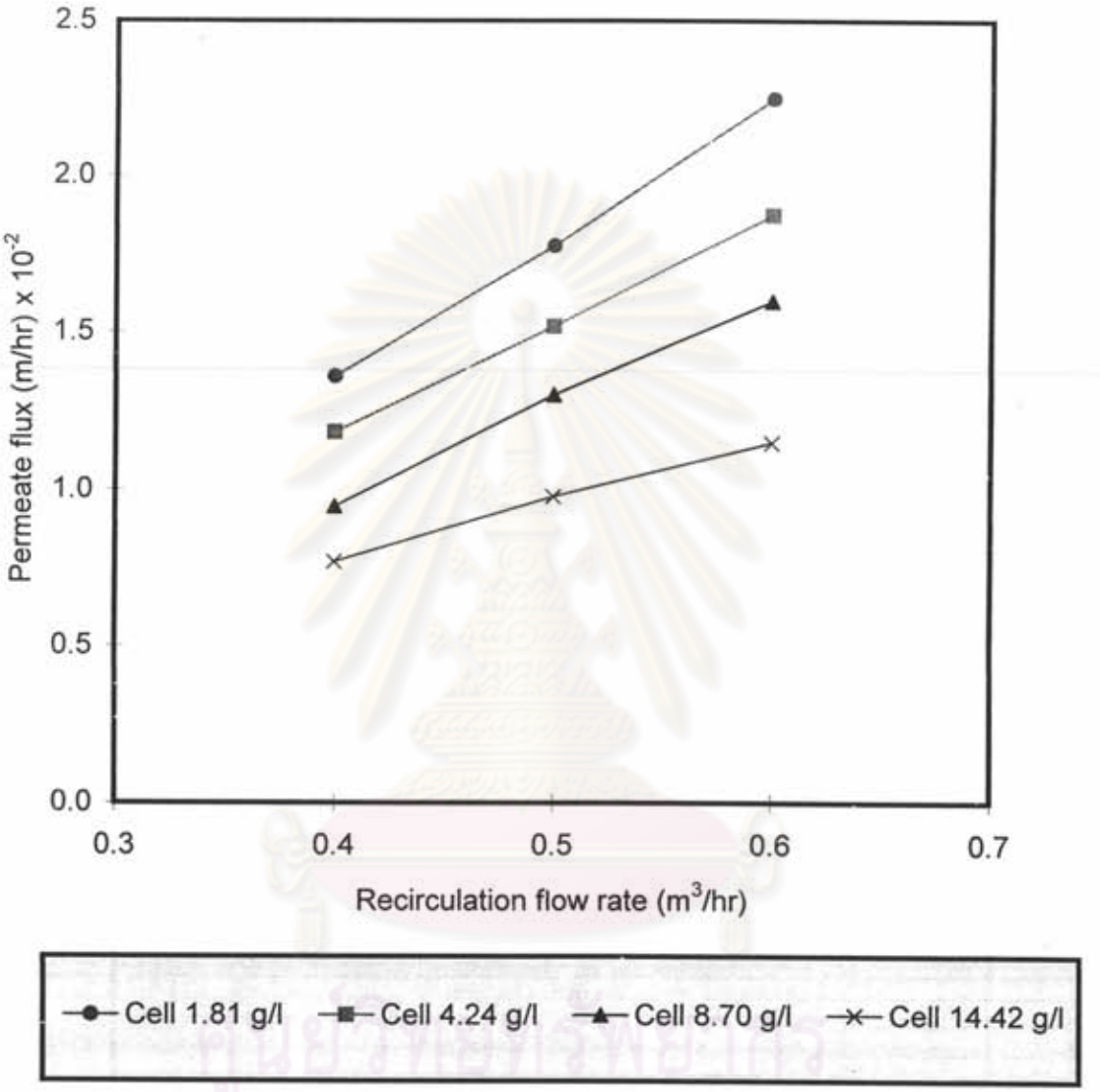



Figure 5-21 Permeate flux of fermentation broth
as a function of recirculation flow rate
- Operating temperature of 37 °C
- Applied pressure of $0.0^{+}$ $kg_f/cm^2$

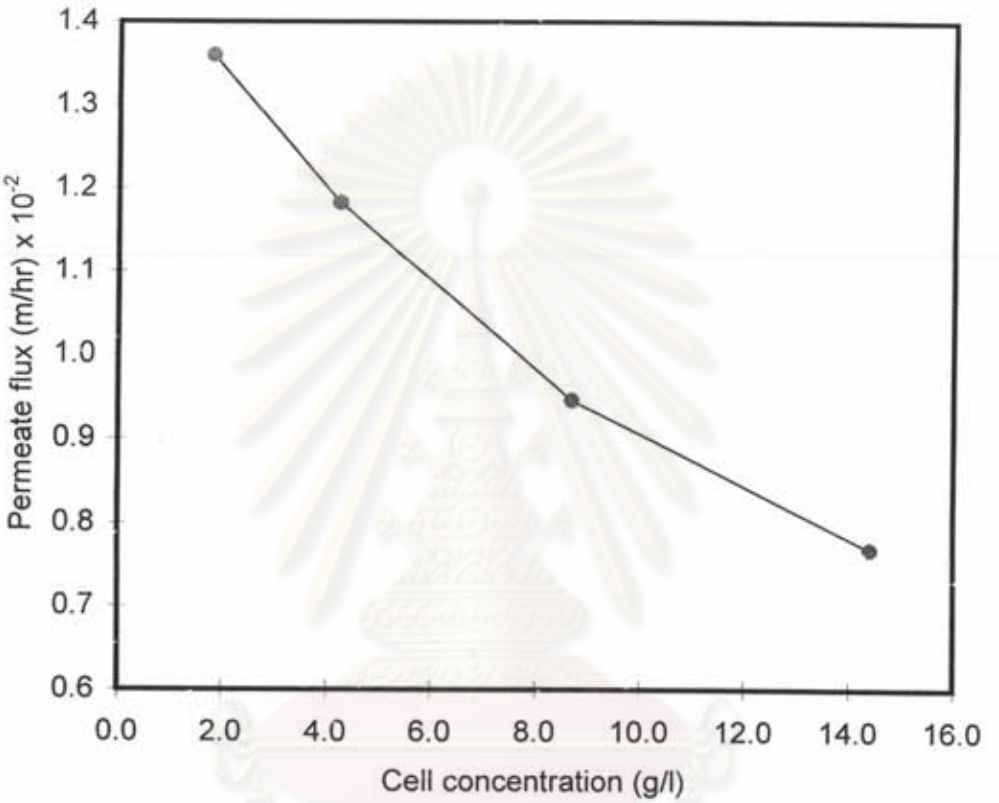





Figure 5-22 Permeate flux of fermentation broth
as a function of cell concentration
- Operating temperature of 37 °C
- Applied pressure of $0.0^{+}$ $kg_f/cm^2$
- Recirculation flow rate of 0.4 $m^3/hr$

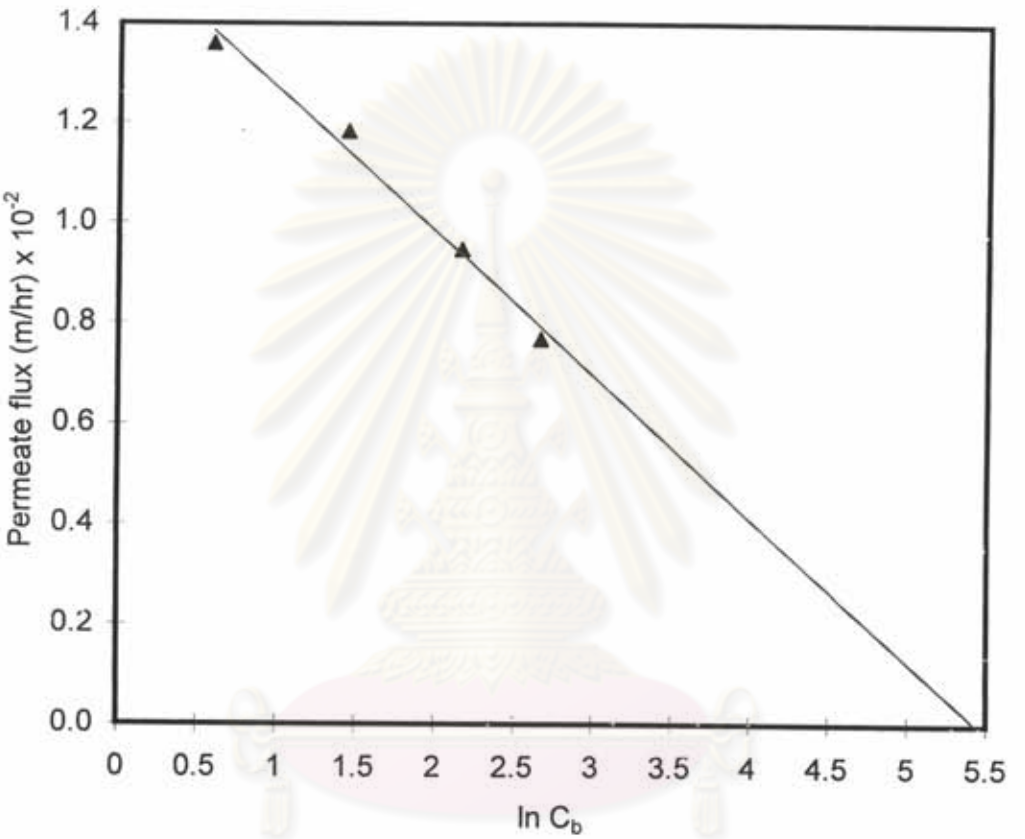





Figure 5-23 Permeate flux of fermentation broth as a function of ln $C_b$

- Operating temperature of 37 °C
- Applied pressure of $0.0^+$ $kg_f/cm^2$
- Recirculation flow rate of 0.4 $m^3/hr$

### 5.5 The effect of dilution rate on growth and protease production in continuous fermentation coupling with microfiltration

The dilution rate of 0.11, 0.20, and 0.30 $hr^{-1}$ were varied via the permeate flow rate to show their effect on growth and protease production.

From Figure 5-24, in the batch-start-up fermentation, the highest cell concentration was achieved at the 40 $^{th}$ hour and the maximum protease was obtained in late stationary phase (0.515 unit/ml). At the same time, glucose depleted from 1.71 g/l to 0.55 g/l. Then the system was switched into a totally recycled batch fermentation, glucose in this period was totally consumed so there was not enough glucose for cell to significantly grow or produce the protease. Next, the system was switched into continuous with microfiltration mode of which retained cells were turned to the fermenter in order to deposit the total cell in fermenter. Obviously, the cell concentration rose up with the dilution rate. At the dilution rate of 0.20, the cell concentration rose up about 3.74-fold (4.63 g/l) from the dilution rate of 0.11. Protease was significantly found at the early log phase (0.103 unit/ml) more than at stationary phase (0.940 unit/ml). It might be concluded that protease production depended on the age of cells and glucose concentration. The glucose depleted from 1.00 g/l to 0.04 g/l so there was enough glucose for cell to grow as well as to produce protease. At the dilution rate of 0.30, the cell concentration rose up about 1.94-fold (9.13 g/l) from the dilution rate of 0.20. Protease was significantly found at the early log phase (0.243 unit/ml) more than at stationary phase (0.043 unit/ml) with the same reason. The relationship between dilution rates among cell concentration, glucose concentration, and protease was available in Figure 5-24. The experiment at dilution rate of 0.3 was stopped because further expected dilution rate could not achieved. The previous study on natto (a non-salted fermented soybean food) producing strain Bacillus subtilis [32] indicated that gamma-polyglutamate (viscous biomaterial) was synthesized by utilization of soybeans which is the same raw material in this thesis. This viscous biomaterial can lower the microfiltration performance and subsequently, lower the dilution rate.

The kinetic parameters were investigated among three dilution rates. The increase of dilution rates brought about the increase of concentration and productivity of cell together with protease according to Figure 5-25. Besides that, the study of the relation between dilution rates and cell yield over glucose ($Y_{X/S}$) also showed the increasing of the parameter with experimental dilution rate as illustrated in Figure 5-26. During the dilution rate of 0.10-0.20 $hr^{-1}$, the cell yield over glucose ($Y_{X/S}$) all increased. However, the slope of the curves of $Y_{P/S}$ and $Y_{P/X}$ decreased while the slope of the $Y_{X/S}$ curve still increased during the dilution rate of 0.20-0.30. The fact that product yield had a declining slope profile with dilution rate indicated the lowering protease production with the dilution rate. In addition, Figure 5-27 showed the relationship among specific growth rate, specific consumption rate, specific product rate and dilution rates. Specific product rate showed high increment during the dilution rate of 0.10-0.20 but showed significantly small increment during the dilution rate of 0.20-0.30. In contrast, specific growth rate and specific consumption rate increased between the dilution rate of 0.10-0.20 but subsequently decreased between the dilution rate of 0.20-0.30. It could be stated that at the dilution rate of 0.20-0.30 the specific growth rate declined because of the age of cell --the binary fission of old cell was less than the fresh one. Hence, the specific consumption rate and specific product rate subsequently decreased.

Due to the problem of old cell, another experiment which was focused on studying the stability and cell bleeding effect of continuous fermentation coupling with microfiltration for protease production was set at the highest practical dilution rate ($D = 0.3\ hr^{-1}$).

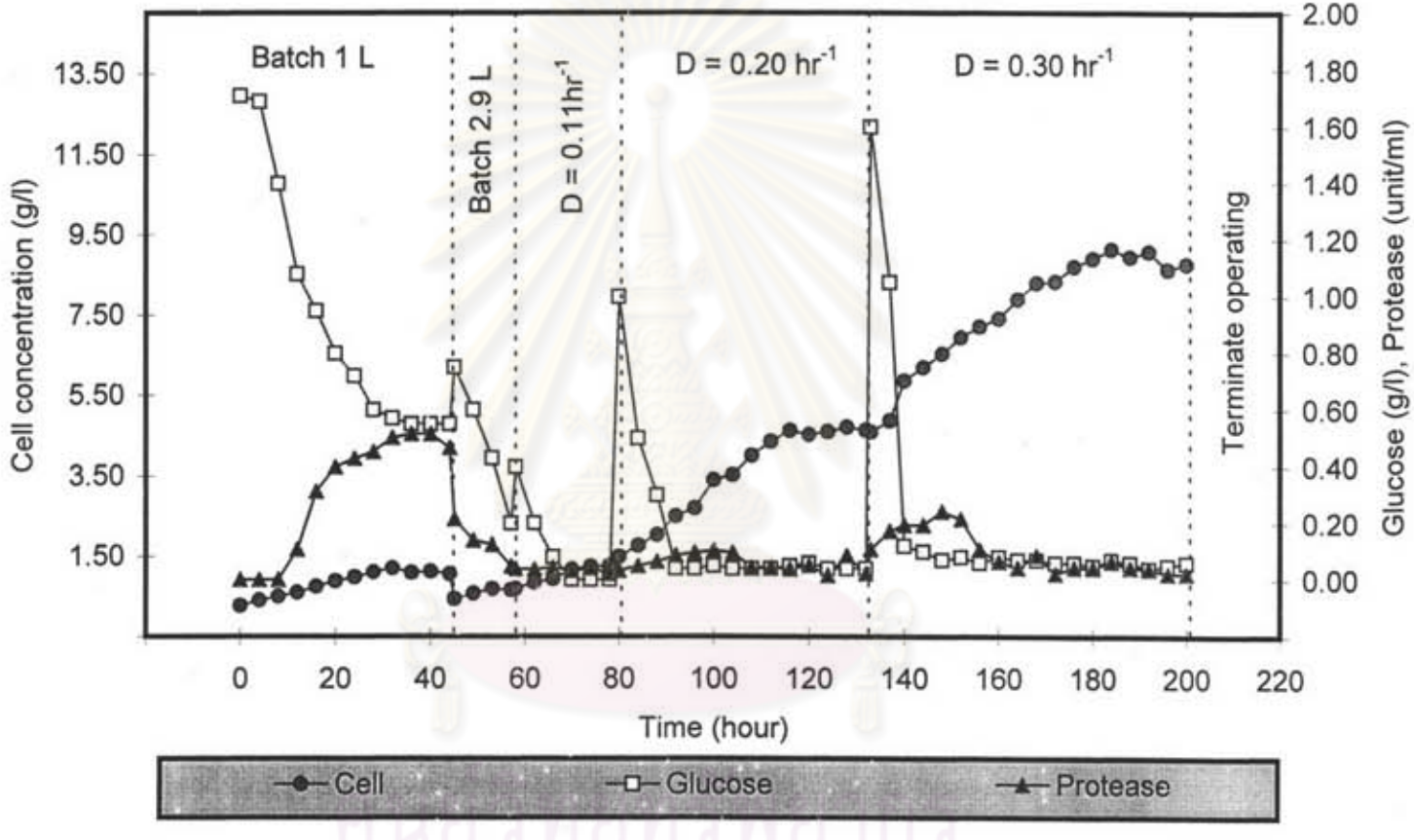



Figure 5-24 The effect of dilution rate on growth and protease production

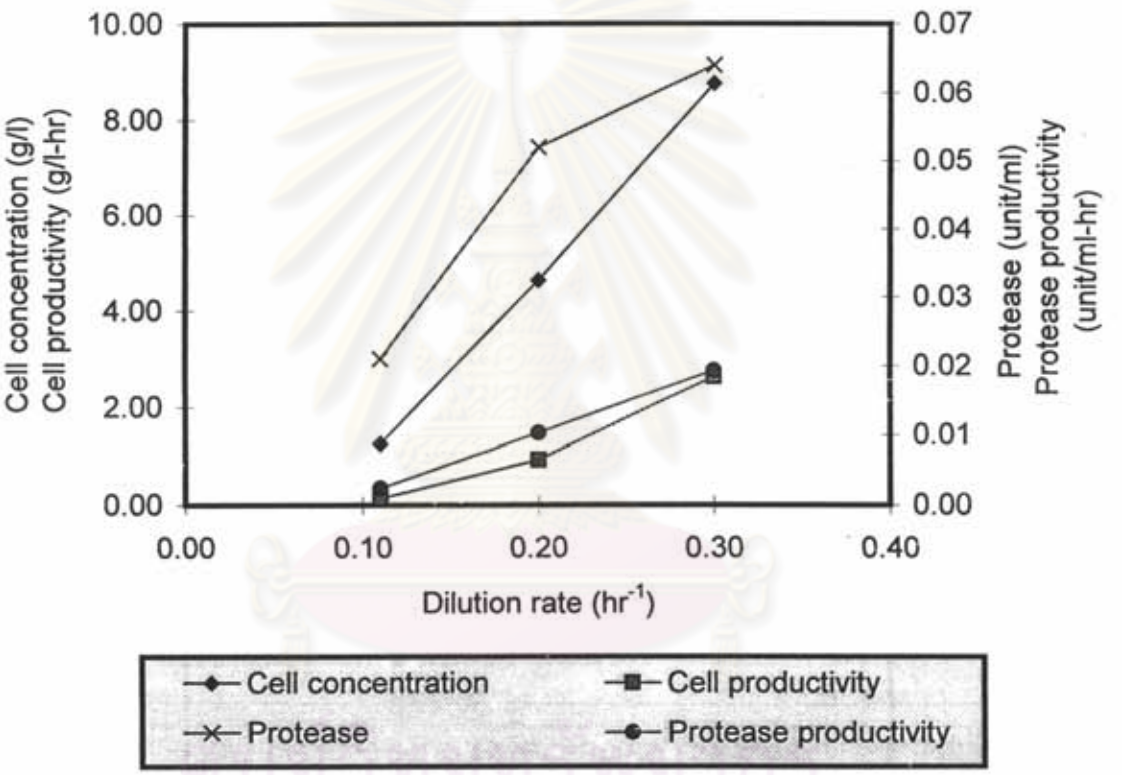



Figure 5-25 The effect of dilution rate on concentration and productivity of cell and protease

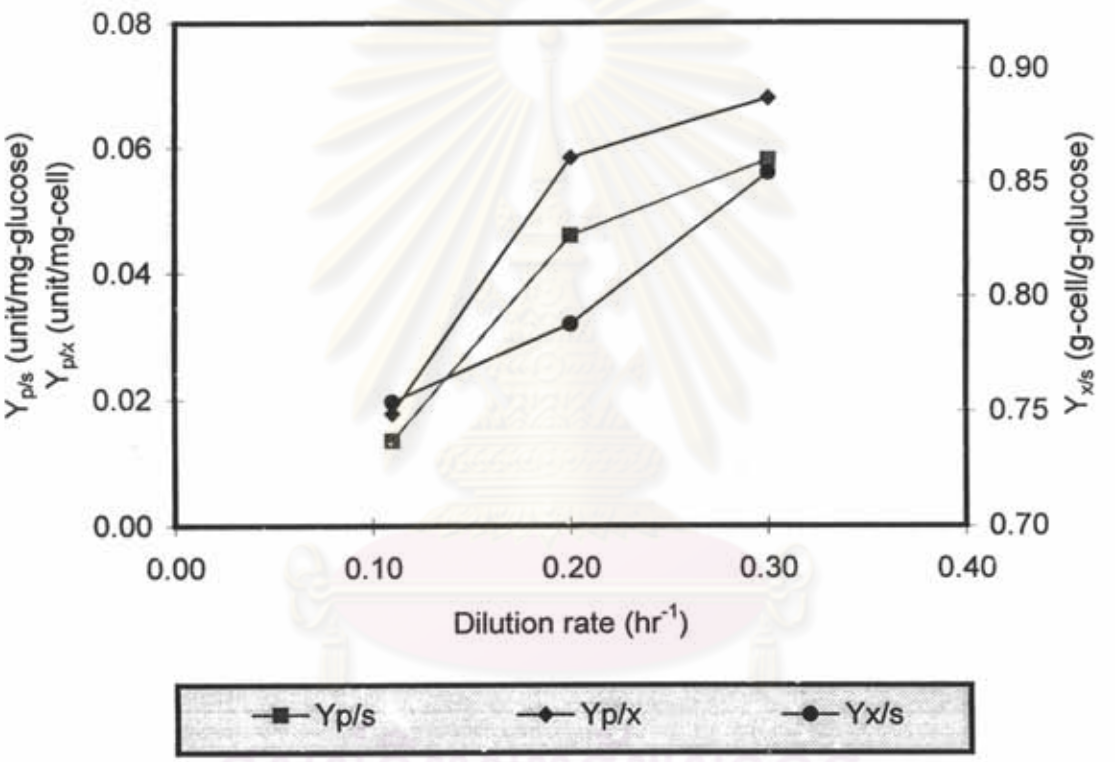



Figure 5-26 The effect of dilution rate on $Y_{P/S}$, $Y_{P/X}$, and $Y_{X/S}$

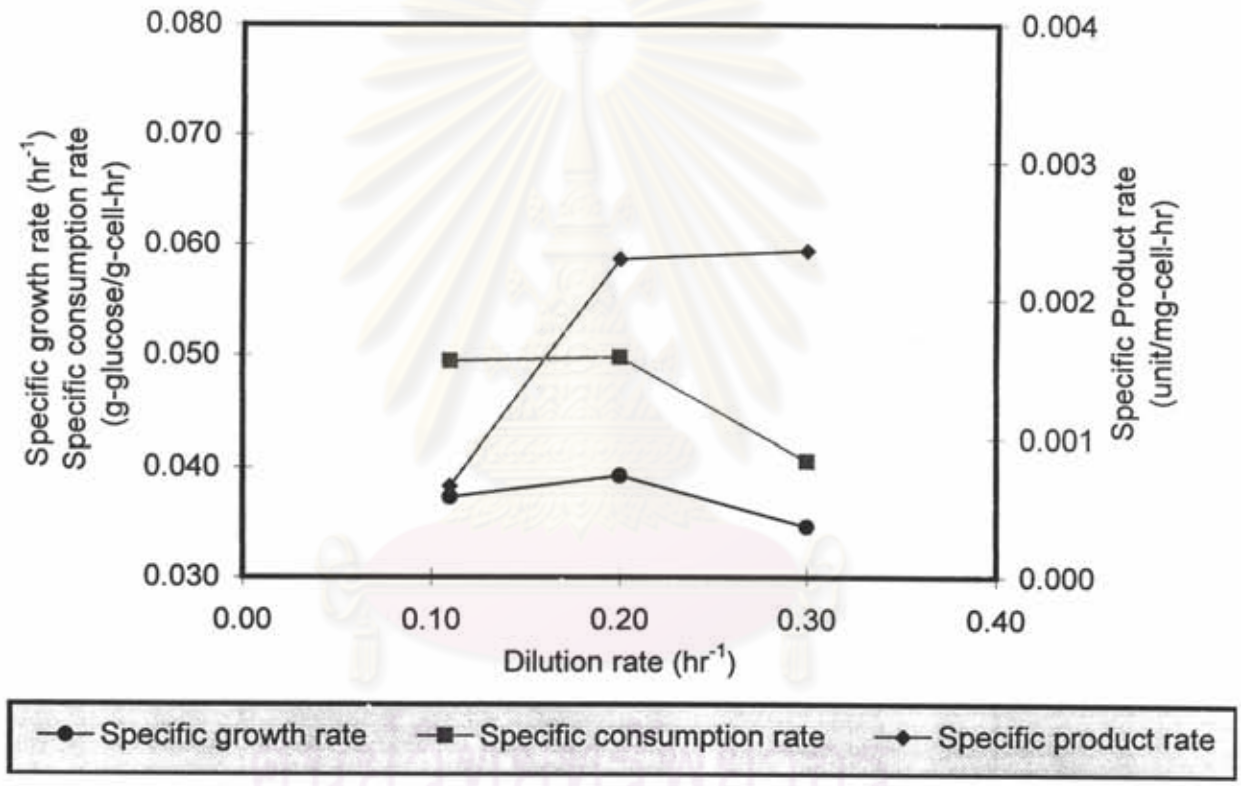



Figure 5-27 The effect of dilution rate on specific growth rate, specific consumption rate, and specific product rate

### 5.6 The stability of protease production at constant suitable dilution

The purpose of this experiment was to clarify the stability of protease production by prolonging cultivation time at the dilution rate of 0.3 $hr^{-1}$ including the influence of twice cell bleeding.

In batch-startup-process, the maximum protease was obtained in late stationary phase where cell concentration reached 0.91 g/l. At the same time, glucose depleted from 1.6 g/l to 43 g/l as shown in Figure 5-28. Obviously, in stationary phase glucose was still consumed by the bacillus for protease and other bioproduct production as same as presented in previous batch fermentation. Then the system was switched into a totally recycled batch fermentation. Glucose was up to 0.8 g/l and continuously depleted while the only cell was promoted by glucose consumption, However, the amount of protease produced decreased insignificantly. Next, the system was switched into continuous with microfiltration mode of which retained cells were totally turned back to the fermenter. The cell concentration rose up about 16.72-fold (9.98 g/l) as glucose depleted from 1.47 to 0.70 g/l and was relatively constant at mid stationary onwards. Protease was significantly found at the early log phase (0.202 unit/ml) and at stationary phase (0.150 unit/ml) with the same reasons as the pervious dilution rate-varying experimental set. Focusing on stability of protease production, it was indicated that there was relatively constant production at stationary phase for the 30-hour experimental interval. Hence cell bleeding process was done to show the effect of fresh culture. First, the culture was bleeded out at the ratio of 0.345 per working volume, cells rose up to a new higher steady state (12.42 g/l) while glucose was consumed. The reasons why it reached a new steady state are because the initial cell is more than that of the beginning of D = 0.3 $hr^{-1}$ and growth inhibitor is filtered and withdrawn by the microfiltration permeate.

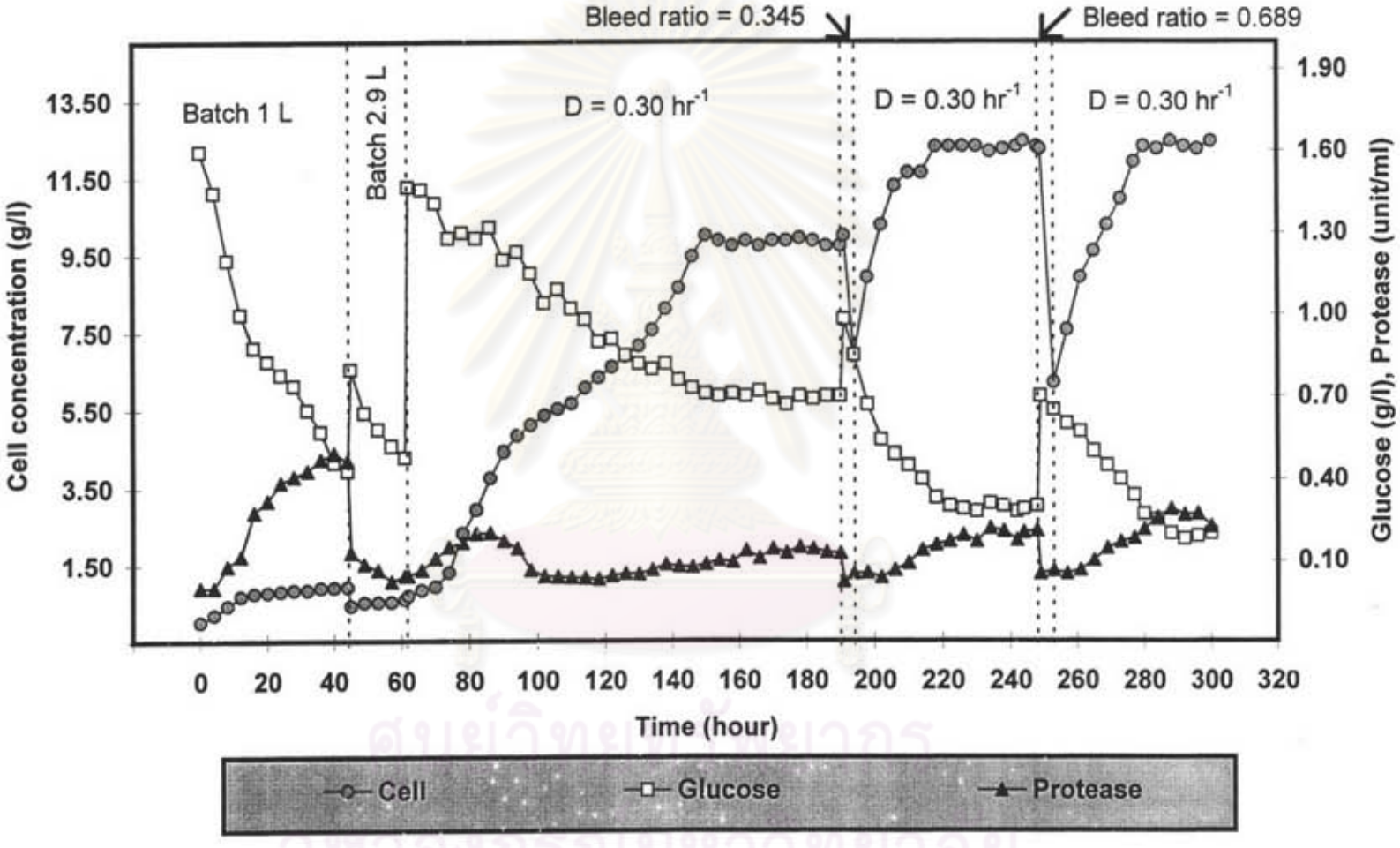



Figure 5-28 The effect of twice cell bleeding effect on growth and protease production at the dilution rate of 0.3 $hr^{-1}$.

A little protease was detected at the early log phase, but significant amount of it was found at stationary phase (0.204 unit/ml). Again, the culture was secondly bleeded out at the ratio of 0.689 per working volume, the same profile protease production was noticed but higher amount (0.290 unit/ml) was achieved whereas cell concentration rose up to the same stationary phase. These twice cell bleeding effect resembled the batch fermentation but a much more cell concentration was achieved with continuous protease production. However, the protease concentration was not much comparing to the normal batch. The criteria to compare these two systems is productivity as mentioned in the Section 5.7.

## 5.7 Protease productivity comparison between batch fermentation and continuous fermentation coupling with microfiltration

The results of comparison between batch fermentation and continuous fermentation coupling with microfiltration were illustrated in Table 5-2 and Figure 5-29. The use of microfiltration with continuous fermentation can improve the protease production. The protease productivity of continuous fermentation coupling with microfiltration which has twice cell bleeding is 1.53-fold higher than that of batch fermentation (based on cultivation time). Note that the productivity of batch fermentation is calculated from protease activity divided by cultivation time or process time whereas the productivity of continuous fermentation is calculated from protease activity multiplied by dilution rate. However, for an industrial approach, the batch productivity is typically based on total process time (including preparation time). The more preparation time spent, the more process time consumed, consequently the batch productivity is definitely much lower than that of continuous fermentation coupling with microfiltration comparing to that is calculated by cultivation time.

Table 5-2 Protease activity and productivity comparison between batch fermentation and continuous fermentation coupling with microfiltration

| | Batch | Continuous with microfiltration | |
|---|---|---|---|
| | | Without cell bleeding | Twice cell bleeding |
| Protease activity (unit/ml) | 0.798 | 0.150 | 0.290 |
| Protease productivity (unit/ml-hr) | 0.057 | 0.045 | 0.087 |

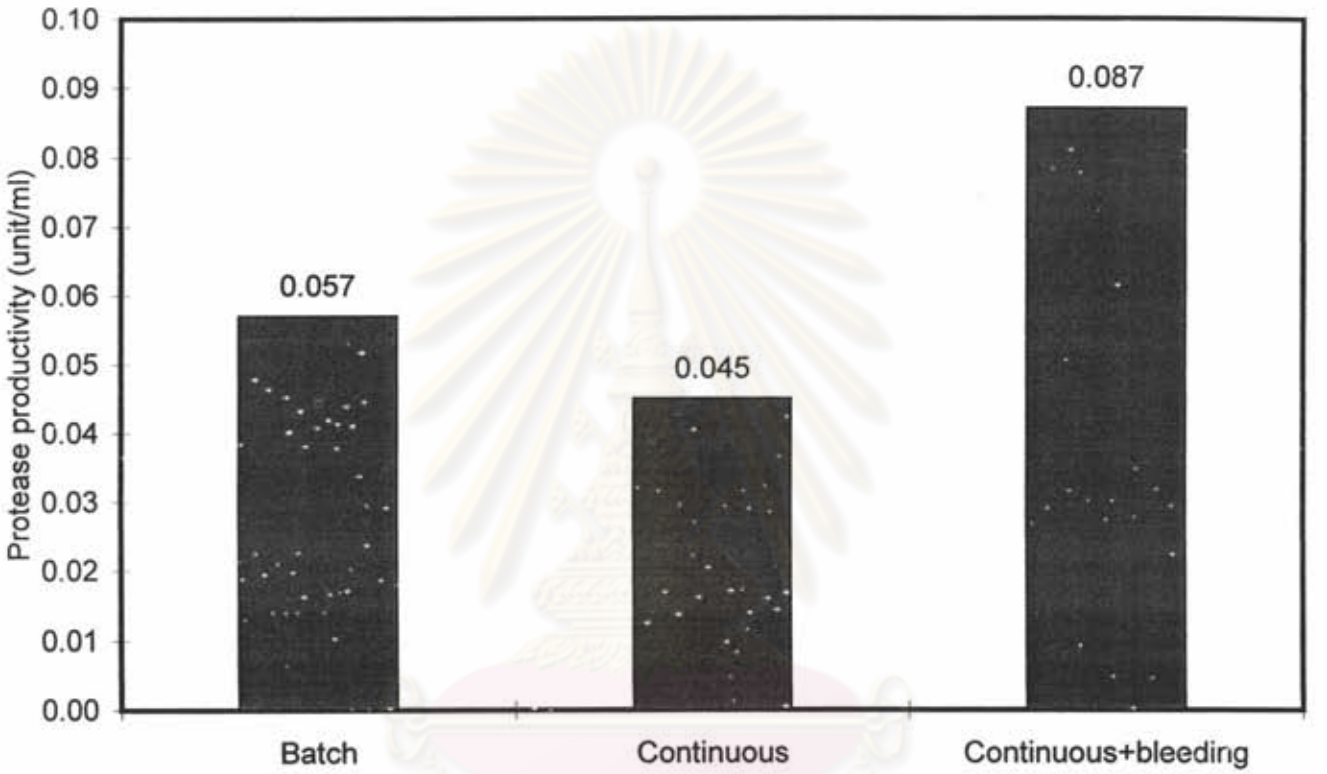



Figure 5-29 Protease productivity comparison between batch fermentation and continuous fermentation coupling with microfiltration