CHAPTER V

RESULTS AND DISCUSSION

5.1 The Study of the Ultrafiltration Membrane Characteristics

Results of the study are reported in Table A1. Permeate flux = Permeate flow rate/Filtration area (0.2 m^2).

5.1.1 The Effect of Applied Pressure on Permeate Flux

With deminaralized water (run A), permeate flux will increase linearly with pressure (see figure 5.1).

At low biomass concentrations (1.70-25.24 g/l in run B, C) permeate flux was increased with increasing pressure from 0.0^+ to 0.8^+ kg_f/cm^2. The correlation was not linear because the resistances also increased with pressure from the effect of concentration polarization.

At higher biomass concentrations (45.4 g/l in run D), when the pressure reached 0.6^+ kg_f/cm^2, the permeate flux was constant with increasing pressure. At biomass concentrations of 64.40 g/l, when the pressure reached 0.2^+ kg_f/cm^2, the permeate flux was also constant with increasing pressure. This was due to the fact that at P = 0.6^+ kg_f/cm^2 in run D and P = 0.2^+ kg_f/cm^2 in run E, a gel layer was formed and increased overall resistance. Further increase in pressure would increase the thickness and the resistance of the gel layer so the flux rate would reach a maximum and become relatively constant with pressure (see figure 5.2).

From the study it was also shown that at high cell concentrations (more than 45.4 g/l), the applied pressure of more than 0.0^+ kg_f/cm^2 caused the occurance of a gel layer and the permeate flux was almost constant with increasing pressure. Moreover from this

study, applied pressures of more than 0.0^{+} kg_f/cm^2 caused rapid fouling of membrane. The membrance fouling would decrease the permeate flux and create difficulty in operating the experiment. Therefore, the selected pressure for application in the cell recycle system was 0.0^{+} kgf/cm^2.

From the experiment, the permeate flux of deminaralized water increased linearly with pressure following the equation $J = PTM/(R_G+R_M)$ (for cleaning water; $R_G = 0$; R_m = Constant). At a pressure of 0.0 kg_f/cm^2 measured from the pressure gauge, the permeate flux was 11.45×10^{-3} m^3/hr (at 0.4 m^3/hr recirculation flow rate, 33 °C). The fact that permeate flux was zero when transmembrane pressure was zero showed that the measuring pressure from the pressure gauge must be incorrect. The correct pressure could be calculated from the plotted line of the deminaralized water permeate flux against pressure (figure 5.1). From figure 5.1, the correct pressure at 0.0 kg_f/cm^2 measured from the pressure gauge was 0.17 kg_f/cm^2 which was the minimum pressure attained from pumping the deminaralized water across the membrane at 0.4 m^3/hr recirculation flow rate. The symbol (+) above the value of the pressure meant that the correct pressure value was the pressure (measured by pressure gauge) plus 0.17 kg_f/cm^2.

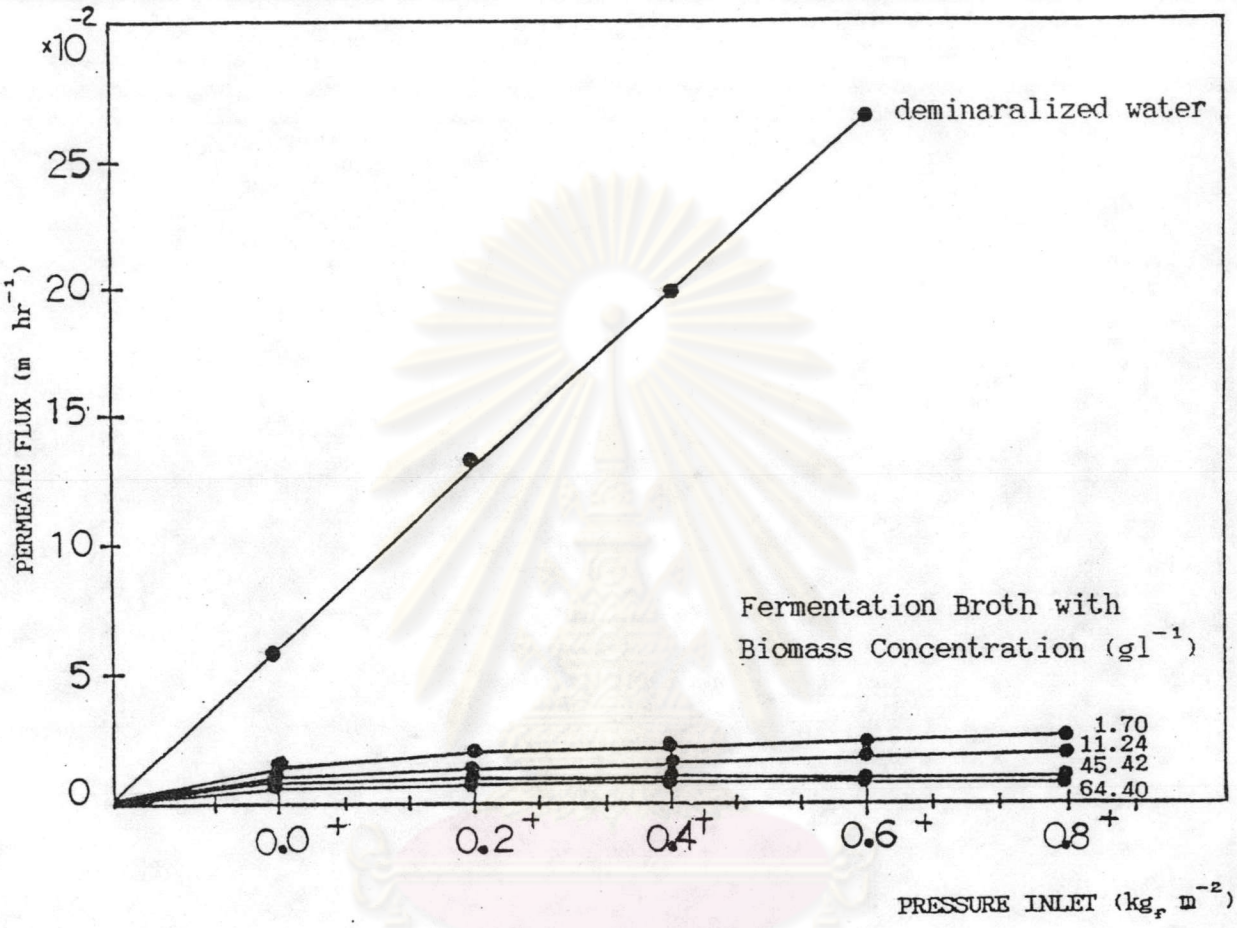

Figure 5.1 The effect of applied pressure on permeate flux of water and fermentation broth

At operating temperature = 33 °C

recirculation flow rate = 0.4 m^3/hr

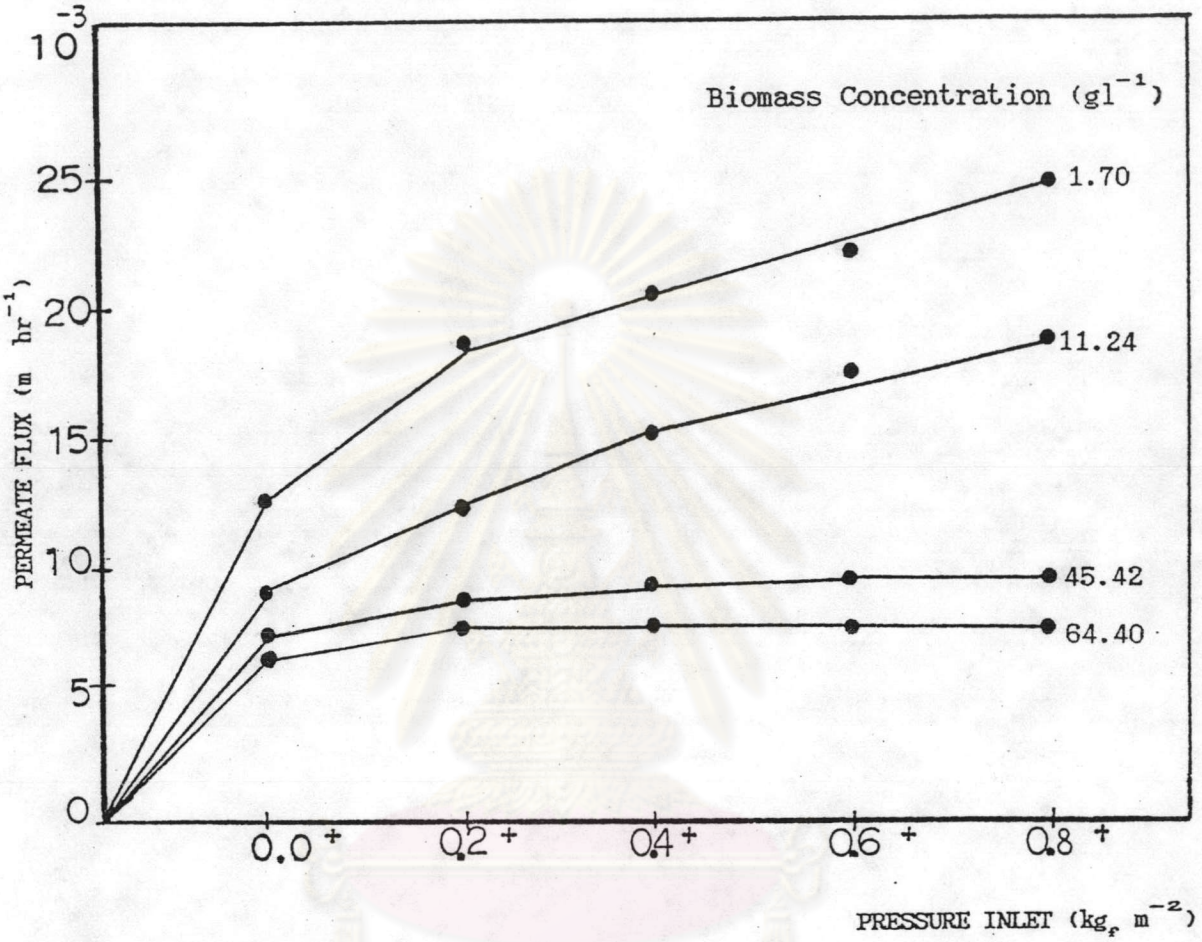

Figure 5.2 Permeate flux of fermentation broth as a function of applied pressure.

At operating temperature = 33 °C
recirculation flow rate = 0.4 m^3/hr
biomass concentration 1.70, 11.24, 45.42 and 64.40 g/l

5.1.2 Effect of recirculation flow rate on permeate flux

From the experiment, (figure 5.3) permeate flux increased with recirculation flow rate. From the ultrafiltration's theory, the mass transfer coefficient (K) increased with the square root of recirculation flow rate in laminar flow, and almost linearly with recirculation flow rate in turbulent flow, and the increasing of recirculation flow rate increased shear force at the membrane surface, so the thickness and resistace of the gel layer decreased with recirculation flow rate. Recirculation flow rate effected the pressure drop across the membrane and thus reduced the transmembrane pressure, but this 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. In the study, the microorganism was highly disintegrated at a 0.5 or 0.6 m^3/hr recirculation flow rate from the highly turbulent condition. Moreover, in the fermentation broth high formation of foam occured from this highly turbulent condition. So the selected recirculation flow rate for application in cell recycle system was set at 0.4 m^3/hr.

5.1.3 Effect of Cell Concentration on Permeate Flux

This study used a specific applied pressure (0.0^+ kg_f/cm^2) and a specific recirculation flow rate (0.4 m^3/hr), From the experiment (fig. 5.4), permeate flux was reduced with increasing biomass concentration. Table 5.1 demonstrates permeate flux against ln C_B. The plots of permeate flux against ln C_B in Figure 5.5 showed that the permeate flux declined linearly with lnC_B. According to gel polarization theory, flux is a function of the bulk solute concentration (C_B) following $J = k \ln(C_G/C_B)$. From the study at a cell concentration

1.7 g/l onwards, gel polarization occured and C_G became constant. From figure 5.5, mass transfer coefficient (K) is the slope in the plot line which was 1.75×10^{-3} m/hr and gel concentration (C_G) calculated from point at 0.0 m/hr permeate flux was 2810.67 g/l.

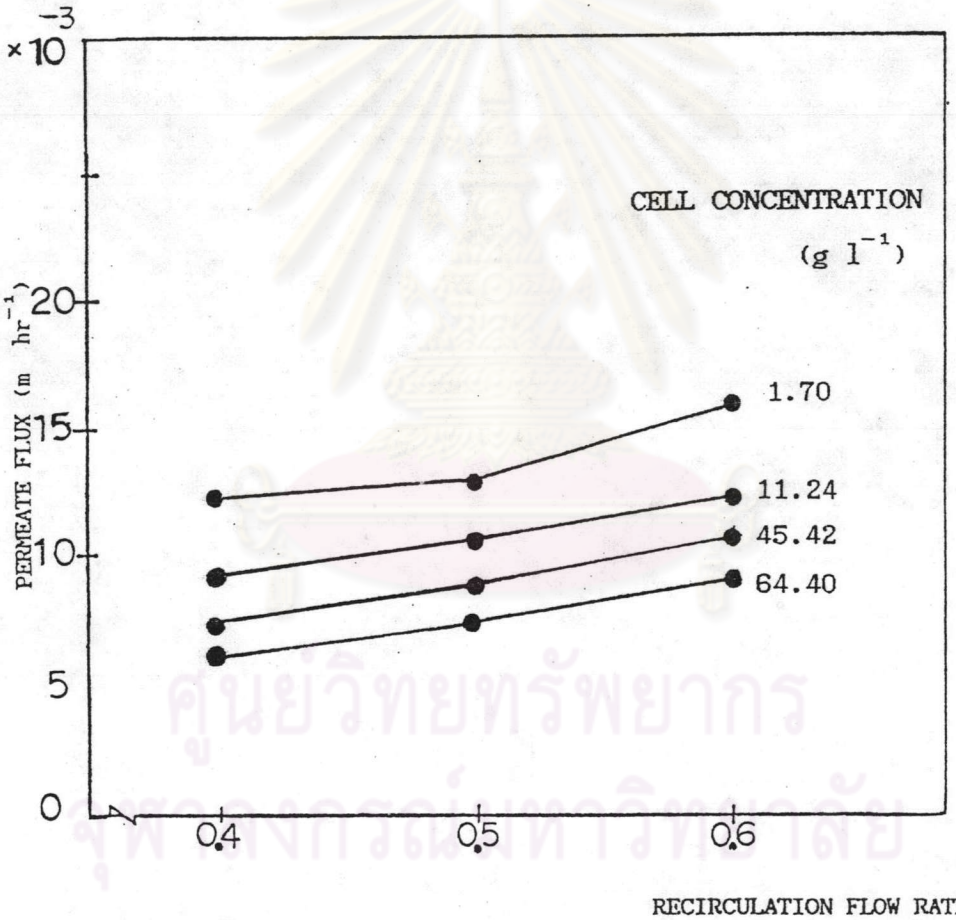

Figure 5.3 Permeate flux as a function of recirculation flow rate
At operating temperature = 33 °C
applied pressure = 0.0⁺ kg_f/cm^2
biomass concentration 1.70, 11.24, 45.42, 64.40 g/l

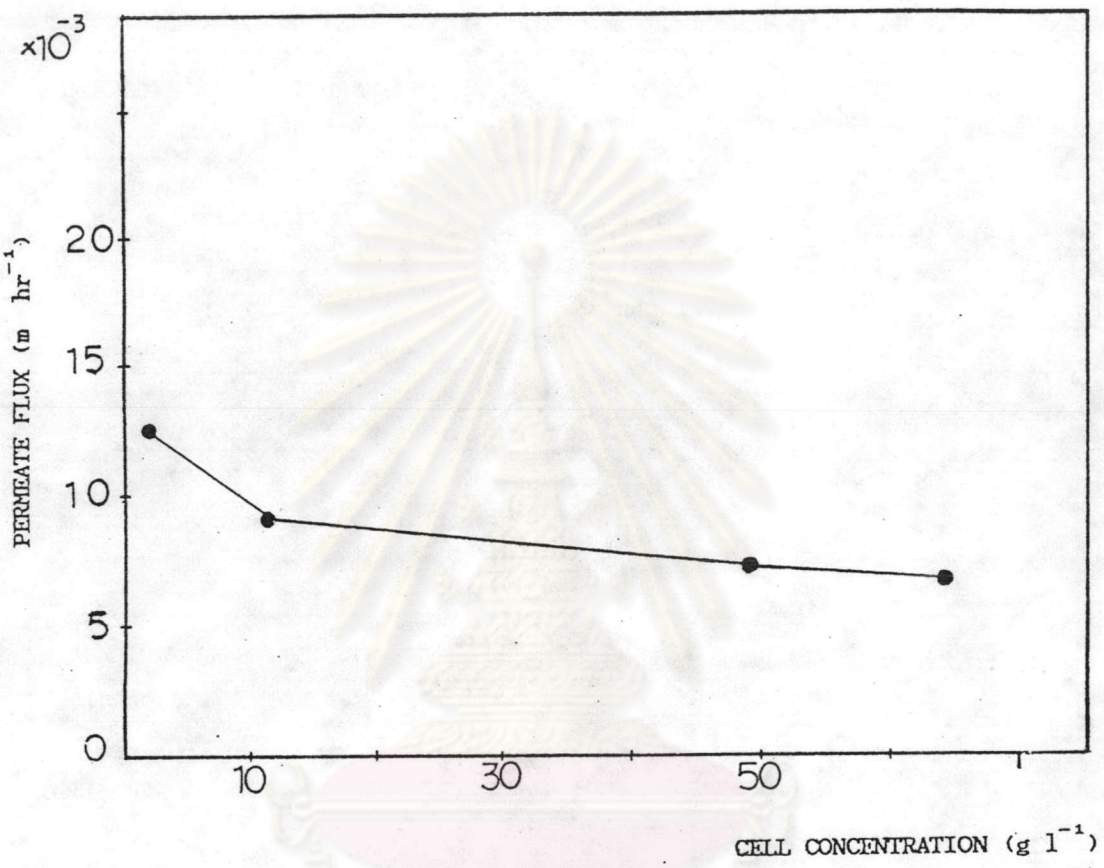

Figure 5.4 Permeate flux as a function of biomass concentration (C_B)

At operating temperature = 33 °C

applied pressure = 0.0^{+} kg_f/cm^2

recirculation flowrate = 0.4 m^3/hr

Table 5.1 Permeate flux against ln C_B at 0.0^+ kg_f/cm^2 applied presure and 0.4 m^3/hr recirculation flow rate with 33 °C operation temperature

ln C_B	0.5306	2.4195	3.8160	4.1651
Permeate flux ($m\ hr^{-1}$)	12.45×10^{-3}	9.15×10^{-3}	7.27×10^{-3}	6.65×10^{-3}

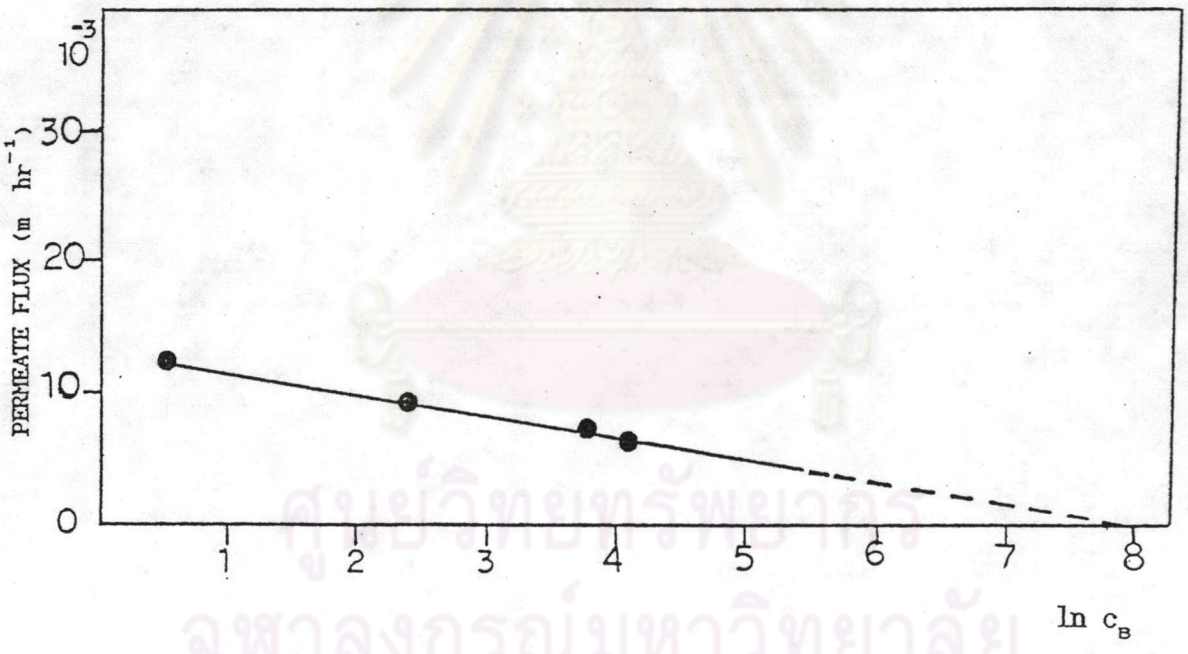

Figure 5.5 Permeate flux as a function of ln c_B
At operating temperature = 33 °C
applied pressure = 0.0^+ kg_f/cm^2
recirculation flowrate = 0.4 m^3/hr

5.2 Application of Ultrafiltration for Cell Recycle in Continuous ABE Fermentation

5.2.1 Effect of Feeding Glucose Concentration and Dilution Rate on Cell Recycle System

5.2.1 A) At Feeding Glucose Concentration 42.4 g/lit (run K)

The results of this experiment has was shown in figure 5.6-5.12, and table 5.2, A2, A7. After the second batch operation, the cell mass reached 3.86 g/l with the maximal specific growth rate and production rate were 0.07, 0.10 $gl^{-1}hr^{-1}$ respectively. The residual glucose was 10.5 g/l, then the first continuous fermentation with total cell recycle was started at a fixed dilution rate of 0.11 hr^{-1}.

The cell mass and glucose consumption began to increase rapidly.12 hr after that, cell mass reached 31.1 g/l with a 8.05 g/l solvent concentration and 4.21 g/l butanol concentration. Glucose consumption was 4.90 $gl^{-1}hr^{-1}$ and glucose concentration in the fermentor was reduced to 4.7 g/l, so, the dilution rate was changed from 0.11 hr^{-1} to 0.22 hr^{-1} (cell will loose activity in the very low glucose concentration (<5 g/l).

About 19 hr of operation at a 0.22 hr^{-1} dilution rate, the cell mass reached 69.0 g/l, the residual glucose was reduced to 3.5 g/l, with 10.41 g/l solvent concentration and 5.89 g/l butanol concentration. The glucose consumption increased to 9.39 $gl^{-1}hr^{-1}$ which was more than the glucose feed rate (9.328 $gl^{-1}hr^{-1}$). Therefore the dilution rate was changed to 0.36 hr^{-1}.

At the start of a 0.36 hr^{-1} dilution rate, the glucose concentration in the system was 8.8 g/l, 3 hr after that, the glucose concentration was reduced to 4.9 g/l and the glucose consumption was

14.8 $gl^{-1}hr^{-1}$. Solvent, and butanol concentrations were reached at 10.94, 6.20 g/l respectively. Then the dilution rate was changed to 0.55 hr^{-1}.

In the step using 0.55 hr^{-1} dilution rate, cell concentration was almost constant. The residual glucose concentration in the system fluctuated from 6.5 to 9.3 g/l and the average solvent, butanol concentrations were 11.03, 6.26 g/l respectively. The average glucose consumption was 19.30 $gl^{-1}hr^{-1}$ (the glucose feed rate at D = 0.55 hr^{-1} was 23.32 $gl^{-1}hr^{-1}$). The maximum cell concentration attained in this experiment was about 80 g/l, while fermentation operation was extremely difficult because of high cell concentration. After running with 0.55 hr^{-1} dilution rate for 15 hr^{-1}. The dilution rate could not be maintained at 0.55 hr^{-1} anymore. Because the permeate flux was less than the point where the 0.55 hr^{-1} dilution rate occured, the experiment was then stopped.

Figure 5.9 shows the behavior of microorganism during operation of run A in form specific growth rate, (μ) specific production rate (ν), and specific acid production rate (ν_{acid}). Table 5.2 shows the productivity and concentration of total solvent and butanol in this experiment. From the datas, the maximum productivity and concentration were achieved from the step where dilution rate was 0.55 hr^{-1} and the maximum solvent and butanol productivitives were 6.06 and 3.44 $gl^{-1}hr^{-1}$ respectively. Product concentrations and total solvent (ethanol + acetone + butanol) concentrations for continuous run A were plotted against time (see figure 5.6, 5.8). The continuous availability of glucose was demonstrated in figure 5.7. In this continuous fermentation, total acids were changed to acetone and butanol, so there was no acid-accumulation in the system.

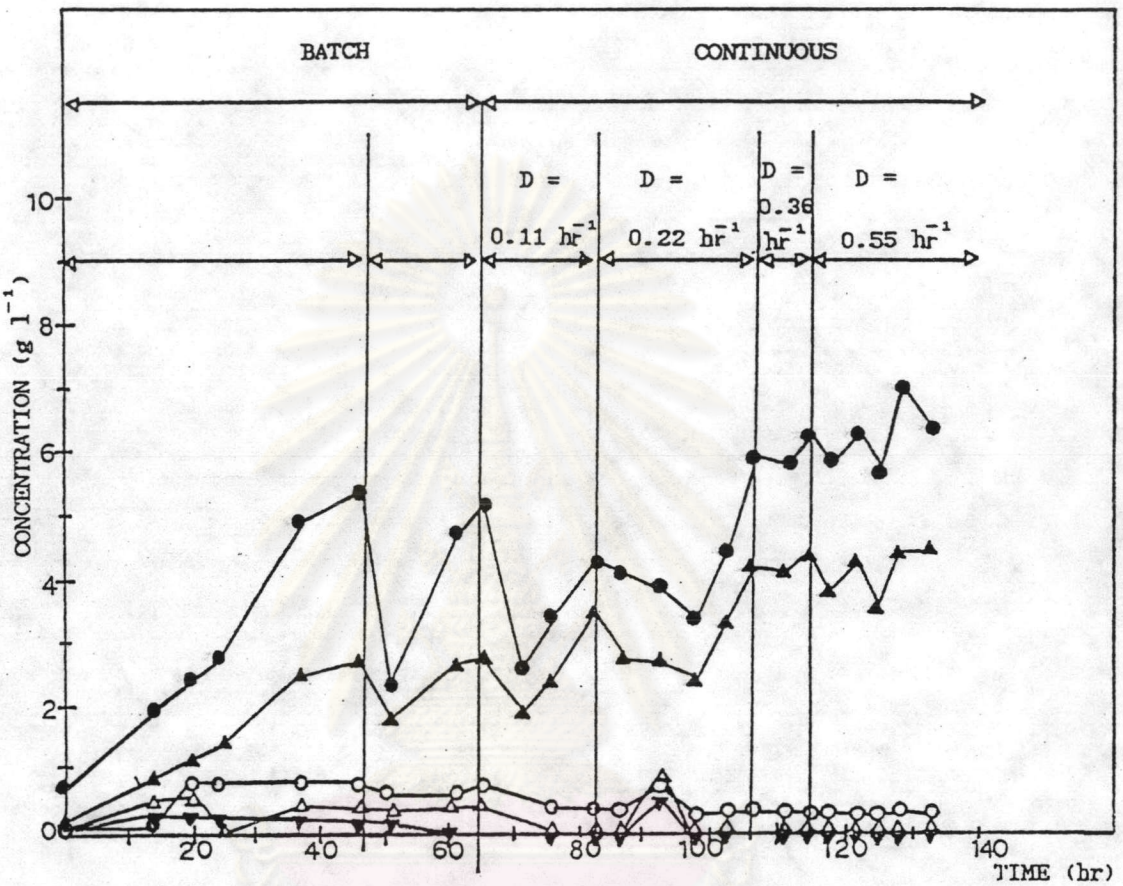

Figure 5.6 Time variation of butanol, acetone, ethanol acetic acid and butyric acid concentration during a total cell recycle system of Cl. acetobutylicum at a feeding glucose concentation 42.4 g/l (run K)

● butanol
▲ acetone
○ ethanol
△ acetic acid
▼ butyric acid

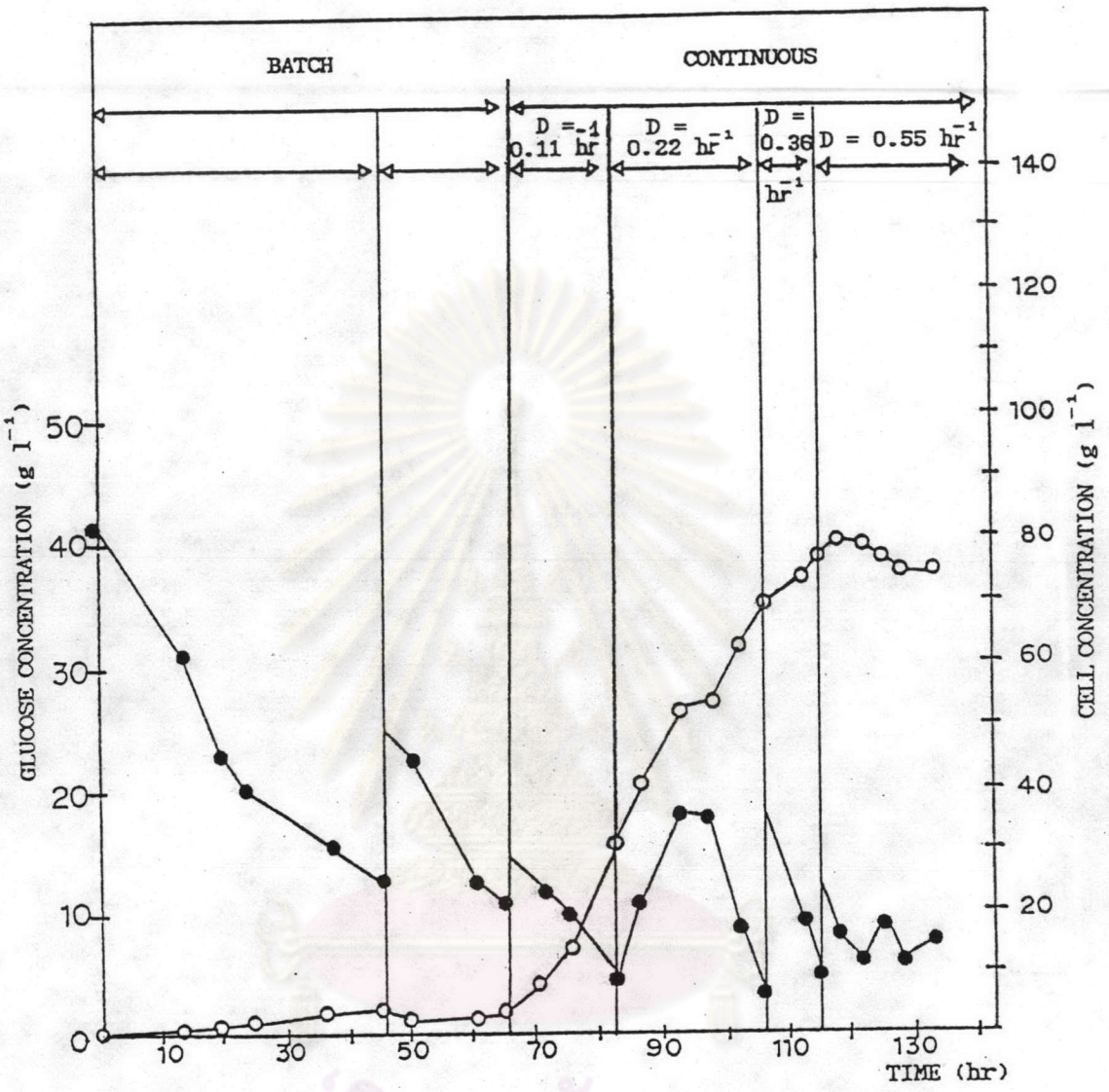

Figure 5.7 Time variation of glucose, biomass concentration during a total cell recycle system of Cl. acetobutylicum at a feeding glucose concentration 42.4 g/l (run K)

● glucose

○ biomass

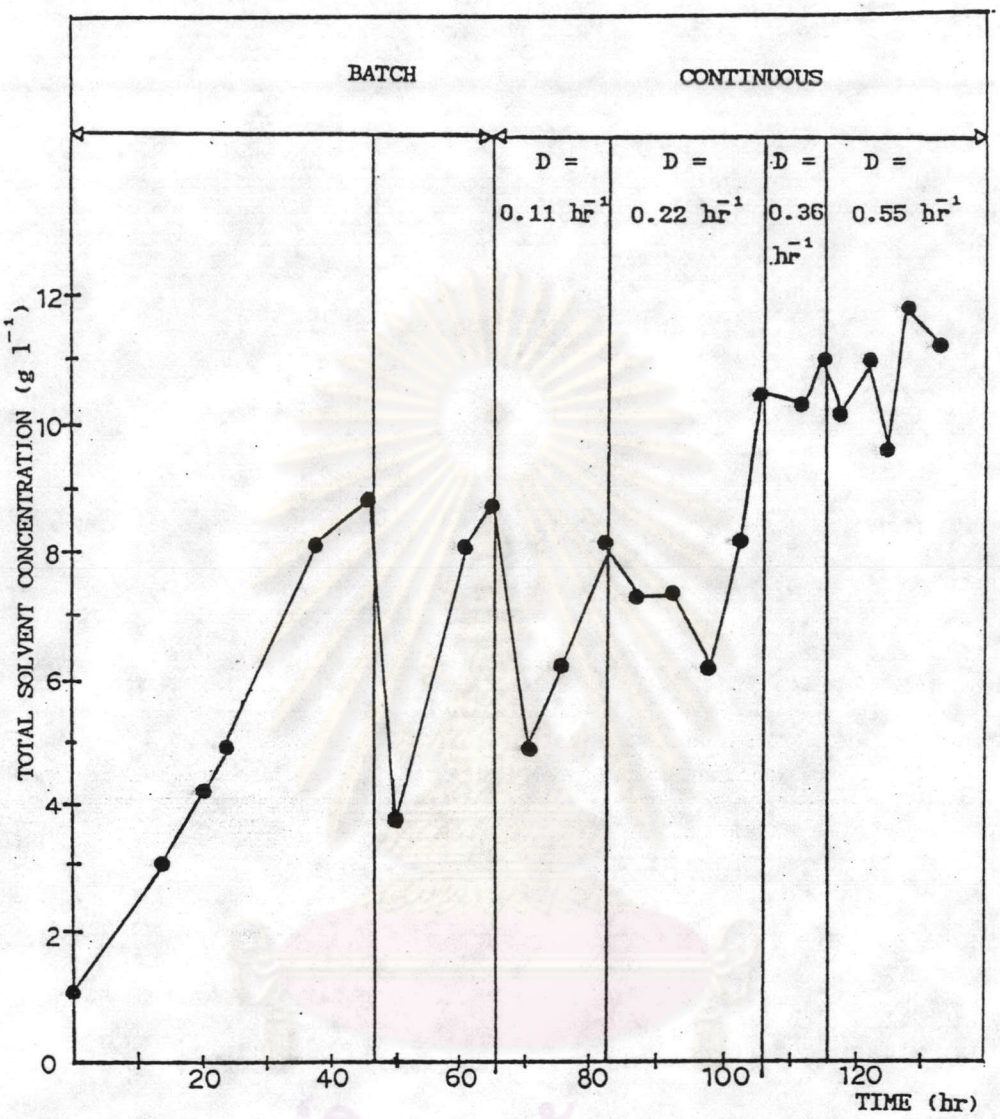

Figure 5.8 Time variation of total solvent concentration during a total cell recycle system of Cl. acetobutylicum at a feeding glucose concentation 42.4 g/l (run K)

● total solvent

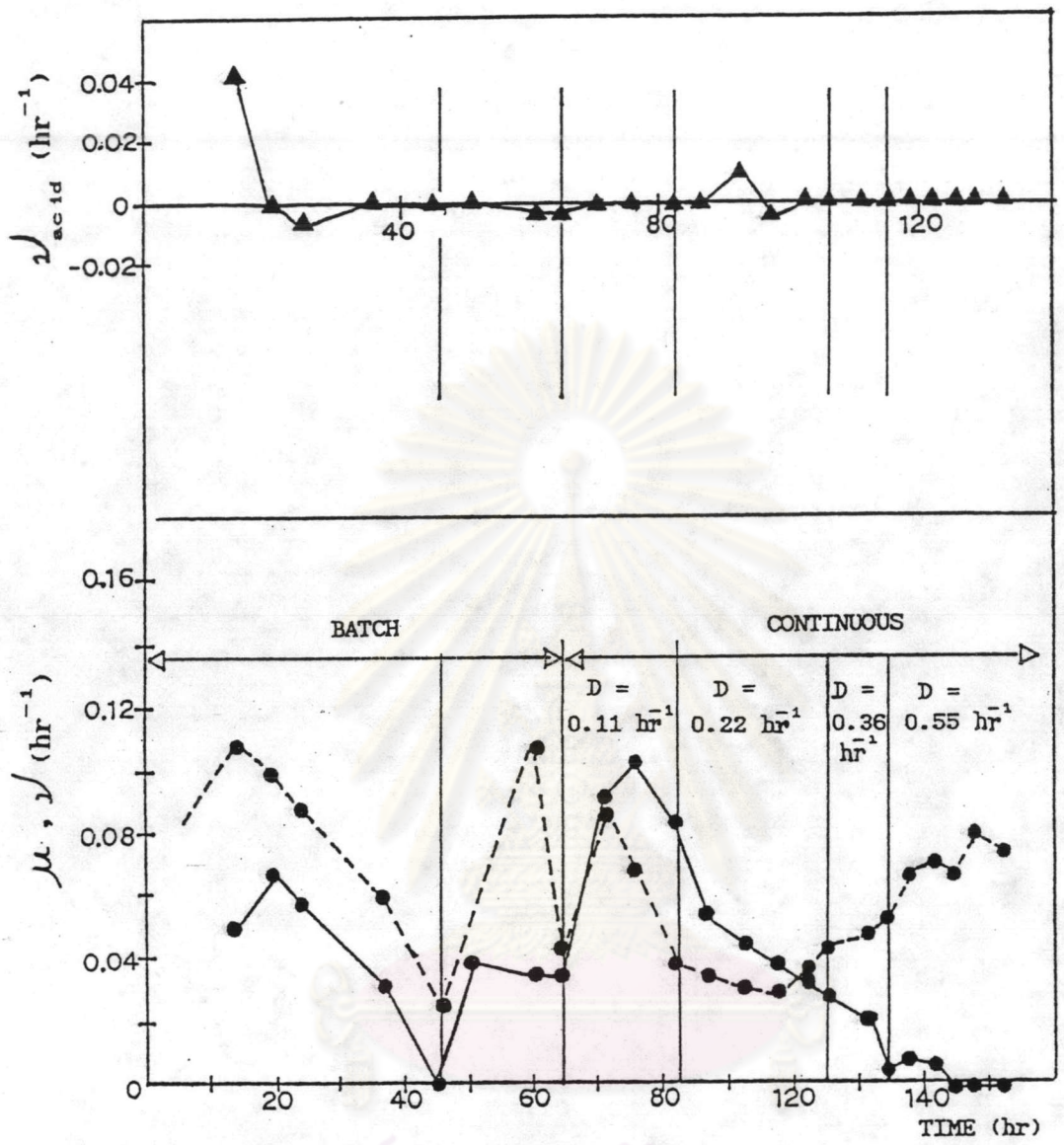

Figure 5.9 Specific growth rate (μ), specific production rate (ν) and specific acid production rate (ν_{acid}) against time (run K)

- ● specific growth rate (μ)
- -●- specific production rate (ν)
- ▲ specific acid production rate (ν acid)

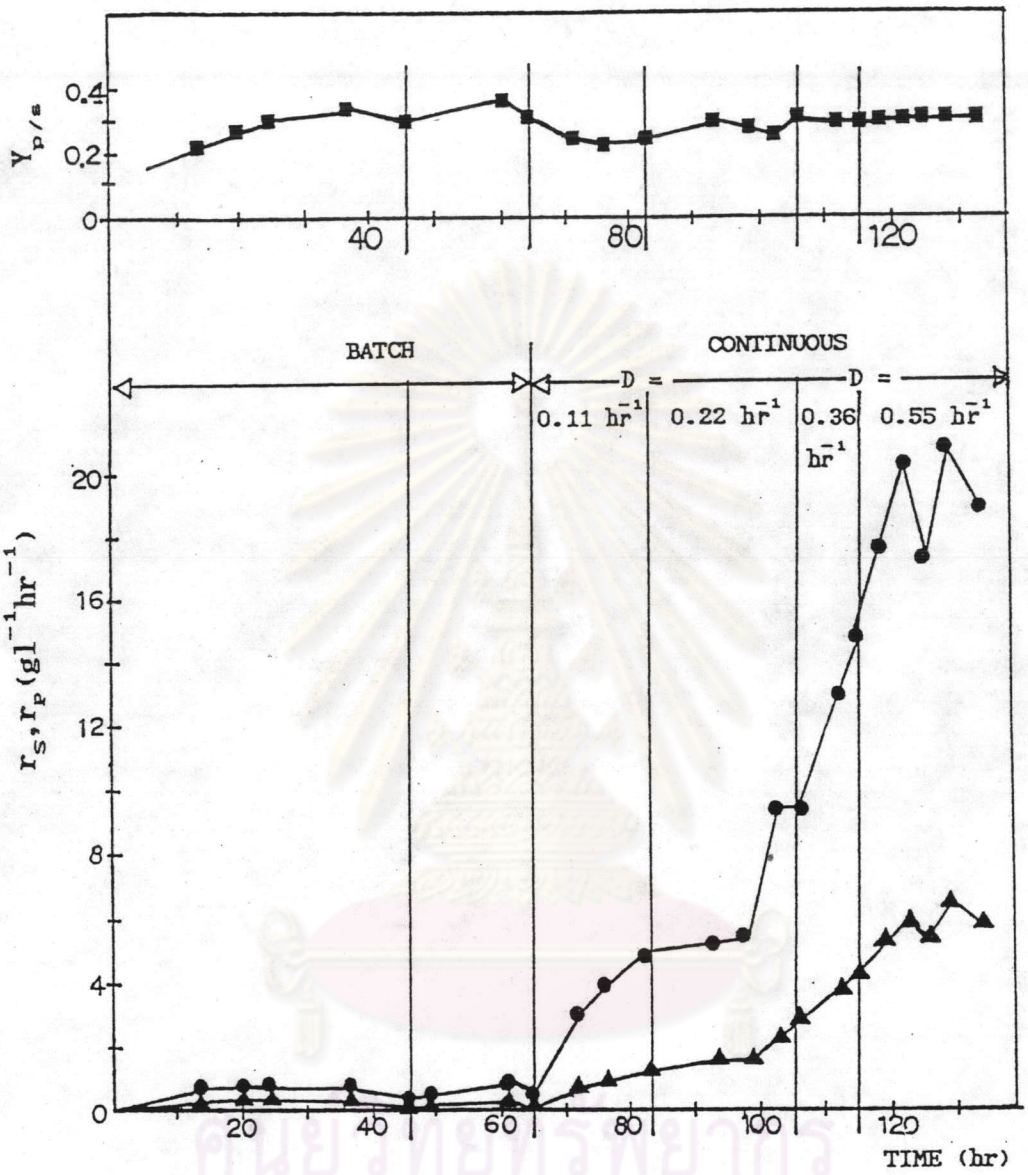

Figure 5.10 Glucose consumption (r_s), total solvent productivity (r_p) and production yield ($y_{p/s}$) against time (run K)

● glucose consumption (r_s)

▲ total solvent productivity (r_p)

■ production yield ($y_{p/s}$)

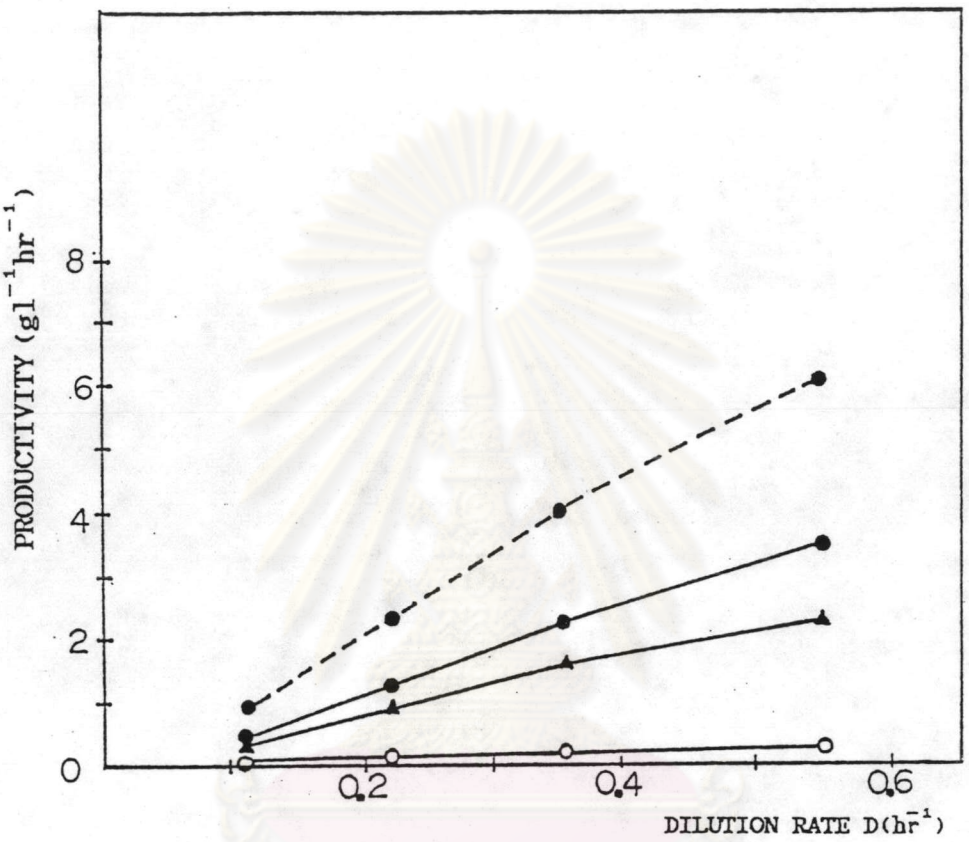

Figure 5.11 Total solvent, butanol, acetone and ethanol productivity as a function of the dilution rate from cell recycle system with 42.4 g/l feeding glucose concentration (run K)

--●-- total solvent

● butanol

▲ acetone

○ ethanol

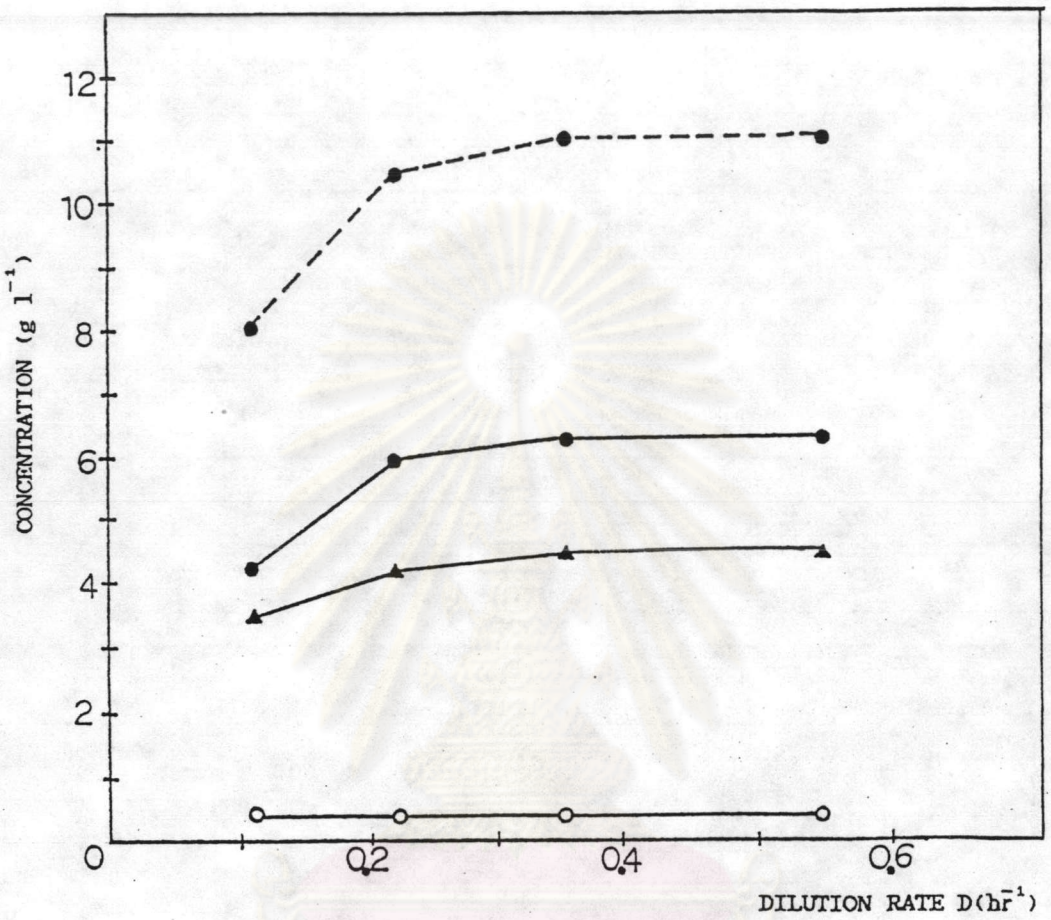

Figure 5.12 Total solvent, butanol, acetone and ethanol concentration as a function of the dilution rate from cell recycle system with 42.4 g/l feeding glucose concentration (run K)

- -●- total solvent
- ● butanol
- △ acetone
- ○ ethanol

Table 5.2 Results of ABC fermentation from cell recycle system with feeding glucose concentration 42.4 g/l (run K)

Dilution rate (hr^{-1})	Productivity ($gl^{-1}hr^{-1}$)		Concentration (gl^{-1})		r_s ($gl^{-1}hr^{-1}$)	$Y_{p/s}$
	Solvent	butanol	Solvent	butanol		
D = 0.11	1.16	0.58	8.05	4.21	4.90	0.24
D = 0.22	2.67	1.71	10.41	5.89	9.39	0.28
D = 0.36	4.15	2.36	10.94	6.20	14.80	0.28
D = 0.55	6.06	3.44	11.03	6.26	19.30	0.31

Remark

*The reported productivities were calculated from the overall production at the end of each stages.

5.2.1 B) At Feeding Glucose Concentration 52.0 g/lit (Run L)

The results of this experiment are shown in figures 5.13-5.17, and tables 5.3, A3, A8. After the second batch operation, the cell mass reached 5.04 g/lit with the maximal specific growth rate and production rate of 0.07 and 0.09 hr^{-1} respectively. The residual glucose concentration was attained at 11.7 g/l.

Then the continuous fermentation with total cell recycle was started at a fixed dilution rate of 0.11 hr^{-1}. After running with this dilution rate for 27 hr, the cell concentrations was rather constant at 55.4 g/l and the residual glucose was reduced to 11.6 g/l. Total solvent and butanol concentrations were 11.98, 6.92 g/l respectively with a glucose consumption rate at 4.64 g/l hr.

According to the results,the cell concentration was rather constant. The dilution rate was changed to 0.22 hr^{-1}. After running at 0.22 hr^{-1} dilution rate for 28 hr the cell concentration and the glucose consumption were increased to 57.4 gl^{-1}, 10.58 $gl^{-1}hr^{-1}$ respectively with 13.07 g/l of total solvent concentration and 7.86 g/l of butanol concentration, the cell concentration was again almost constant. Therefore the dilution rate was changed to 0.36 hr^{-1}.

With the 0.36 hr^{-1} dilution rate, the cell concentration was slowly increased to 68.7 g/l in 21 hours, and then the cell concentration dropped to 64.5 g/l, the residual glucose, the butanol concentration and the total solvent concentration were fluctuated from 10.8 to 15.4, 6.4 to 7.3 and 10.8 to 12.7 g/l respectively. The glucose consumption was set between 11.2-15.3 g/l hr. High cell concentration reduced permeate flux of the recycling unit lower than the point that the experiment operated with 0.36 hr^{-1} dilution rate could be done, so the experiment was stopped.

Figure 5.16 shows the behavior of the microorganism during operation of run B in terms of specific growth rate (μ), specific production rate (ν) and specific acid production rate (ν_{acid}). Table 5.3 shows the productivity and concentration of total solvent and butanol as function of dilution rate. Product concentrations for continuous run B were plotted against time (see figure 5.13) and total solvents (ethanol + acetone + butanol) concentrations is demonstrated in figure 5.15. The continuous availability of glucose and the glucose consumption are demonstrated in figures 5.14, 5.17 respectively. From table 5.3 the maximal productivity of 4.31 $gl^{-1}hr^{-1}$ was achieved at a 0.36 hr^{-1} dilution rate while the maximal butanol concentration (7.86 gl^{-1}) and maximal solvent concentration (13.07 g/l) were achieved at the 0.22 hr^{-1} dilution rate. In this continuous fermentation, total acid was also changed to acetone and butanol, so there were no acid accumulation in the system.

Comparing glucose consumption between run K and L (at 42.4 g/l and 52.0 g/l feeding glucose concentrations) shows that at the same dilution rate the glucose consumption hardly increased with feeding glucose concentration. There was higher residual glucose in run L because the amount of feeding glucose in run L was not used for fermentation. This could be explained by the effect of butanol on glucose uptake. Moriera and et al (23) reported that at high concentrations (more that 7 g/l), butanol could inhibite the rate of glucose uptake into the cell, thus the glucose consumption rate was limited by butanol concentration.

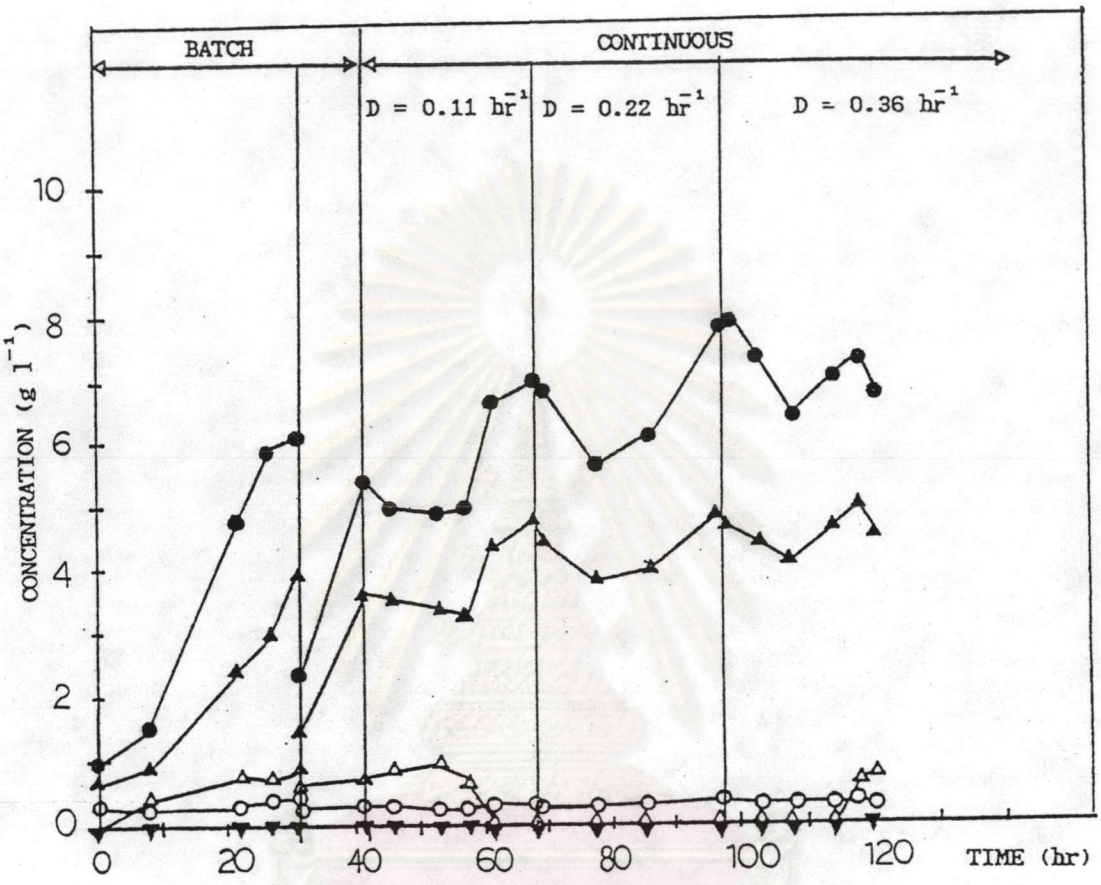

Figure 5.13 Time variation of butanol, acetone, ethanol acetic acid and butyric acid concentration during a total cell recycle system of Cl. acetobutylicum at a feeding glucose concentation 52.0 g/l (run L)

- ● butanol
- ▲ acetone
- ○ ethanol
- △ acetic acid
- ▼ butyric acid

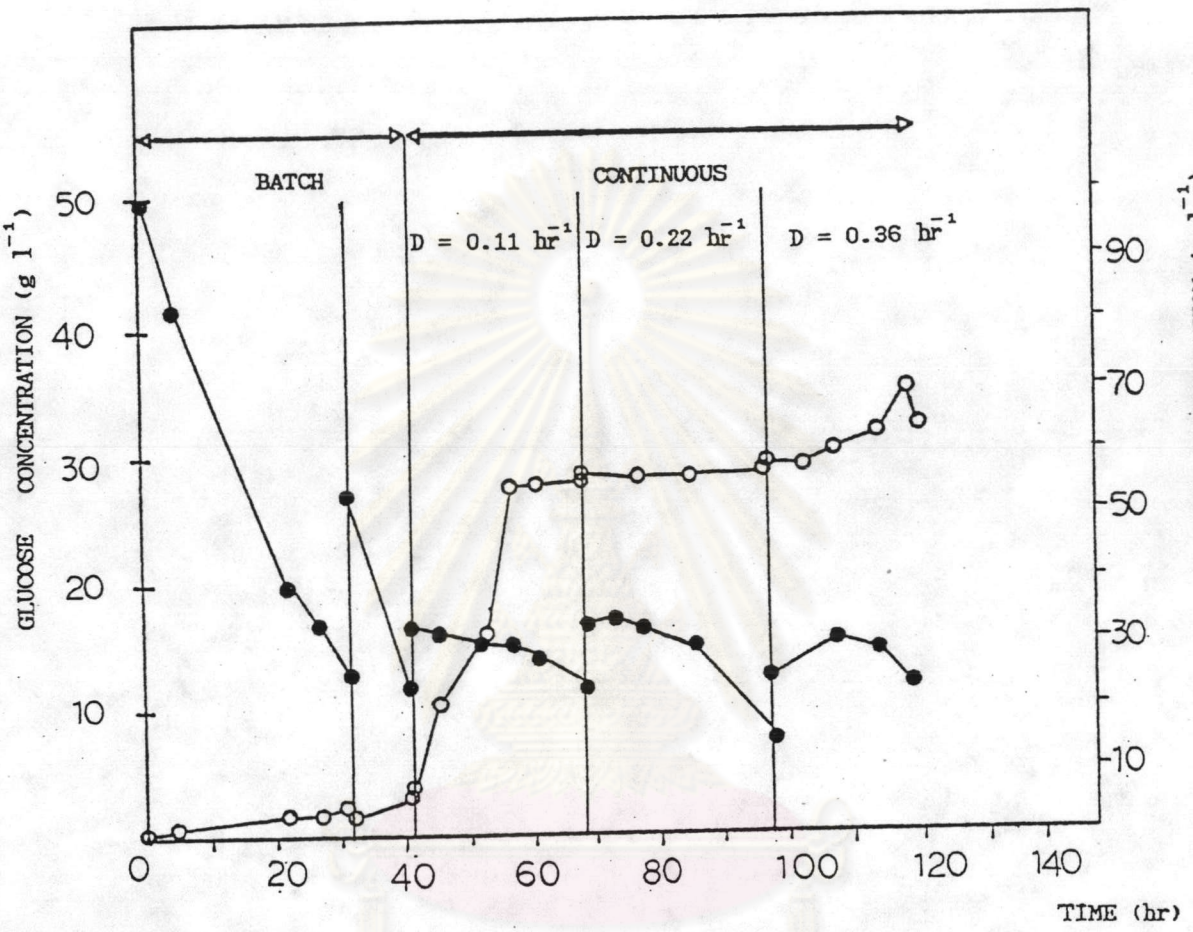

Figure 5.14 Time variation of glucose, biomass concentration during a total cell recycle system of Cl. acetobulylicum at a feeding glucose concentration 52.0 g/l (run L)

● glucose

○ biomass

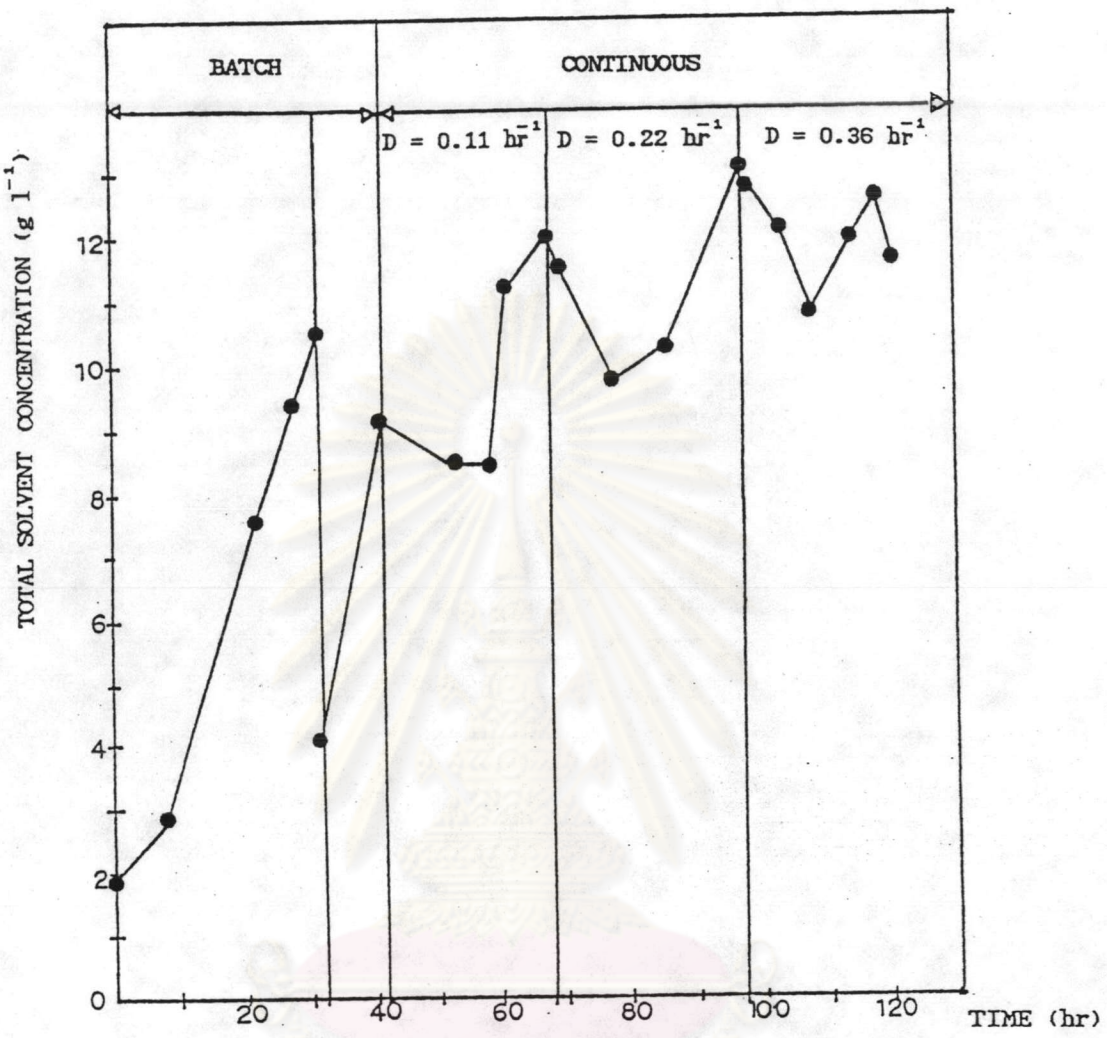

Figure 5.15 Time variation of total solvent concentration during a total cell recycle system of Cl. acetobutylicum at a feeding glucose concentation 52.0 g/l (run L)

● total solvent

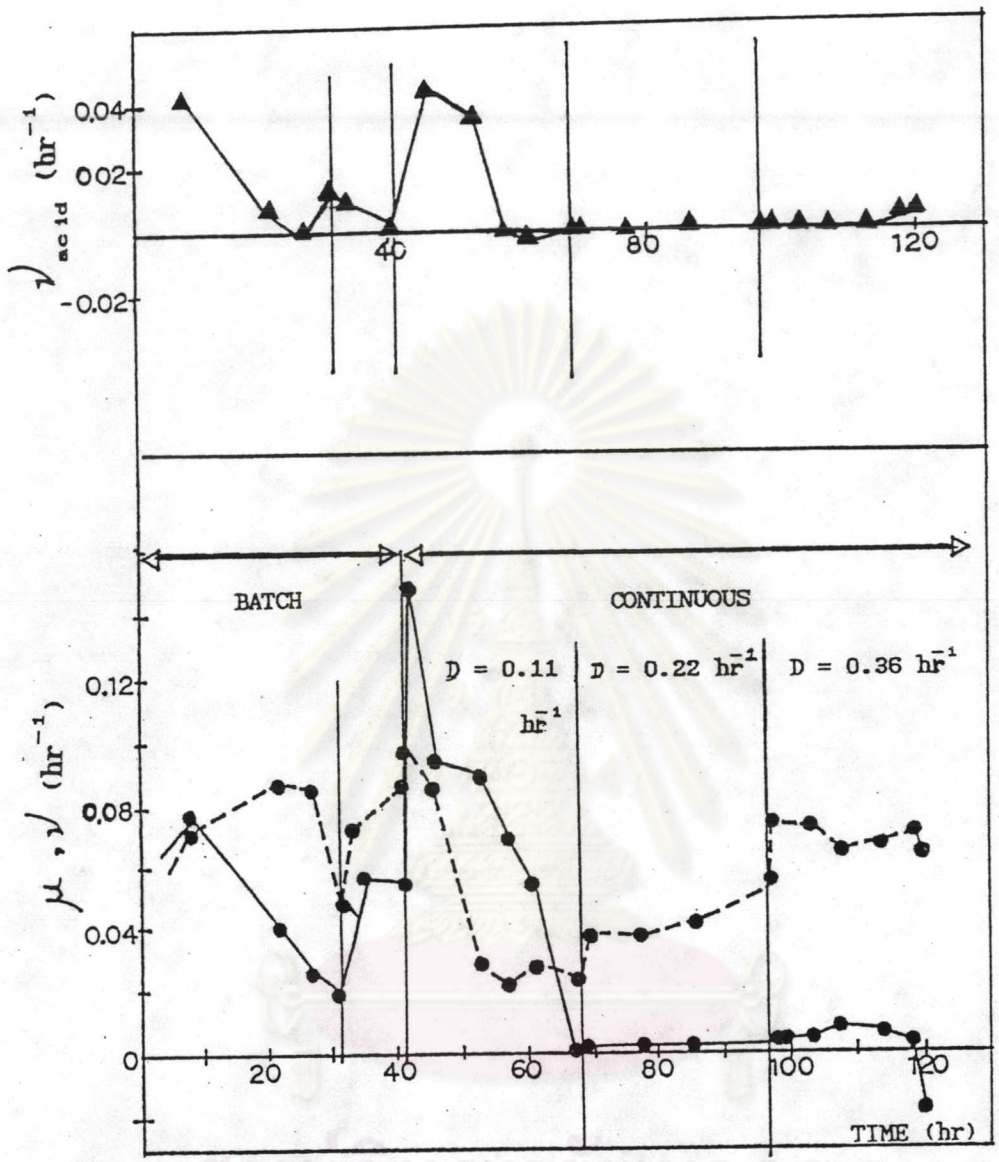

Figure 5.16 Specific growth rate (μ), specific production rate (ν) and specific acid production rate (ν_{acid}) against time (run L)

- ● specific growth rate (μ)
- --●-- specific production rate (ν)
- ▲ specific acid production rate (ν acid)

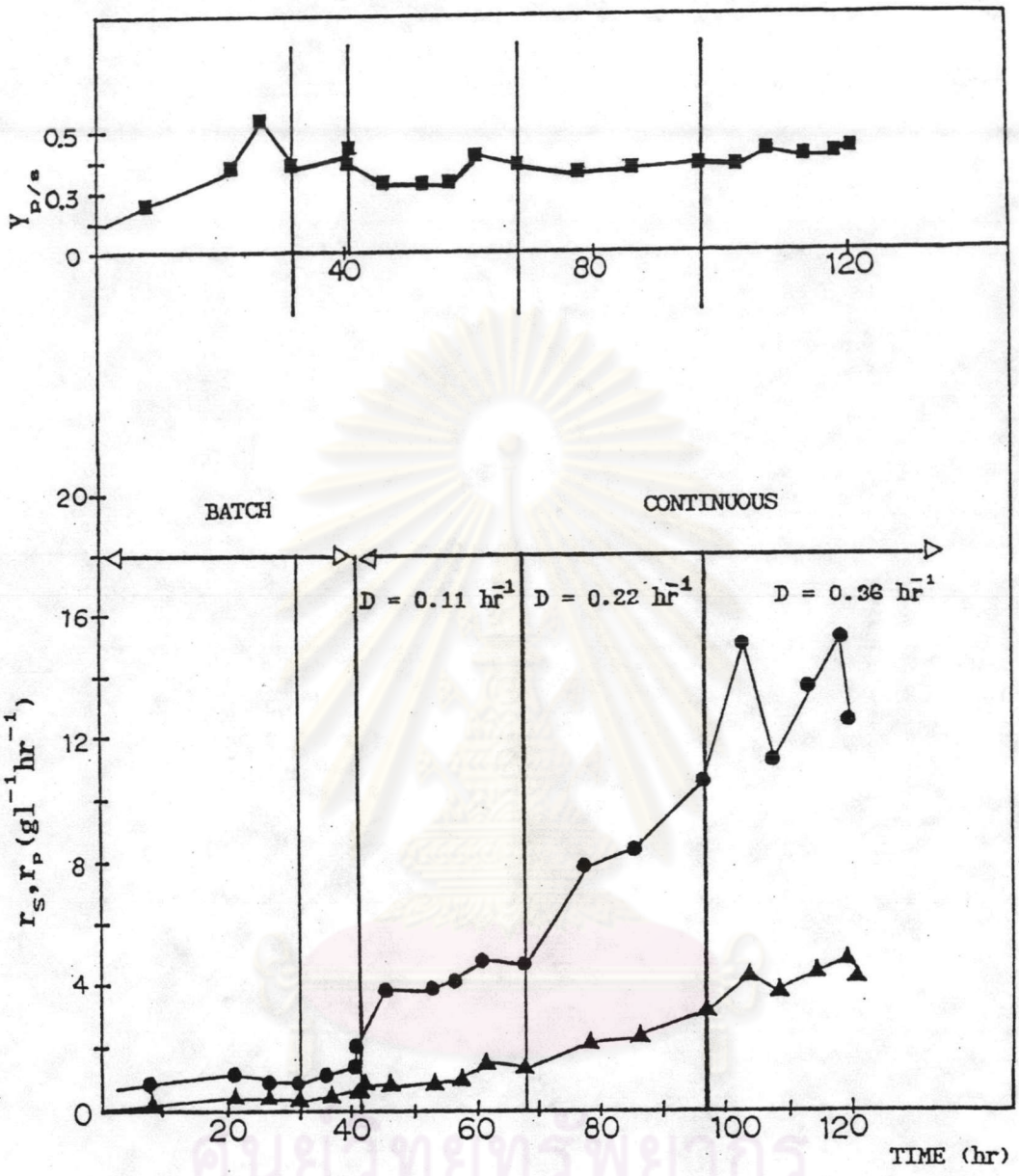

Figure 5.17 Glucose consumption (r_s), total solvent productivity (r_p) and production yield ($y_{p/s}$) against time (run L)

- ● glucose consumption (r_s)
- ▲ total solvent productivity (r_p)
- ■ production yield ($y_{p/s}$)

Table 5.3 Results of ABE fermentation from cell recycle system with feeding glucose concentration 52.0 g/l run L

Dilution rate (hr^{-1})	Productivity ($gl^{-1}hr^{-1}$)		Concentration (g/l)		r_s ($gl^{-1}hr^{-1}$)	$Y_{p/s}$
	solvent	butanol	solvent	butanol		
D = 0.11	1.36	0.80	11.98	6.92	4.64	0.29
D = 0.22	3.13	1.89	13.07	7.86	10.58	0.30
D = 0.36	4.31	2.56	11.98	7.11	13.54	0.32

Remark

*The reported productivities were calculated from the overall production at the end of each stages.

5.2.1 C) At Feeding Glucose Concentration 64.8 g/l (run M)

The results of this experiment are shown in figure 5.19-5.22 and table 5.4, A4, A9. After 38 hr of the second batch operation, the cell mass was almost constant at 4.1 gl^{-1} with the maximal specific growth rate and production rate at 0.06, and 0.10 hr^{-1} respectively; then first continuous fermentation with total cell recycle started at a fixed dilution rate of 0.11 hr^{-1}.

At a 0.11 hr^{-1} dilution rate the cell concentration was increased to 60 gl^{-1} in 43.5 hr^{-1} and the cell concentration remained constant at 60 g/l. The residual glucose was 40 g/l and the average total solvent and butanol concentrations were 8.50 and 4.76 g/l repectively with 0.94 g/l of solvent productivity. The glucose consumption was rather constant at 2.80 g/l hr and then the dilution rate was changed into 0.22 hr^{-1}.

After running at a 0.22 hr^{-1} dilution rate for 14.5 hr^{-1}, the cell concentration increased to 65 g/l and then the cell concentration fluctuated between 65-62 g/l with total solvent and butanol concentration at 4.22 gl^{-1} and 2.38 gl^{-1} respectively. The glucose consumption rate was nearly constant at 2.81 g/l hr and the solvent productivity was 0.91 g/l hr.

Comparison of the glucose consumption and the solvent productivity between 0.11 hr^{-1} and 0.22 hr^{-1} dilution rate, showed that increasing of dilution rate could not improve the productivity and the glucose consumption. The experiment was then stopped at a 0.22 hr^{-1} dilution rate.

In this experiment, there was higher acid accumulation than previous experiments (runs K, L). The low productivity and low glucose consumption rate were achieved while the cell concentration was rather high. All of the results showed that the microorganisms reduced the activity of fermentation of glucose to produce solvent. The low productivity might come from the very excessive residual glucose from operation with very high feeding glucose concentration that made the microorganisms tend to use the majority of consumption glucose to produce cells but reduce the activity of consuming glucose for producing solvent. Another factor that might reduce activity to produce solvent was the effect of burned glucose. From many fermentation processes, the amount of burned glucose could reduce the activity of microorganism to produce solvent. The burning of glucose occured while keeping high glucose concentration broth at high temperatures for a long time.

The behavior of this microorganism at a specific growth rate, a specific production rate and a specific acid production rate are shown in figure 5.21 while the glucose consumption, the solvent productivity and the production yield are shown in figure 5.22, the productivity and concentration against dilution rate is shown in table 5.4.

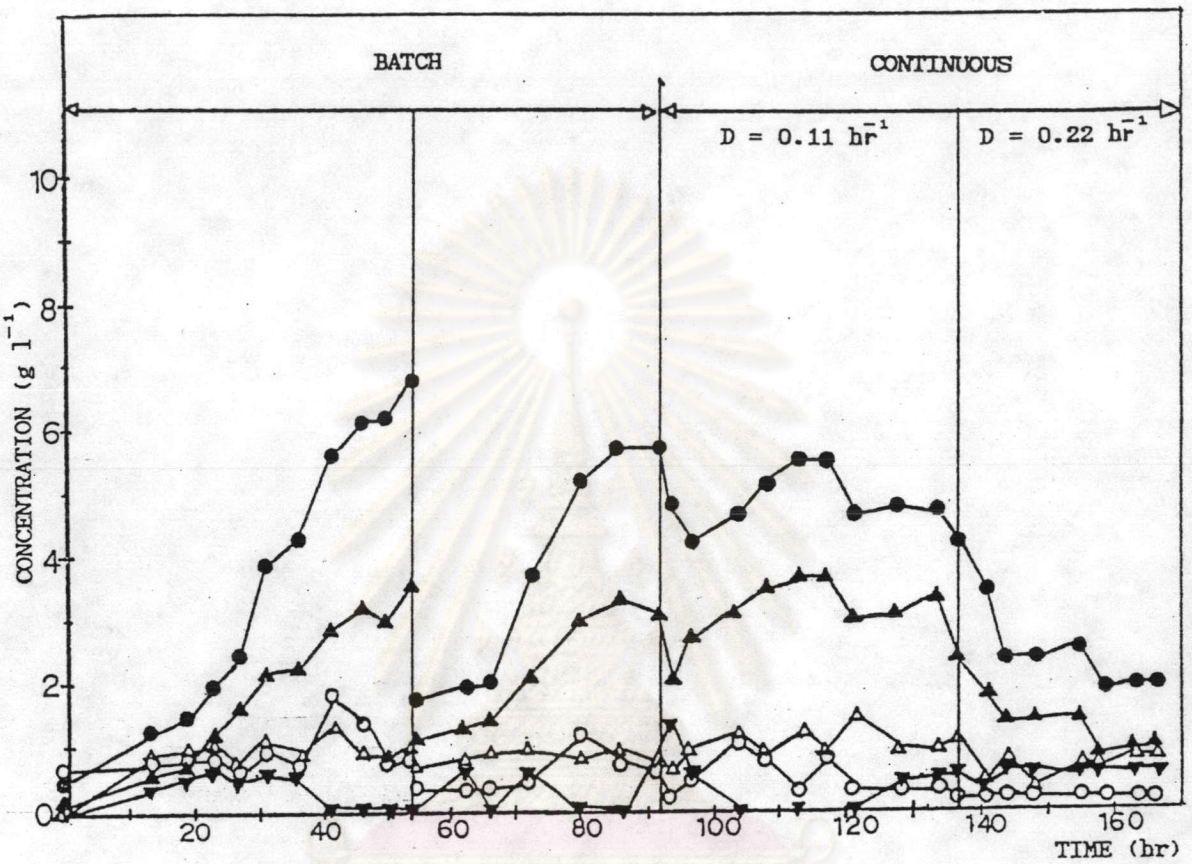

Figure 5.18 Time variation of acetic acid and butyric acid concentration during a total cell recycle system of Cl. acetobutylicum at a feeding glucose concentation 64.8 g/l (run M)

- ● butanol
- ▲ acetone
- ○ ethanol
- △ acetic acid
- ▼ butyric acid

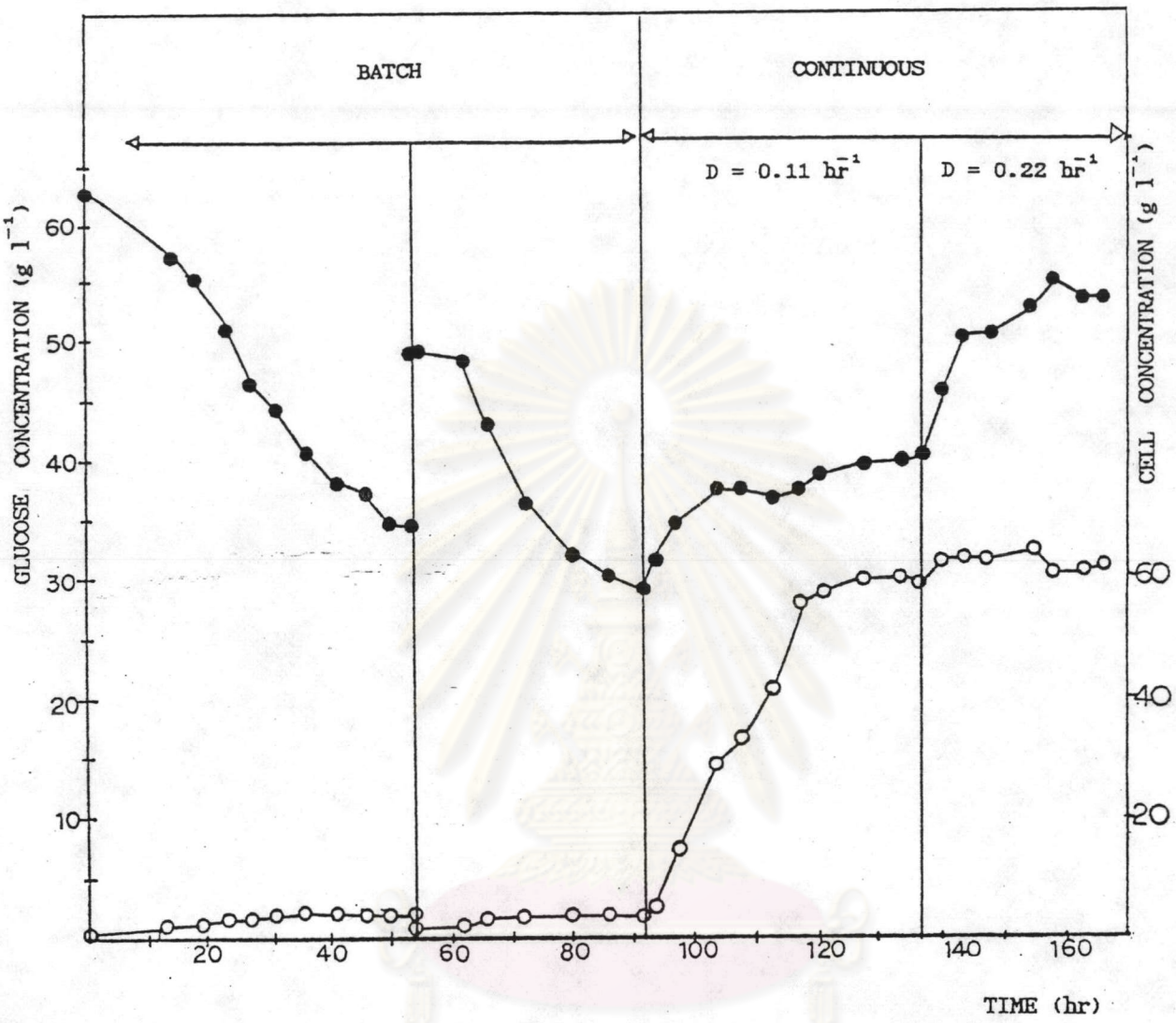

Figure 5.19 Time variation of glucose, biomass concentration during a total cell recycle system of Cl. acetobulylicum at a feeding glucose concentration 64.8 g/l (run M)

● glucose

o biomass

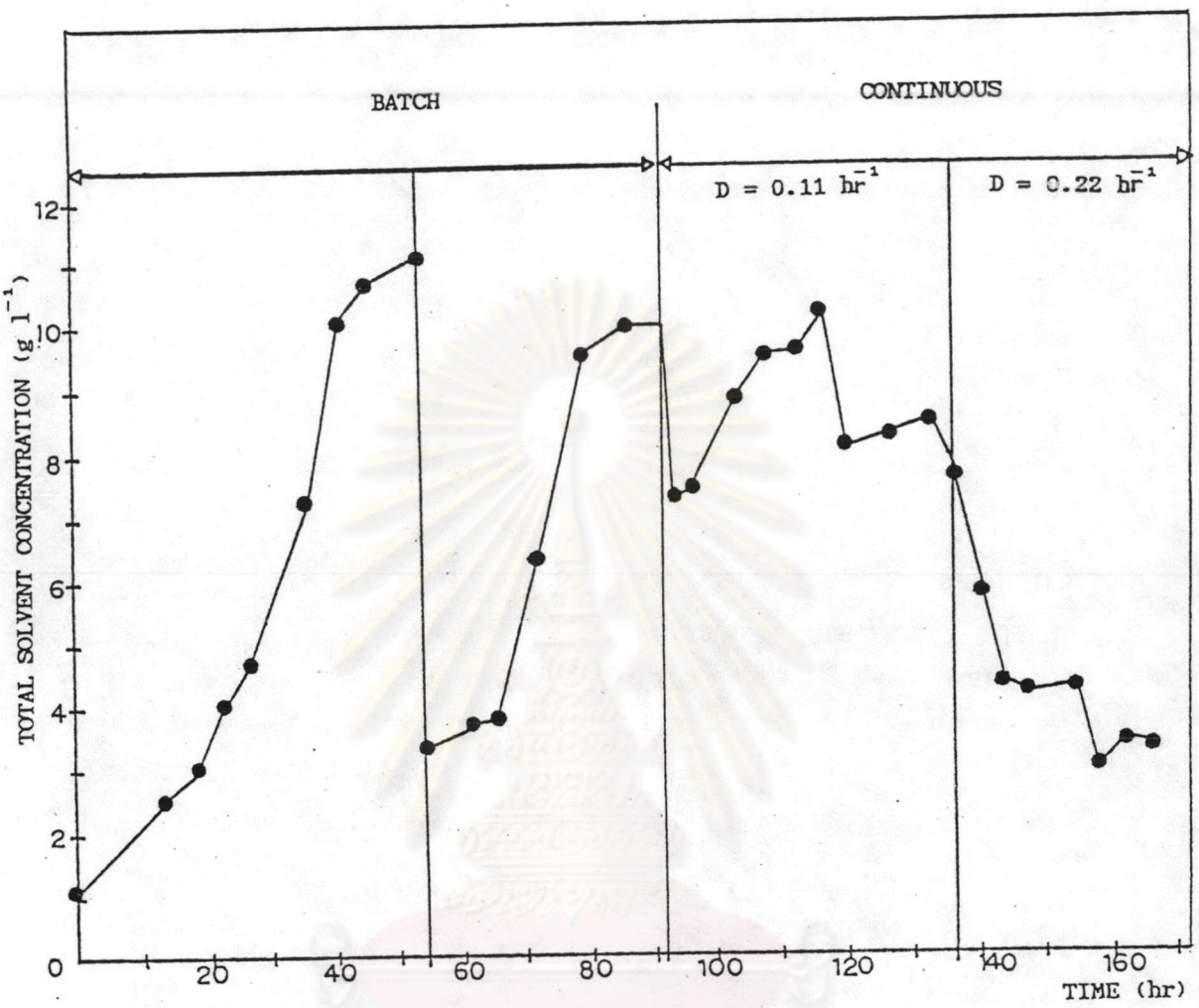

Figure 5.20 Time variation of total solvent concentration during a total cell recycle system of Cl. acetobutylicum at a feeding glucose concentation 64.8 g/l (run M)

● total solvent

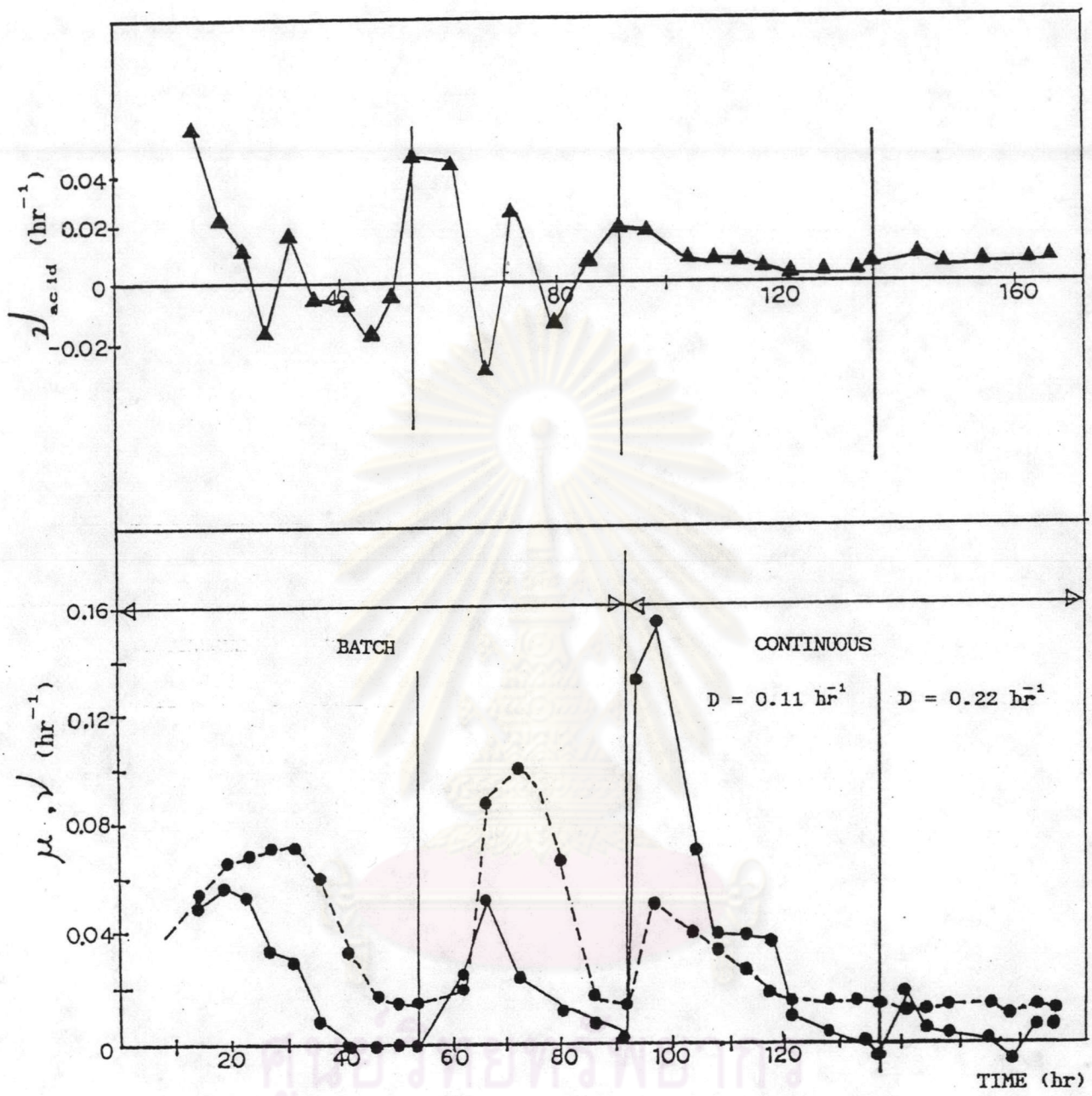

Figure 5.21 Specific growth rate (μ), specific production rate (ν) and specific acid production rate (ν_{acid}) against time (run M)

- ● specific growth rate (μ)
- –●– specific production rate (ν)
- ▲ specific acid production rate (ν_{acid})

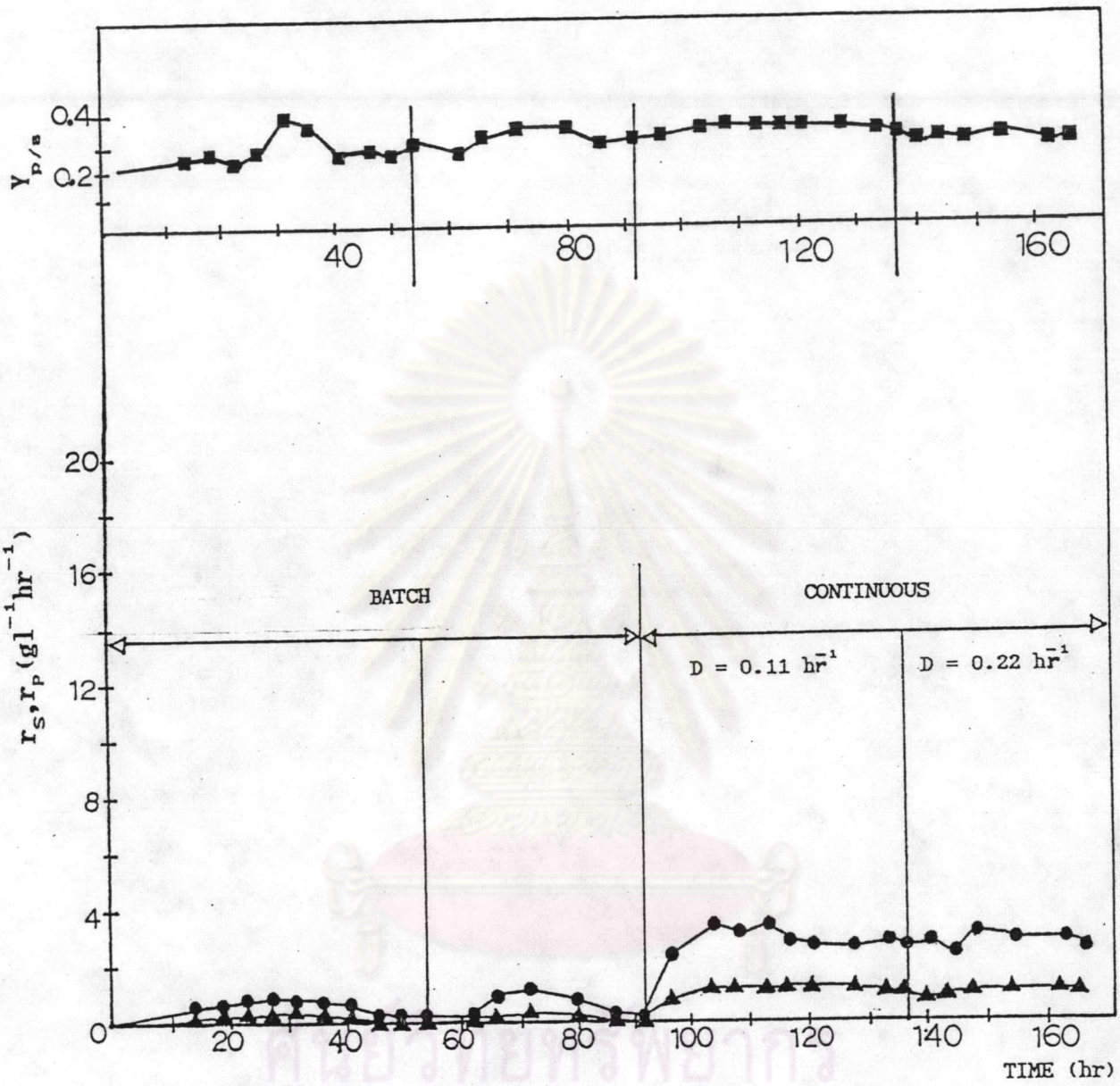

Figure 5.22 Glucose consumption (r_s), total solvent productivity (r_p) and production yield ($y_{p/s}$) against time (run M)

● glucose consumption (r_s)

▲ total solvent productivity (r_p)

■ production yield ($y_{p/s}$)

Table 5.4 Results of ABE fermentation from cell recycel system with feeding glucose concentration 64.8 g/l run M

Dilution rate (hr^{-1})	Productivity ($gl^{-1}hr^{-1}$)		Concentration ($gl^{-1}hr^{-1}$)		r_s ($gl^{-1}hr^{-1}$)	$Y_{P/S}$
	solvent	butanol	solvent	butanol		
D = 0.11	0.94	0.52	8.50	4.76	2.80	0.33
D = 0.22	0.91	0.52	4.12	2.38	2.81	0.31

Remark

*The reported productivities were calculated from the overall production at the end of each stages.

5.3 Experiment of Cell Recycle System with Constant Dilution Rate

5.3.1 At 42.3 gl^{-1} Feeding Glucose Concentration and 0.55 hr^{-1} Constant Dilution Rate. (Run N)

The experiment of the cell recycle system with total cell recycle at a 42.3 g/l feeding glucose concentration was operated at a fixed dilution rate of 0.55 hr^{-1} from the start of the continuous operation to compare the productivity and the glucose consumption rate with the experiment of run A (in run A the dilution rate was increased from 0.11 to 0.22, 0.36 and 0.55 hr^{-1} respectively. following the glucose consumption).

The results of this experiment are shown in figures 5.23-5.27 and tables A5, A10. After the second batch operation, the cell mass reached 4.1 g/l with the maximal specific growth rate and production rate of about 0.07 hr^{-1} and 0.10 hr^{-1} respectively. The residual glucose concentration was reduced to 11.8 gl^{-1},then the first continuous fermentation with total cell recycle was started at a fixed dilution rate of 0.55 hr^{-1}.

After changing from batch operation to continuous operation with total cell recycle, the cell concentration and the glucose consumption were increased rapidly. The cell concentration reached 36.12 g/l with a 10.70 g/l solvent concentration and a 5.65 g/l butanol concentration in 14 hours of operation. The residual glucose was 9.0 g/l with a glucose consumption rate of 19.35 $gl^{-1}hr^{-1}$ and a 0.31 production yield. Then the fermentation operation was extremely difficult because of high cell concentration. The dilution rate could not be fixed at 0.55 hr^{-1} anymore, because the permeate flux was less than the point where a dilution rate of 0.55 hr^{-1} could occur. Then, the dilution rate was decreased and the residual glucose was also

decreased to less than 5 g/lit. After about 10 hours, a part of the fermentation broth was aseptically drained and replace by feeding medium. The cell concentration was reduced to 31.28 g/l and the dilution rate fixed at 0.55 hr^{-1} again, Then the second continuous fermentation with total cell recycle at 0.55 hr^{-1} dilution rate was started.

From the reported data in figures 5.23-5.25, the curve of the cell concentration, the residual glucose, the solvent concentration against time of the second operation were similar to that of the first operation performed at a 0.55 hr^{-1} dilution rate. After 13 hours of operation, the cell concentration reached 43.7 g/l. The solvent and butanol concentrations were 9.5 and 5.0 g/l respectively. The glucose consumption was 18.9 g/l. Then the dilution rate could not be fixed at 0.55 hr^{-1} again because of high cell concentration of fermentation broth, so the experiment was stopped. However from the result, the solvent and butanol production of the second operation was less than that of the first operation which might come from the loss of cell activity at very low glucose concentration for about 6 hours.

The comparison of the cell recycle system with 42.4-42.3 g/l feeding glucose concentration between run K and run N is shown reported in table 5.8. The results of run N was almost the same as the results of run K at a 0.55 hr^{-1} dilution rate. The solvent productivity of run N (5.98 $gl^{-1}hr^{-1}$) was only 1.32% different from run K at a 0.55 hr^{-1} dilution rate (6.06 $gl^{-1}hr^{-1}$) due to the difference in the dilution rate at the start of the operation. From the results that the productivity of run N was close to the productivity of run K (at the same dilution rate). There was no need to repeat the experiment in order to check the productivity with constant dilution rate at 50 and 60 gl^{-1} feeding glucose concentration.

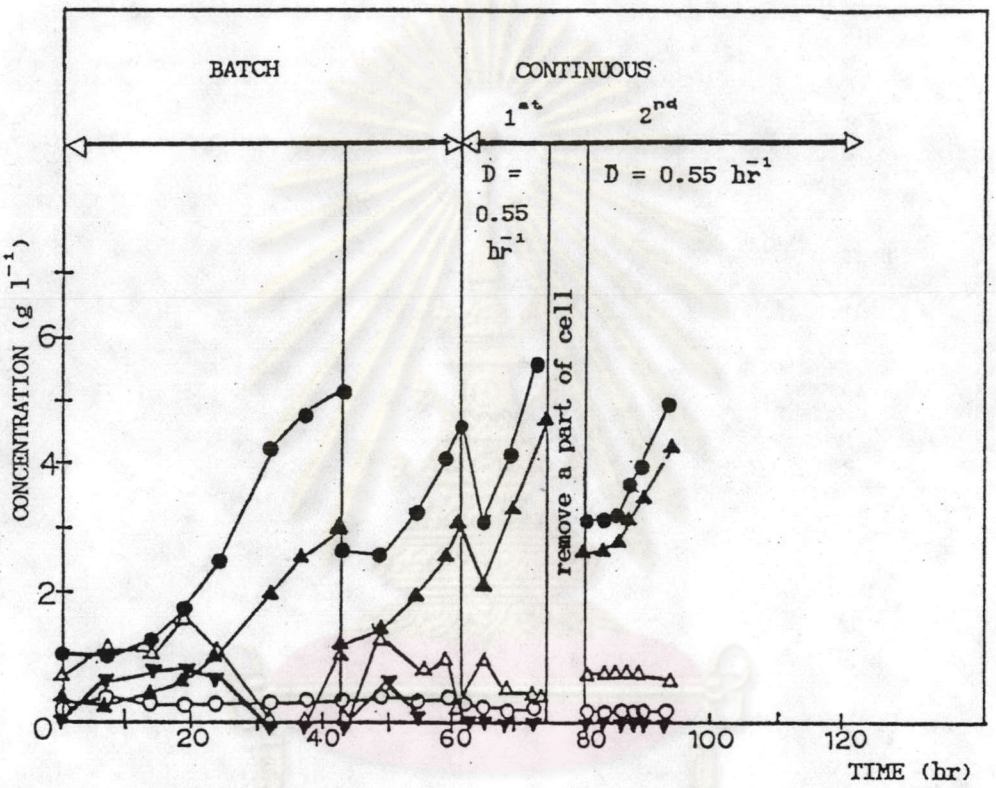

Figure 5.23 Time variation of butanol, acetone, ethanol, acetic acid and butyric acid concentration during a total cell recycle system of Cl. acetobutylicum at a constant 0.55 hr^{-1} dilution rate with 42.3 g/l feeding glucose concentration (run N)

- ● butanol
- ▲ acetone
- O ethanol
- △ acetic acid
- ▼ butyric acid

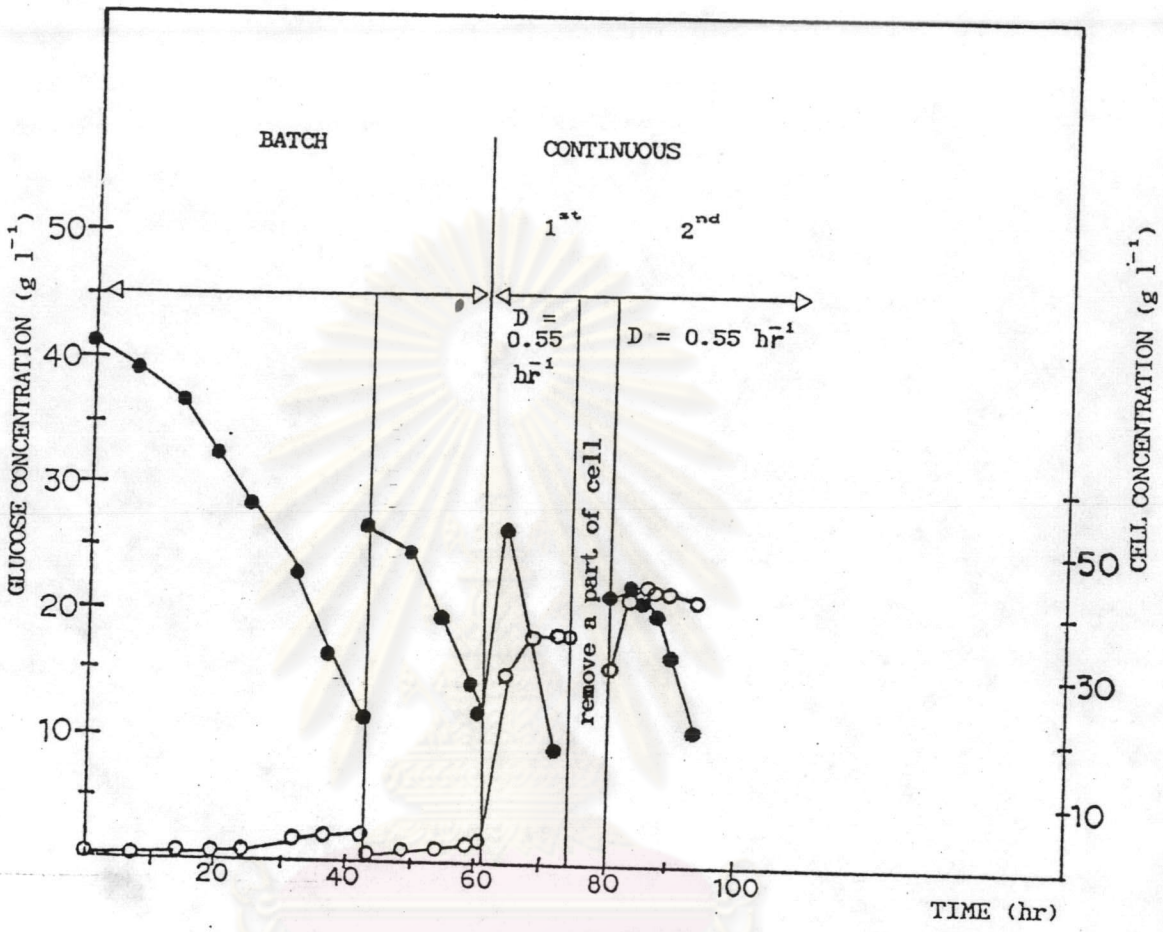

Figure 5.24 Time variation of glucose, biomass concentration during a total cell recycle system of Cl. acetobutylicum at a constant 0.55 hr^{-1} dilution rate with 42.3 g/l feeding glucose concentration (run N)

● glucose

○ biomass

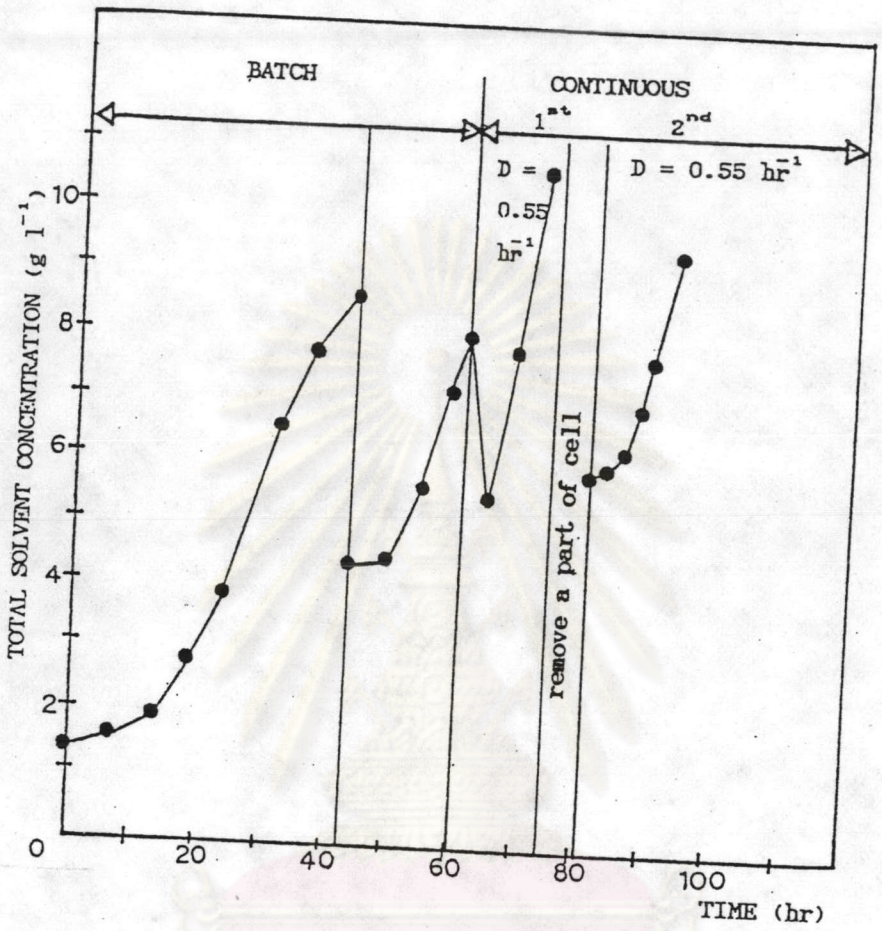

Figure 5.25 Time variation of total solvent concentration during a total cell recycle system of Cl. acetobutylicum at a constant 0.55 hr^{-1} dilution rate with 42.3 g/l feeding glucose concentration (run N)

● total solvent

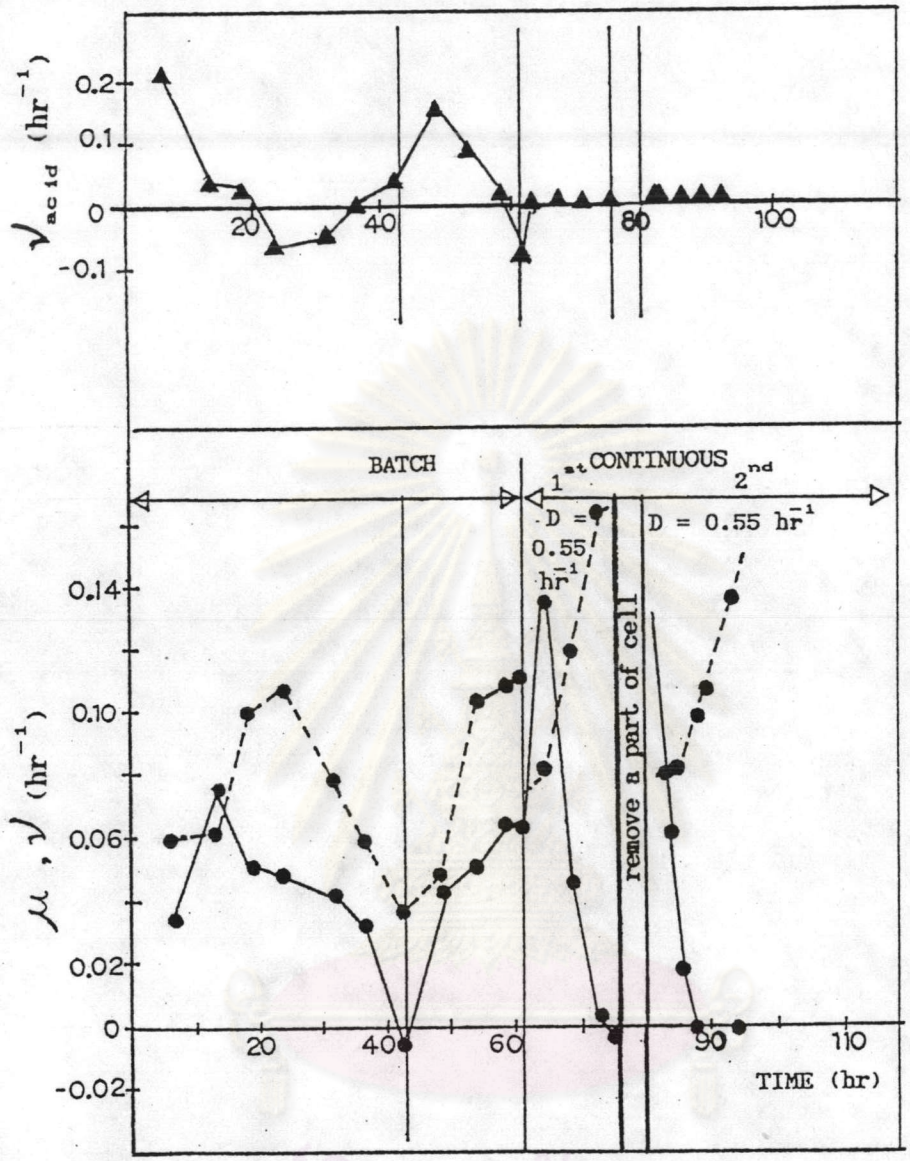

Figure 5.26 Specific growth rate (μ), specific production rate (ν) and specific acid production rate(ν_{acid}) against time (run N)

● specific growth rate (μ)

–●– specific production rate (ν)

▲ specific acid production rate (ν acid)

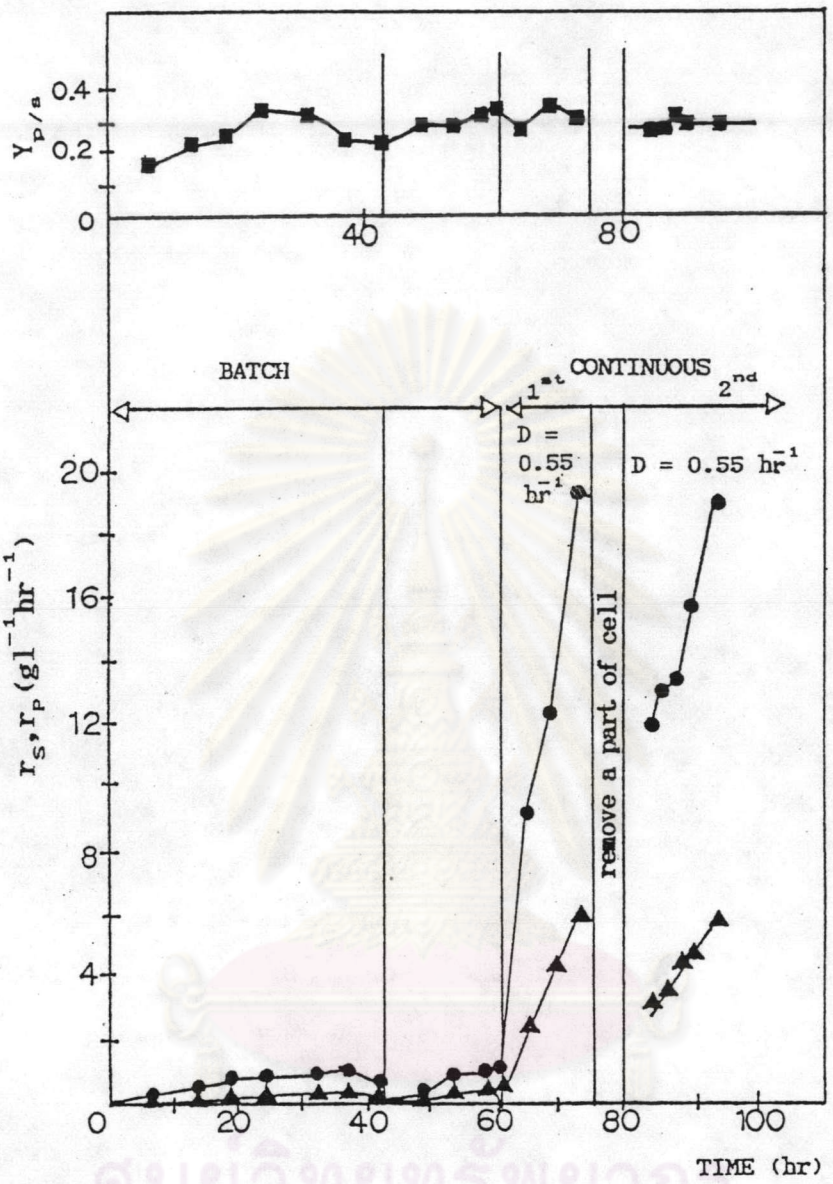

Figure 5.27 Glucose consumption (r_s), total solvent productivity (r_p) and production yield ($y_{p/s}$) against time (run N)

- ● glucose consumption (r_s)
- ▲ total solvent productivity (r_p)
- ■ production yield ($y_{p/s}$)

Table 5.5 Comparision of ABE fermentation from cell recycle systems with feeding glucose concentration 42.4 and 42.3 g/l in run K and run N respectively

Run	Dilution rate (hr^{-1})	Productivity ($gl^{-1}hr^{-1}$)		Concentration (g/l)		r_s	$Y_{p/s}$
		solvent	butanol	solvent	butanol	($gl^{-1}hr^{-1}$)	
A	0.11	1.16	0.58	8.05	4.21	4.90	0.24
	0.22	2.67	1.71	10.41	5.89	9.39	0.28
	0.36	4.15	2.36	10.94	6.20	14.80	0.28
	0.55	6.06	3.44	11.03	6.26	19.30	0.31
D^{1st}	0.55	5.98	3.40	10.70	5.65	19.35	0.31
D^{2nd}	0.55	5.67	3.40	9.50	5.01	19.00	0.30

Remark

*The reported productivities were calculated from the overall production at the end of each stages.

5.3.2 At 43.6 gl^{-1} Feeding Glucose Concentration and 0.65 hr^{-1} Constant Dilution Rate (Run O)

The experiment of the cell recycle sytem with a 43.6 gl^{-1} feeding glucose concentration and a 0.65 hr^{-1} dilution rate was operated to observe the productivity at the dilution rate above the maximal dilution rate (0.55 hr^{-1}) limited by fouling of the U.F. membrane.

The results of the experiment are shown in figures 5.28-5.32 and table 5.9. After the second batch operation, the cell mass reached 3.01 gl^{-1} with the maximal specific growth rate and production rate of about 0.10 hr^{-1} and 0.13 hr^{-1} respectively. The residual glucose concentration was reduced to 13.6 gl^{-1} then the continuous fermentation with total cell recycle was started with a fixed dilution rate of 0.65 hr^{-1}.

After changing from batch to continuous with total cell recycle operation, the cell concentration was increased rapidly. The cell concentration reached 40.40 gl^{-1} in 21 hours of operation with a 3.65 $gl^{-1}hr^{-1}$ glucose consumption and a 0.88 a $gl^{-1}hr^{-1}$ solvent productivity. Then the operation was extremely difficult because of the high cell concentration. The dilution rate could not be fixed at 0.65 hr^{-1} because the permeate flow rate was less than the point where 0.65 hr^{-1} dilution rate could occur. Then the continuous operation was stopped. At a result the solvent productivity was very low, the batch fermentation was operated to check the activity of the microorganisms.

From the experiment, the increase in dilution rate above the maximal point (0.55 hr^{-1} dilution rate) limited by fouling of the U.F. membrane could not improve solvent productivity. Moreover, the very excessive residual glucose from operation with very high dilution rate made the microorganisms use majority of consumption glucose to

generate cells and reduce the activity of consuming glucose for solvent production. So the glucose consumption and solvent productivity was very low comparing with run N (0.55 hr^{-1} dilution rate). At 21 hours of the operation 74.58% and 24.13% of the glucose consumed was used for producing cells and solvent respectively. The behaviour of the microorganisms against time is shown in figure 5.31 in the form of specific growth rate and specific production rate. A batch fermentation was operated to confirm that the microorganisms did not loose the activity to produce solvent. After 60 hours of batch operation, the cells consumed 97.71% of glucose in the medium, 7.18% and 32.46% of consumed glucose was converted to cells and solvent respectively. The final solvent concentration in the broth was 13.26 gl^{-1} with 0.23 $gl^{-1}hr^{-1}$ of solvent productivity. The result of the batch operation proved that the microorganisms did not loose the activity for producing solvent. So the low productivity in continuous operation came from the reducing of cell activity at very excessive residual glucose. The glucose consumption, solvent productivity and production yield are shown in figure 5.30 and table 5.9.

From the results that the productivity was not improved by increasing dilution rate above the maximum point limited by membrane fouling from the too excessive residual glucose condition, there was no need to make the experiments of this increasing dilution for the cases of 50 and 60 gl^{-1} feeding glucose concentration.

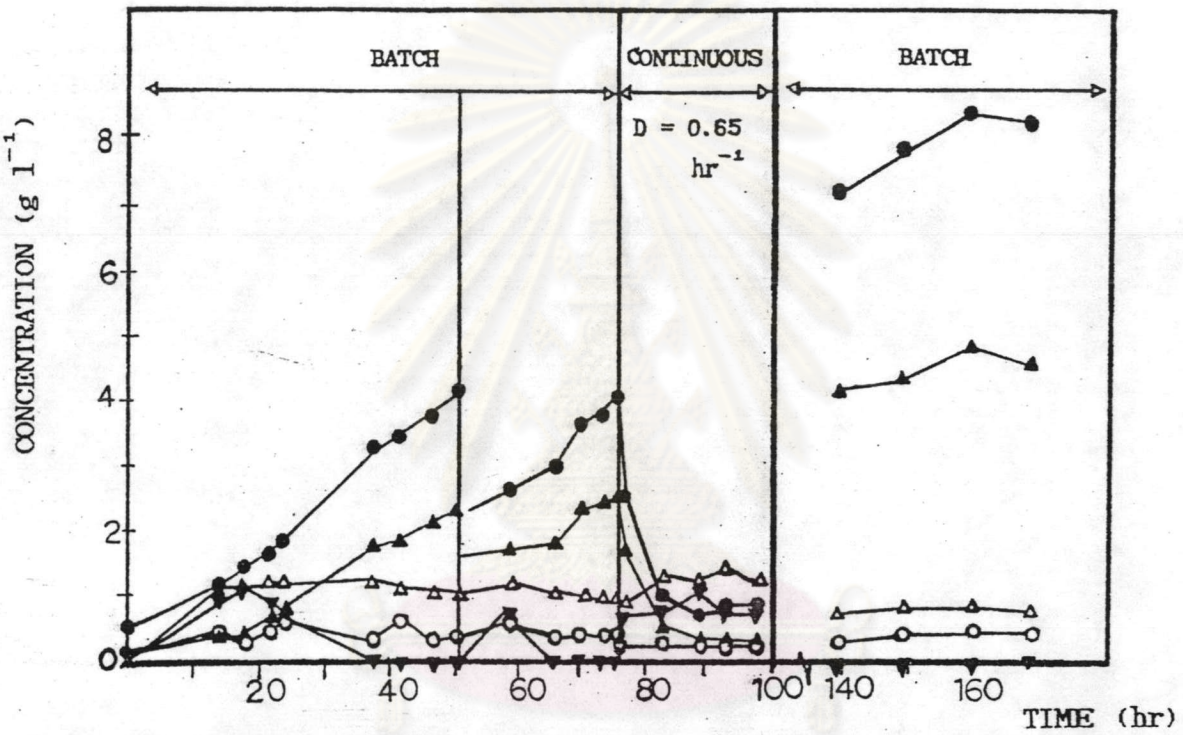

Figure 5.28 Time variation of butanol, acetone, ethanol, acetic acid and butyric acid concentration during a total cell recycle system of Cl. acetobutylicum at a constant 0.65 hr^{-1} dilution rate with 43.6 g/l feeding glucose concentration (run O)

- ● butanol
- ▲ acetone
- o ethanol
- △ acetic acid
- ▼ butyric acid

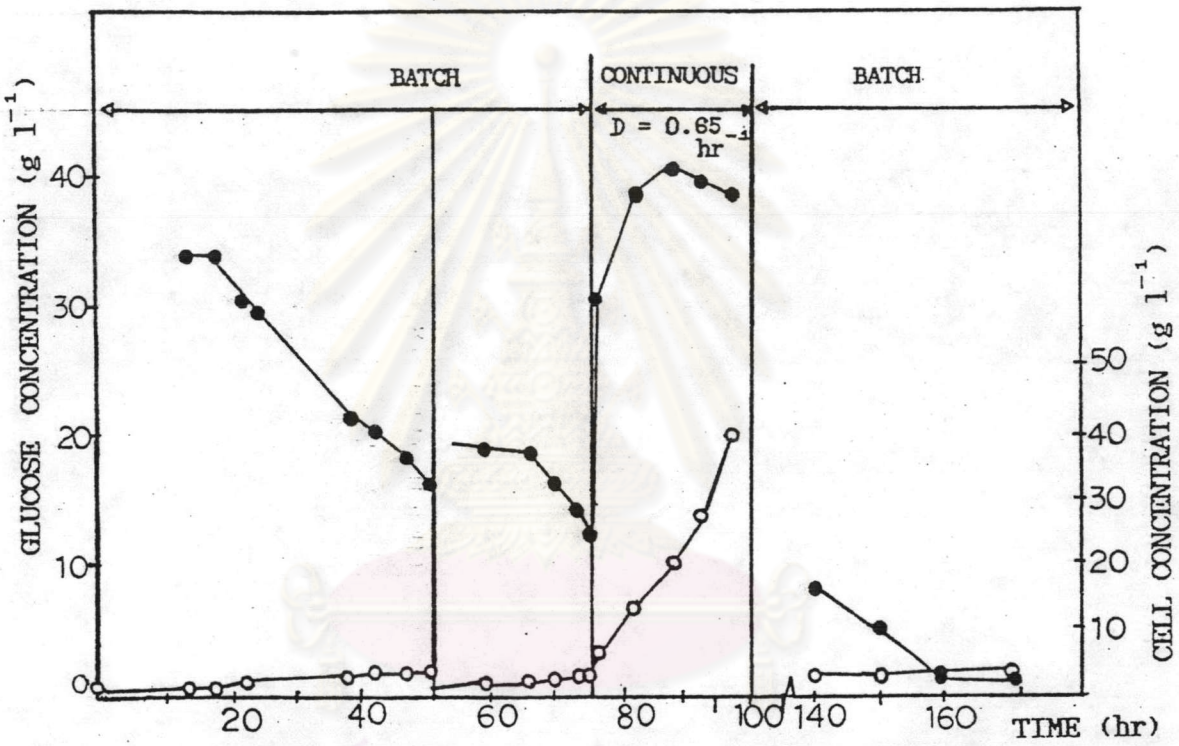

Figure 5.29 Time variation of glucose, biomass concentration during a total cell recycle system of Cl. acetobutylicum at a constant 0.65 hr^{-1} dilution rate with 43.6 g/l feeding glucose concentration (run O)

● glucose

O biomass

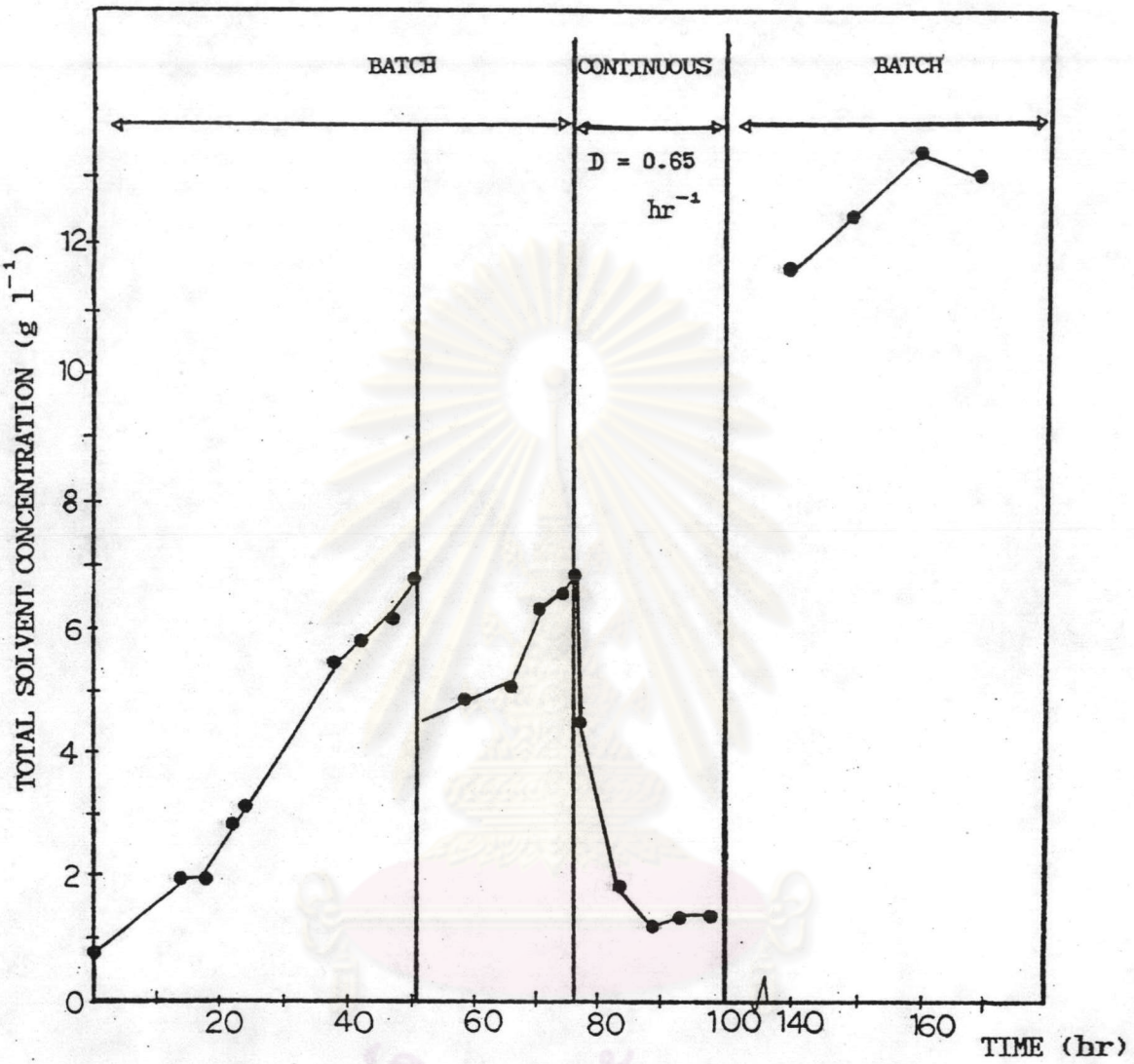

Figure 5.30 Time variation of total solvent concentration during a total cell recycle system of Cl. acetobutylicum at a constant 0.65 hr^{-1} dilution rate with 43.6 g/l feeding glucose concentration (run 0)

● total solvent

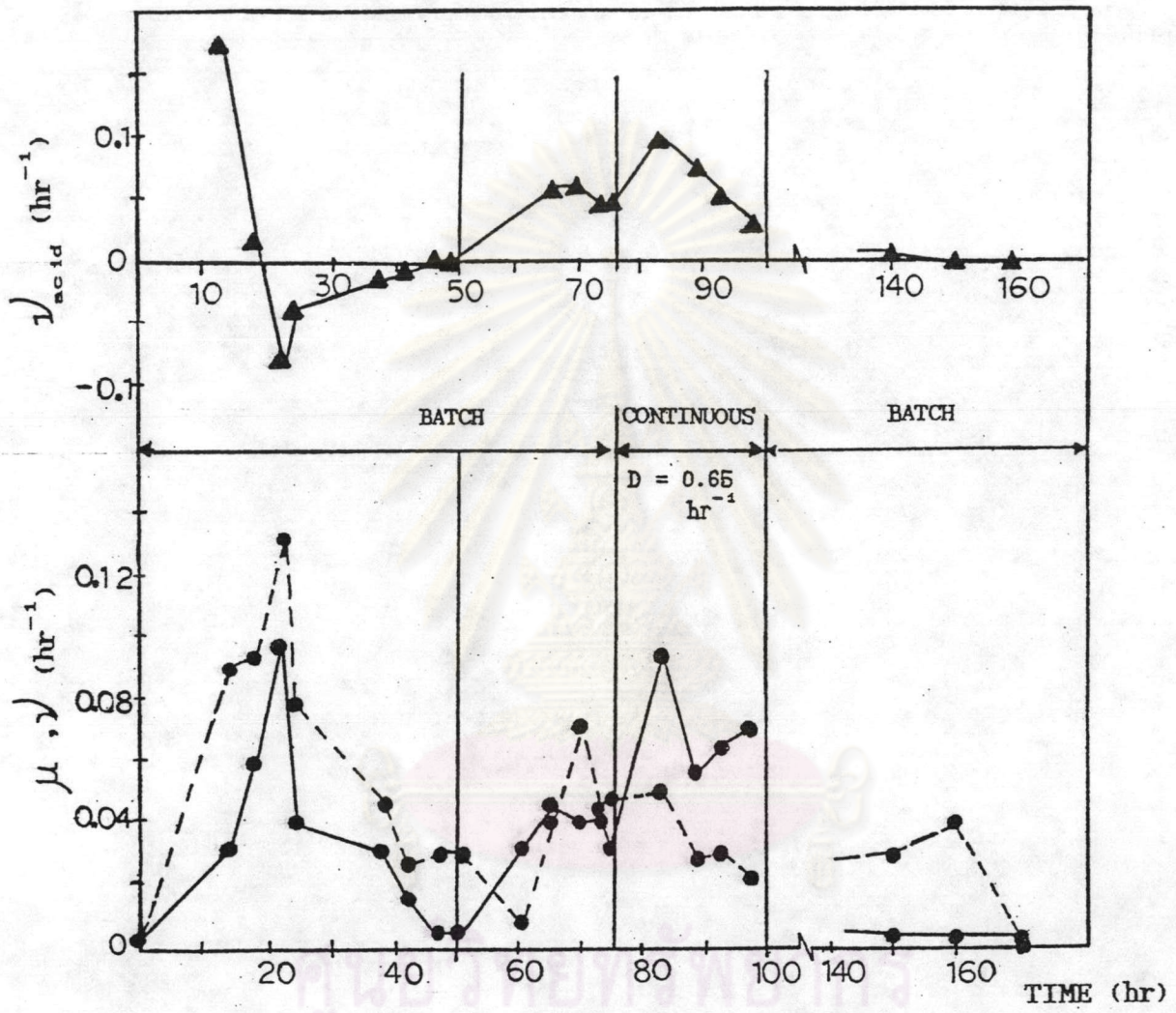

Figure 5.31 Specific growth rate (μ), specific production rate (ν) and specific acid production rate (ν_{acid}) against time (run 0)

- ● specific growth rate (μ)
- –●– specific production rate (ν)
- ▲ specific acid production rate (ν_{acid})

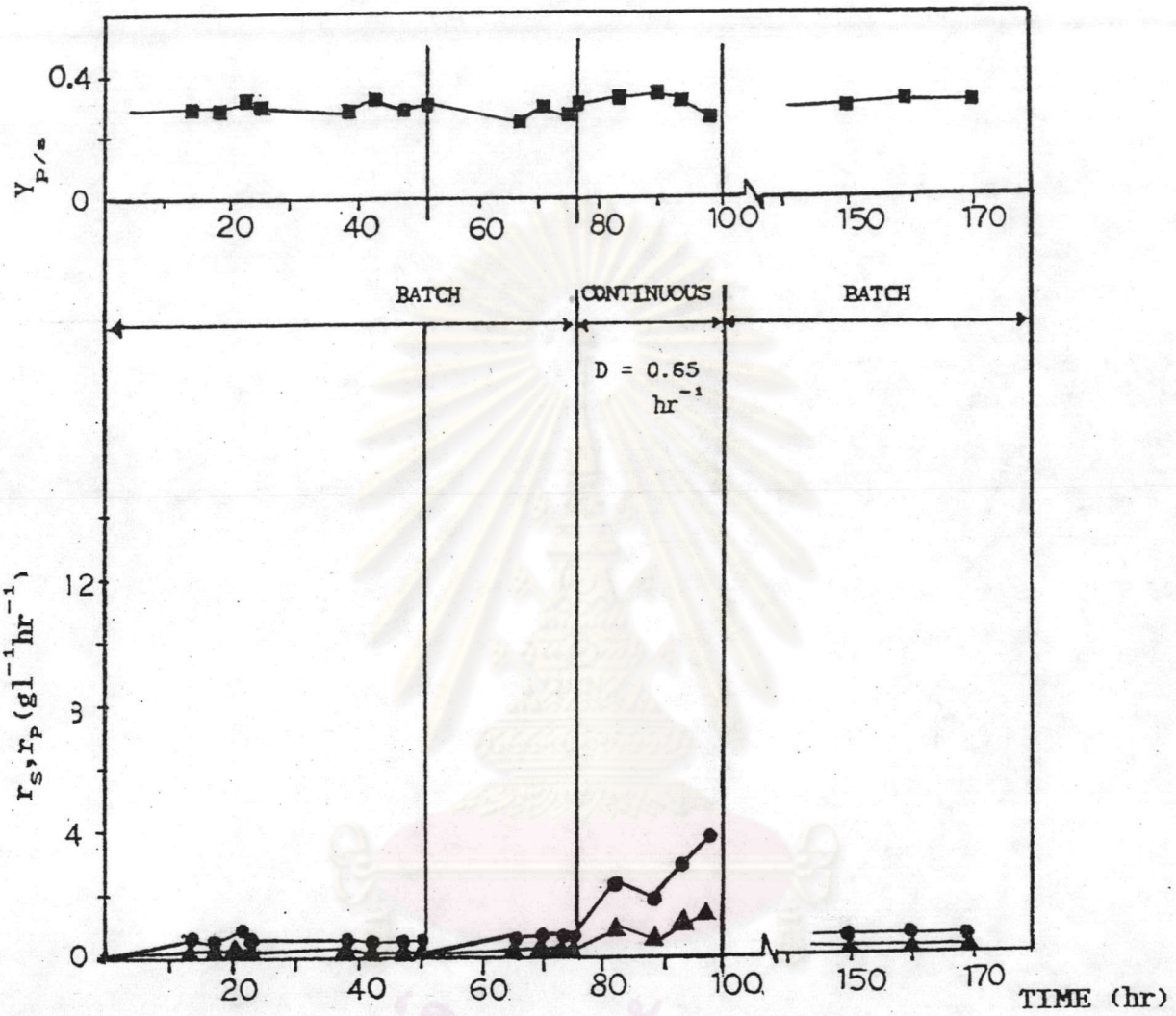

Figure 5.32 Glucose consumption (r_s), total solvent productivity (r_p) and production yield ($y_{p/s}$) against time (run O)

● glucose consumption (r_s)

▲ total solvent productivity (r_p)

■ production yield ($y_{p/s}$)

Table 5.6 Results of ABE fermentation from cell recycle system with feeding glucose concentration 43.6 g/l run O

Dilution rate (hr^{-1})	Productivity ($gl^{-1}hr^{-1}$)		Concentration (g/l)		r_s	$Y_{p/s}$
	solvent	butanol	solvent	butanol	($gl^{-1}hr^{-1}$)	
0.65	0.88	0.56	1.34	0.85	3.65	0.24

Remark

*The reported productivities were calculated from the overall production at the end of the stage

5.4 Optimization of the Cell Recycle System

The comparison of total solvent productivity and butanol productivity are reported in tables 5.5 and 5.6. The comparison of glucose consumption is shown in table 5.7

From the results, the maximal solvent productivity (6.06 $gl^{-1}hr^{-1}$) and the maximal glucose consumption rate (19.30 $gl^{-1}hr^{-1}$) with the 0.31 production yield were achieved in the cell recycle system with a 42.4 g/l feeding glucose concentration at 0.55 hr^{-1} dilution rate (in run K). The solvent concentration achieved was 11.03 gl^{-1} and was consisted of 6.26 gl^{-1} of butanol, 4.40 gl^{-1} of acetone and 0.37 gl^{-1} of ethanol. There was no acid acummulation. A dry weight concentration of 80 g/l was attained. Therefore, the operation with 42-44 g/l of feeding glucose concentration at a 0.55 hr^{-1} dilution rate was the optimal condition for this cell recycle system.

Table 5.7 Comparision of solvent productivity ($gl^{-1}hr^{-1}$) from cell recycle process with feeding glucose concentrations 42.4, 52.0 and 64.8 g/l

Feeding glucose concentration (g/l)	Experiment with increasing dilution rate from 0.11 hr^{-1} to 0.22, 0.36 and 0.55 hr^{-1} respectively			
	D=0.11 hr^{-1}	D=0.22 hr^{-1}	D=0.36 hr^{-1}	D=0.55 hr^{-1}
42.40	1.16	2.67	4.15	6.06
52.00	1.36	3.13	4.31	Stop reaction
64.80	0.94	0.91	Stop reaction	

Feeding glucose concentration (g/l)	Experiment with constant dilution rate from the start of the continuous operation	
	D = 0.55 hr^{-1}	D = 0.65 hr^{-1}
42.00-44.00	5.98	0.88

Table 5.8 Comparision of Butanol productivity (gl^{-1} h^{-1}) from cell recycle process with feeding glucose concentrations 42.4, 52.0 and 64.8 g/l

Feeding glucose concentration (g/l)	Experiment with increasing dilution rate from 0.11 hr^{-1} to 0.22, 0.36 and 0.55 hr^{-1} respectively			
	D=0.11 hr^{-1}	D=0.22 hr^{-1}	D=0.36 hr^{-1}	D=0.55 hr^{-1}
42.40	0.58	1.71	2.36	3.44
52.00	0.80	1.89	2.56	Stop reaction
64.80	0.52	0.52	Stop reaction	

Feeding glucose concentration (g/l)	Experiment with constant dilution rate from the start of the continuous operation	
	D = 0.55 hr^{-1}	D = 0.65 hr^{-1}
42.00-44.00	3.40	0.56

Table 5.9 Comparision of glucose consumption ($gl^{-1}hr^{-1}$) from cell recycle process with feeding glucose concentrations 42.5, 52.0 and 64.8 g/l

Feeding glucose concentration (g/l)	Experiment with increasing dilution rate from 0.11 hr^{-1} to 0.22, 0.36 and 0.55 hr^{-1} respectively			
	D=0.11 hr^{-1}	D=0.22 hr^{-1}	D=0.36 hr^{-1}	D=0.55 hr^{-1}
42.4	4.90	9.39	14.80	19.30
52.0	4.64	10.58	13.54	Stop operating
64.8	2.80	2.81	Stop operating	

Feeding glucose concentration (g/l)	Experiment with constant dilution rate from the start of the continuous operation	
	D = 0.55 hr^{-1}	D = 0.65 hr^{-1}
42.00-44.00	19.00	3.65

5.5 Comparision of Cell Recycle System with Other Processes

5.5.1 Batch Operation (run P)

The experiment of the batch fermentation with feeding glucose concentration at 43.8 g/l was operated to compare with the cell recycle process. The result are shown in figures 5.33-5.35. Figure 5.36 shows the behavior of microorganism during operation of run P in the form of specific growth rate (μ), specific production rate (ν) and specific acid production rate (ν_{acid}). The maximal solvent concentration (12.41 g/l) occured after 49.0 hours of fermentation with 6.20 g/l of butanol and 4.34 g/l of cell. The productivity of this batch process was 0.25 $gl^{-1}hr^{-1}$, while the glucose cosumption rate was 0.86 $gl^{-1}hr^{-1}$ with 0.29 production yield. In the comparison with the maximal productivity of the cell recycle process (see table 5.10), the solvent productivity of the cell recycle process was 24.5 times higher than the batch process.

5.2.4.2 Other process

Comparison of the results obtained from run K (the cell recycle with 42.4 g/l feeding glucose concentration at 0.55 hr^{-1} dilution rate) and the data obtained from the literature, the volumetric solvent productivity was higher than that of most published data (see table 5.11).

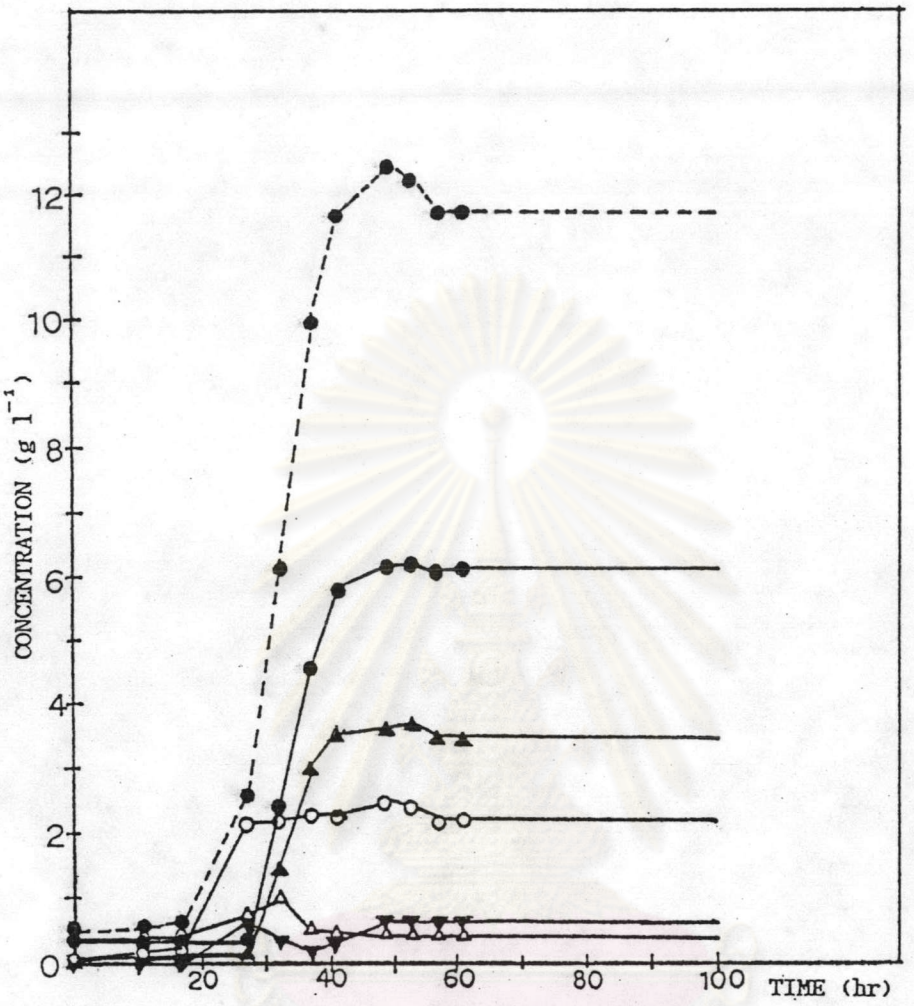

Figure 5.33 Time variation of butanol, acetone, ethanol, acetic acid, butyric acid and total solvent concentration during a batch fermentation of Cl. acetobulylicum at a feeding glucose concentration 43.8 g/l (run P)

● butanol
▲ acetone
O ethanol
Δ acetic acid
▼ butyric acid
-●- total solvent

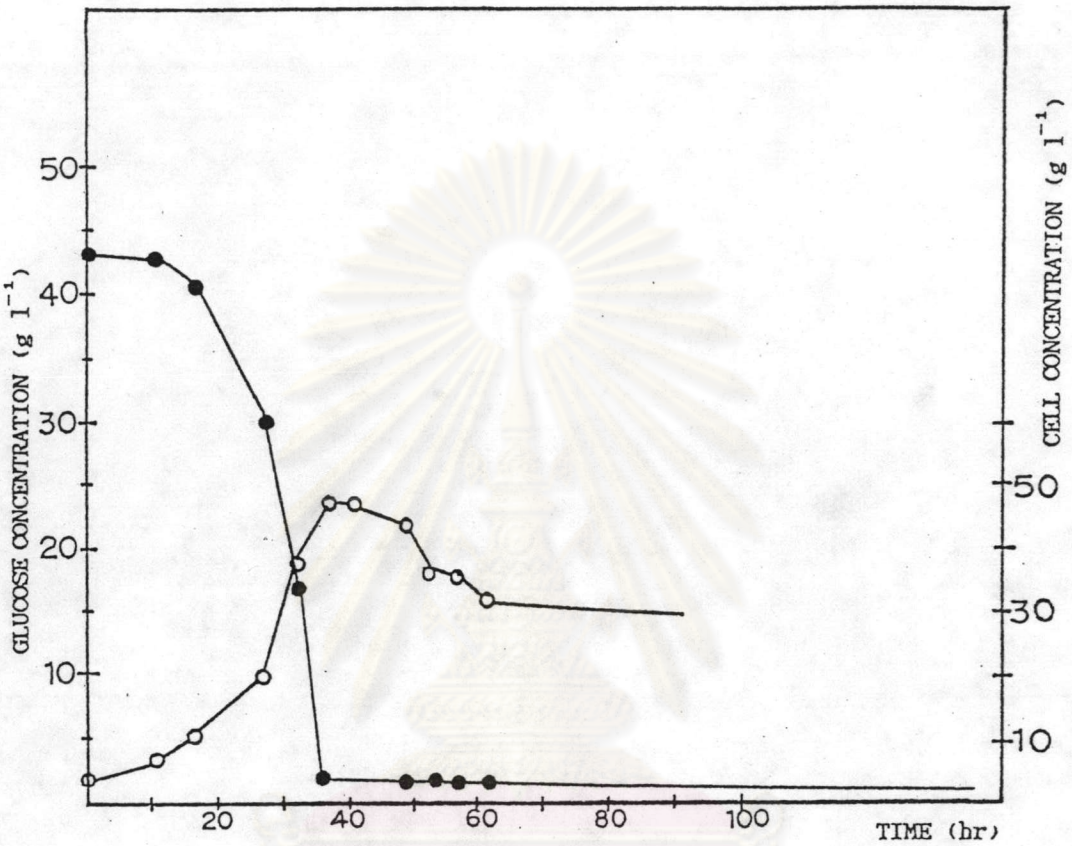

Figure 5.34 Time variation of glucose, biomass concentration during a batch fermentation of Cl. acetobulylicum at a feeding glucose concentation 43.8 g/l (run P)

● glucose

○ biomass

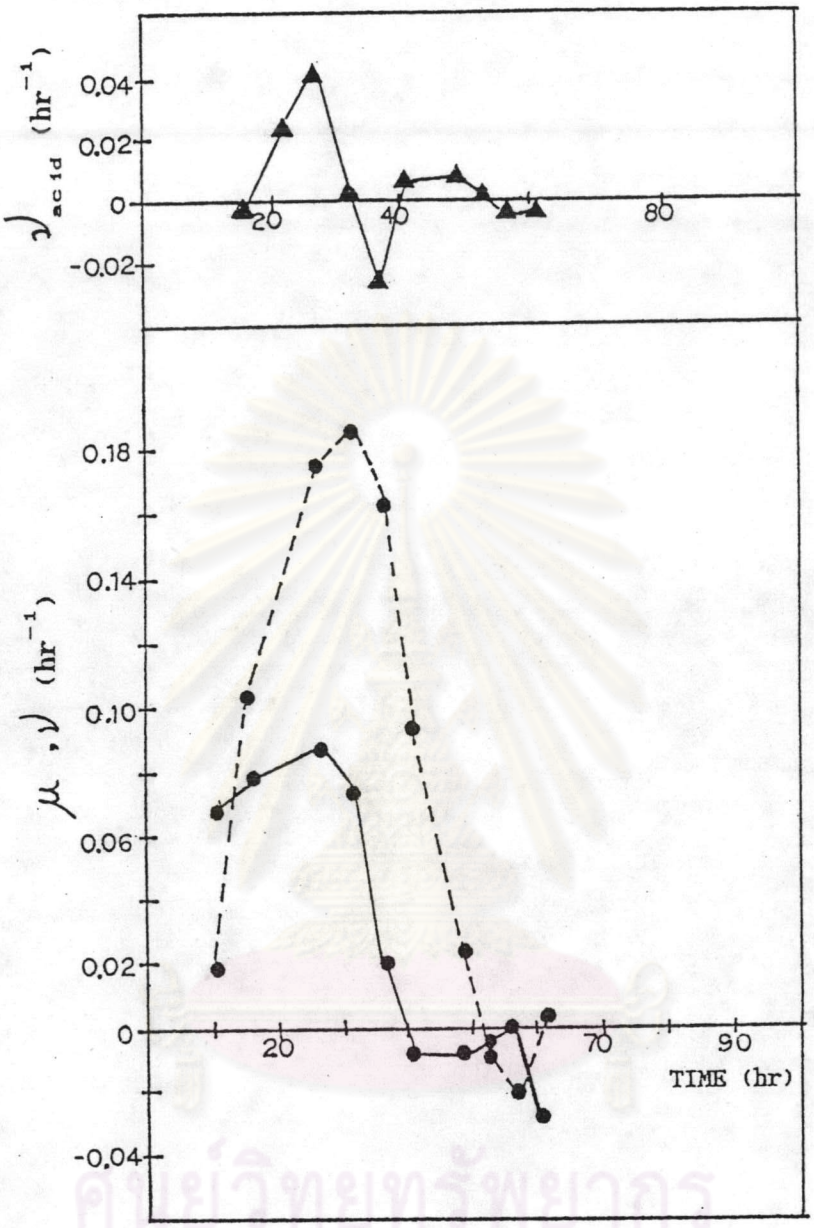

Figure 5.35 Specific growth rate (μ), specific production rate (ν) and specific acid production rate (ν_{acid}) against time (run P)

- ● specific growth rate (μ)
- -●- specific production rate (ν)
- ▲ specific acid production rate (ν acid)

Table 5.10 Comparison of ABE fermentation from run K and run P

RUN	Dilution rate (hr^{-1})	Productivity ($gl^{-1}hr^{-1}$)		Concentration (gl^{-1})		r_s ($gl^{-1}hr^{-1}$)	$Y_{p/s}$
		Solvent	butanol	Solvent	butanol		
A	D = 0.11	1.16	0.58	8.05	4.21	4.90	0.24
	D = 0.22	2.67	1.71	10.41	5.89	9.39	0.28
	D = 0.36	4.15	2.36	10.94	6.20	14.80	0.28
	D = 0.55	6.06	3.44	11.03	6.26	19.30	0.31
F	(batch process)	0.25	0.13	12.41	6.20	0.86	0.29

Remark

*The reported productivities were calculated from the overall production at the end of each stages.

Table 5.11 Comparing the results obtained from run A (at 0.55 hr^{-1} dilution rate) with datas obtained from the literatures

Reference	x (g/l)	ABE (g/l)	r_{PABE} (g/lh)	y_{ABE} (g/g)	Remarks
23	3.5	18	< 0.6	0.32	Industrial batch fermentation from molasses
23	-	23.8	0.6	0.315	Butanol toterant mutant of Cl. acetobutylicum, batch fermentation on hydrolyzed jerusalem artichokes
23	-	2.4	0.8	0.30	continuous immobilized cell of Cl. butylicum carbon subtrate : glucose
23	1.8	5.6	0.72	0.26	Phosphate-limited chemostat carbon substrate : glucose
23	-	11.5	0.75	0.26	Immobilized Cl. butylicum cells in two-stage chemostat; carbon substrate : glucose
23	5.0	12.3	2.70	0.27	Cl. acetobutylicum ATCC 824; chemostat fed with rich glucose medium (15 g/l yeast extract)
24	23.3	10.3	4.10	0.30	Cl. acetobutylicum ATCC 824; continuous run/under phosphate limitation/cellulose triacetate membrane, partial cell recycle; carbon substrate : glucose
23	maximum 94 minimum 30	maximum 20.5 minimum 9.3	4.34	0.27	Cl. acetobutylicum, ATCC 824; continuous run/tubular membrane (one tube), total cell recycle carbon substrate : glucose
Run K	80.0	11.0	6.06	0.30	Cl. acetobutylicum ATCC 824; continuous run/tubular membrane (19 tube), total cell recyole carbon substrate; glucose