

CHAPTER V

RESULTS AND DISCUSSION

5.1 The Study of Control of Biomass Concentration in a Continuous Fermentation.

The experimental results of this study are shown in Figures 5.1-5.6. These experiments were divided into 3 steps that was started by a 1-L batch fermentation, a 3.175-L batch , and a continuous process, respectively. The continuous fermentation were carried out at various dilution rates and biomass concentrations.

After the batch operation, the biomass reached 9.01 g/l with glucose residual of 19 g/l. Then the first continuous fermentation with total cell recycle was started. The specific growth rate and the solvent productivity at the maximum biomass concentration of 11.47 g/l were obtained at 0.91 hr^{-1} and 0.106 g/l-hr. , respectively.

Next, the continuous glucose feeding was started until biomass concentration reached 20 g/l. The biomass concentration was kept constant by turbidity controller. (FSC 402, METTLER TOREDO)

Residual glucose, biomass concentration, and a butanol concentration were constant at steady state. The system was maintained for 18 hours.

The first dilution rate was fixed at 0.1 hr^{-1} . Butanol concentration and solvent productivity were 5.228 g/l and 0.953 g/l-hr , respectively. Glucose consumption was 4.30 g/l-hr and glucose concentration in the fermentor was reduced to 7 g/l , so, the dilution rate was changed from 0.1 hr^{-1} to 0.13 hr^{-1} . (cell would loose activity at the very low glucose concentration level ($<5 \text{ g/l}$)).

At the fixed dilution rate of 0.13 hr^{-1} , the solvent productivity was 1.025 g/l-hr . From the dilution rate of 0.1 to 0.13 hr^{-1} , the solvent productivity rose with increasing dilution rate. Therefore, the dilution rate was increased to 0.19 hr^{-1} .

At the dilution rate of 0.19 hr^{-1} , the solvent productivity and the residual glucose were 1.385 g/l-hr and 17 g/l , respectively. From Figure 5.5 it was noted that solvent productivity increased with dilution rate. As a result, in order to increase solvent productivity, dilution rate should be increased. But, if the dilution rate was increased, the retention time decreased. Then biomass might not be in the growth phase because the time in fermentor was not enough for the bacteria to consume substrate. For this reason, we should increase the biomass concentration to 40 g/l .

After biomass concentration was increased up to 40 g/l, the dilution rate of 0.19 hr^{-1} did not reach the steady state system because of glucose lacking. Therefore, the dilution rate was changed from 0.19 hr^{-1} to 0.3 hr^{-1} .

Butanol concentration of 5.24 g/l and solvent productivity of 2.713 g/l-hr were attained at the dilution rate of 0.3 hr^{-1} . Glucose consumption rate was 12.60 g/l-hr and residual glucose concentration in the fermentor was 8 g/l. The higher solvent productivity was expected at higher dilution rate. Therefore, the dilution rate was then changed to 0.5 hr^{-1} .

At the fixed dilution rate of 0.5 hr^{-1} , the residual glucose was 17 g/l with 6.43 g/l solvent concentration and 3.62 g/l butanol concentration. Solvent productivity and butanol productivity were 3.13 and 1.79, respectively. Owing to the high residual glucose in fermentation broth, the biomass concentration should change to 60 g/l.

At biomass concentration of 60 g/l and the dilution rate of 0.3 hr^{-1} could not be operated due to glucose limitation. Solvent productivity the fermentation of 4.62 g/l-hr was obtained at dilution rate of 0.5 hr^{-1} while the residual glucose was 11 g/l.

The moderate residual glucose concentration (11 g/l) indicated that the dilution rate could be further increased. But it was limited by permeation flux. Therefore, the system could not operate at the dilution rate that was higher than 0.5 hr^{-1} . The most

suitable way was to change the biomass concentration from 60 g/l to 80 g/l at 0.5 hr⁻¹ of dilution rate.

In this system, the maximum solvent productivity of 5.89 g/l-hr and the maximum butanol productivity of 3.39 g/l-hr were obtained at dilution rate of 0.5 hr⁻¹ and biomass concentration of 80 g/l.

At the dilution rate of 0.5 hr⁻¹ and biomass concentration of 40, 60, and 80 g/l, respectively, solvent productivity increased as biomass concentration increased. Therefore the biomass concentration of 100 g/l should be operated.

From Figure 5.8 (Muenduen Phisalponge,1989), the relation between permeation flux and $\ln C_b$ showed that at biomass concentration (C_b) of 100 g/l, dilution rate must be lower than 0.5 hr⁻¹ due to the effect of gel polarization. So we can not operate at biomass concentration of 100 g/l with dilution rate of 0.5 hr⁻¹.

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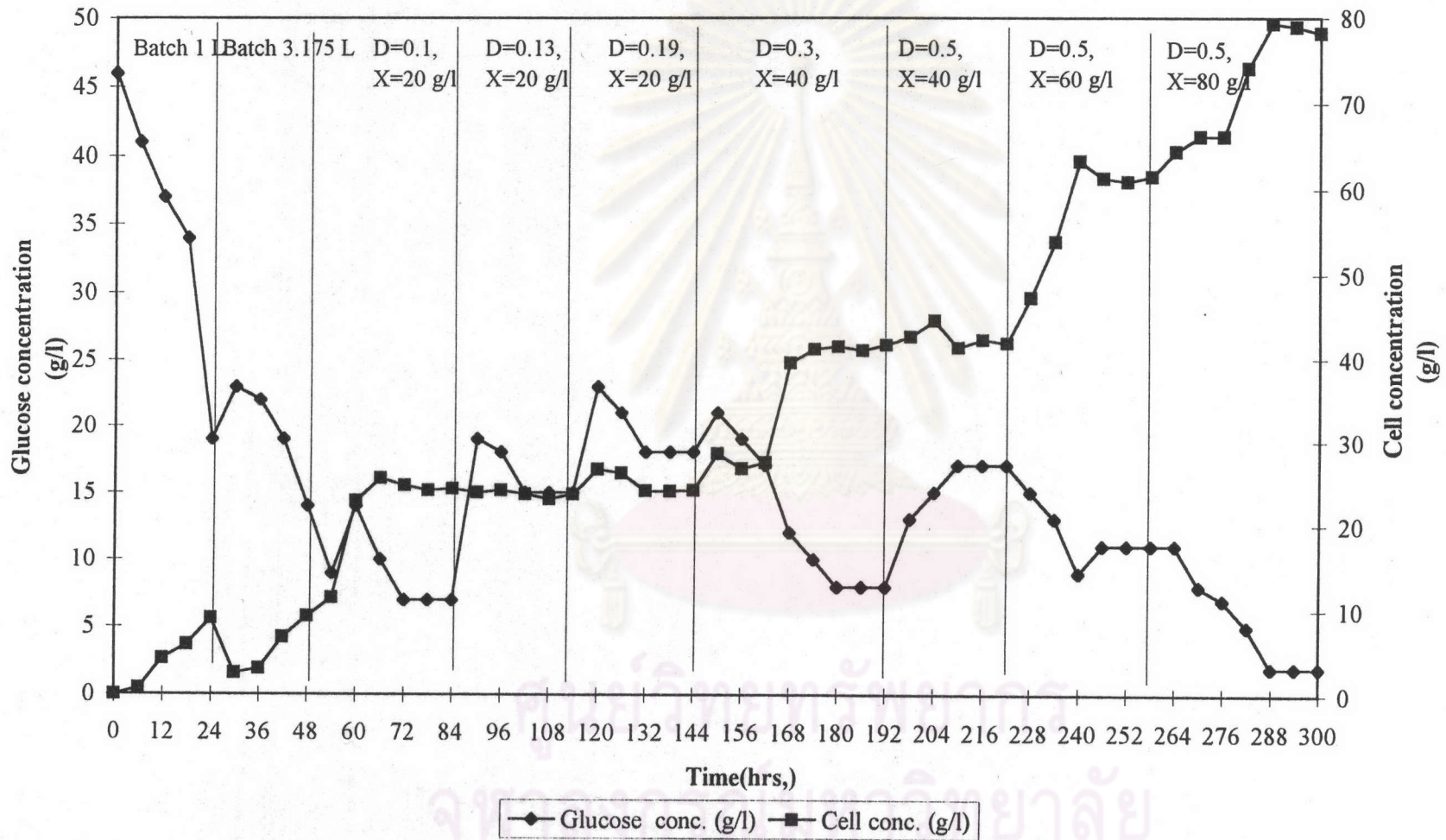


Figure 5.1 Time variation of glucose concentration

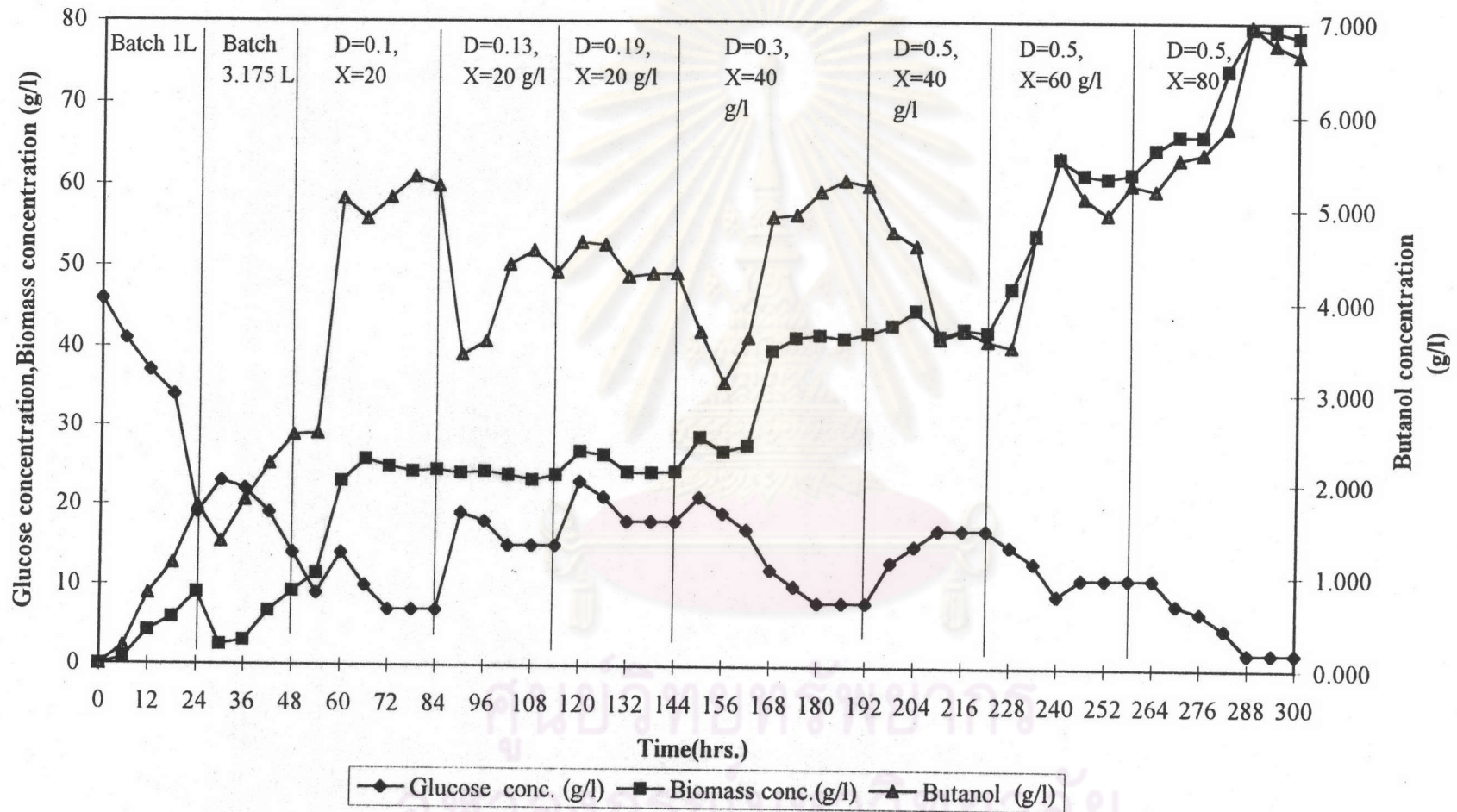


Figure 5.2 Time variation of glucose concentration, biomass concentration, and butanol concentration.

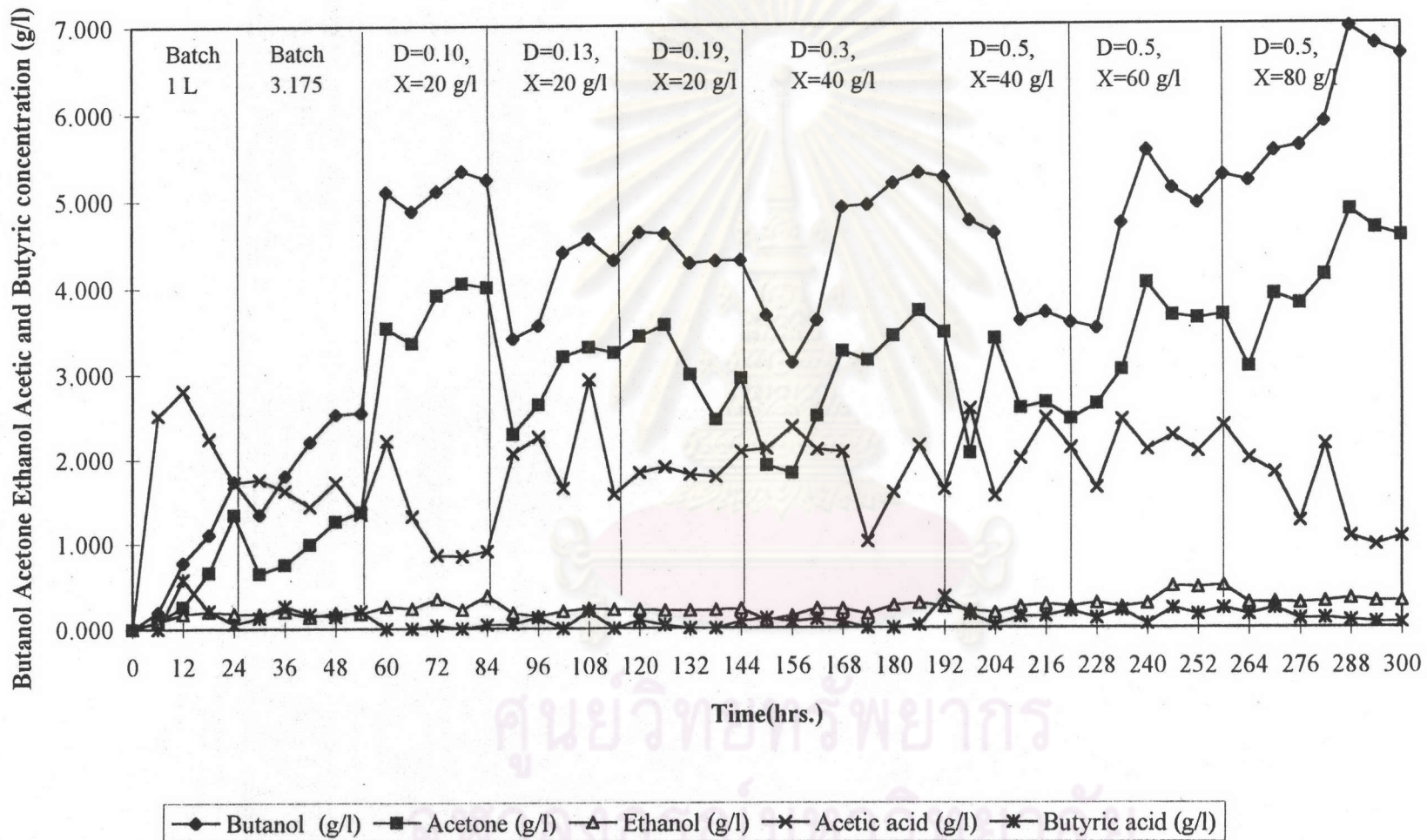


Figure 5.3 Time variation of butanol, acetone, ethanol, acetic acid, and butyric acid

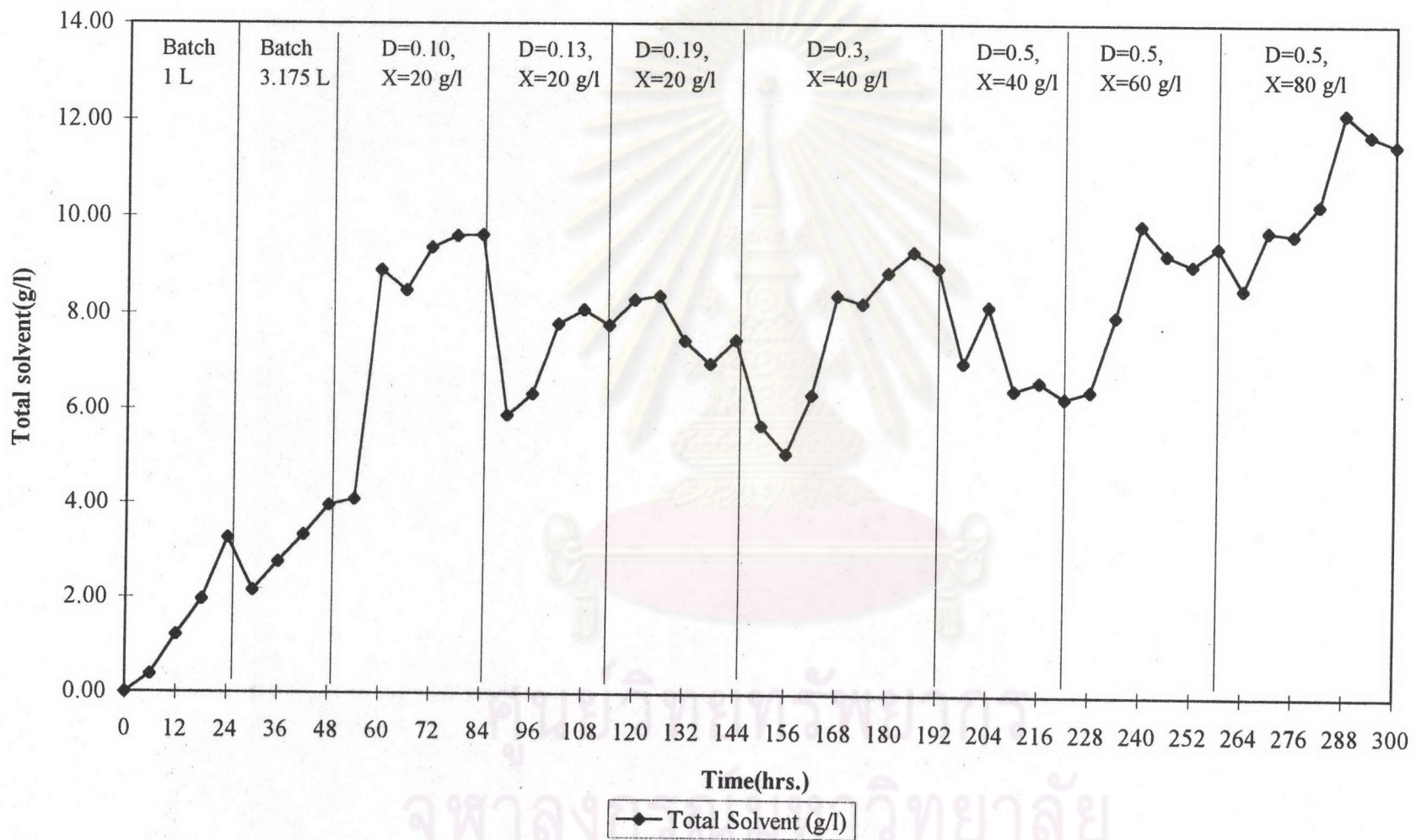


Figure 5.4 Time variation of total solvent

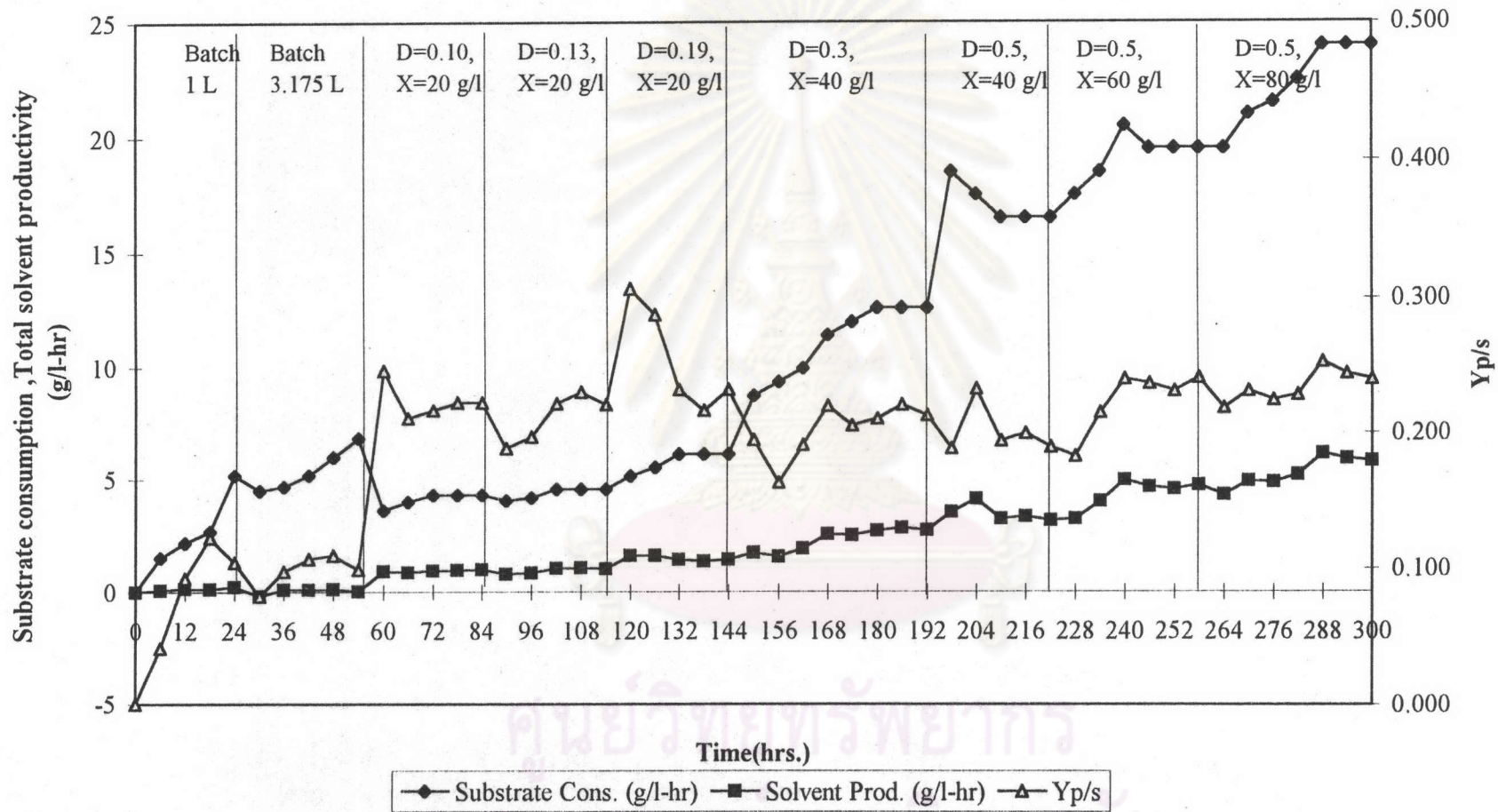


Figure 5.5 Time variation of substrate consumption rate, solvent productivity, and Yp/s

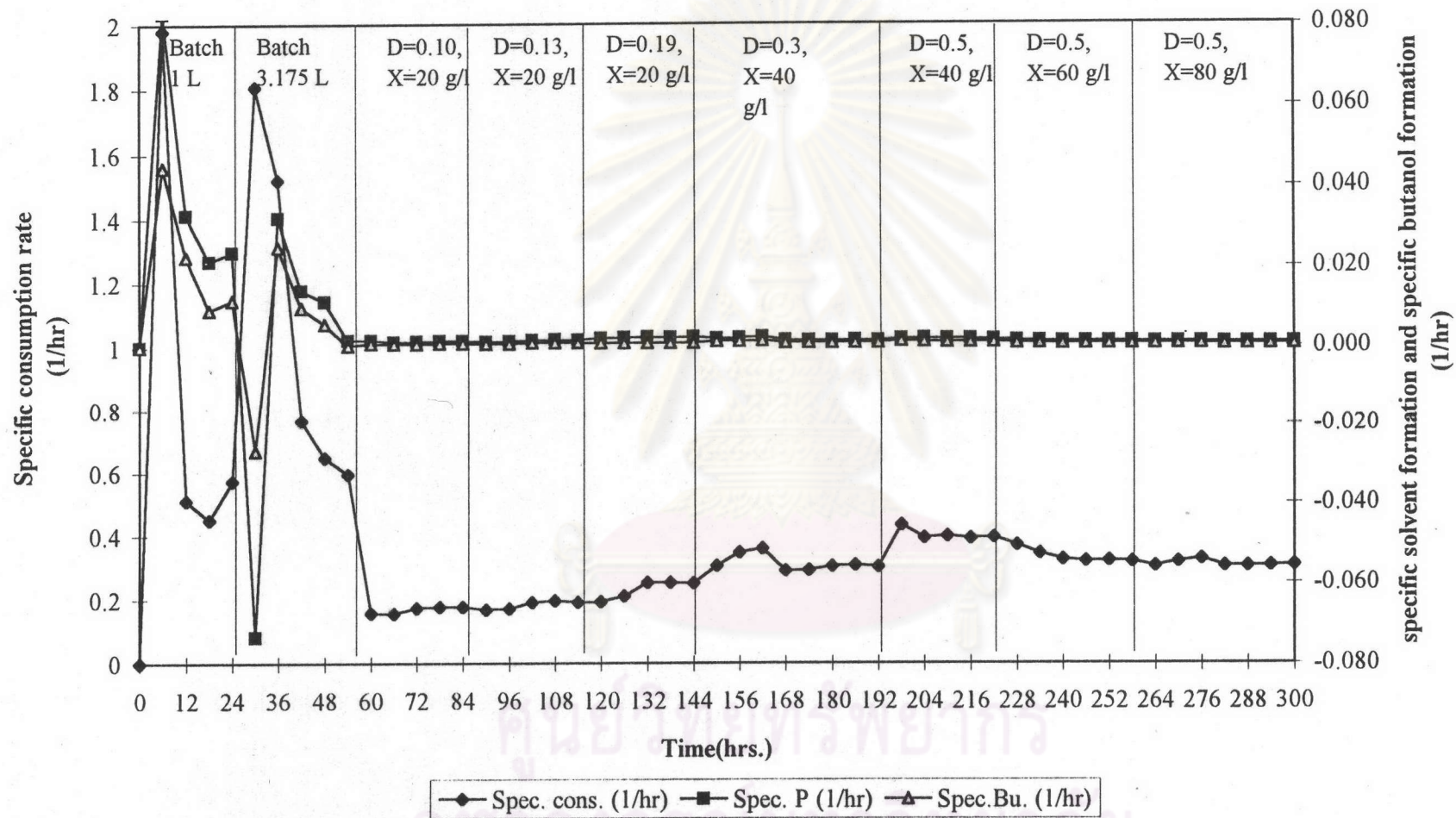


Figure 5.6 Time variation of specific consumption rate, specific solvent formation, and specific butanol formation

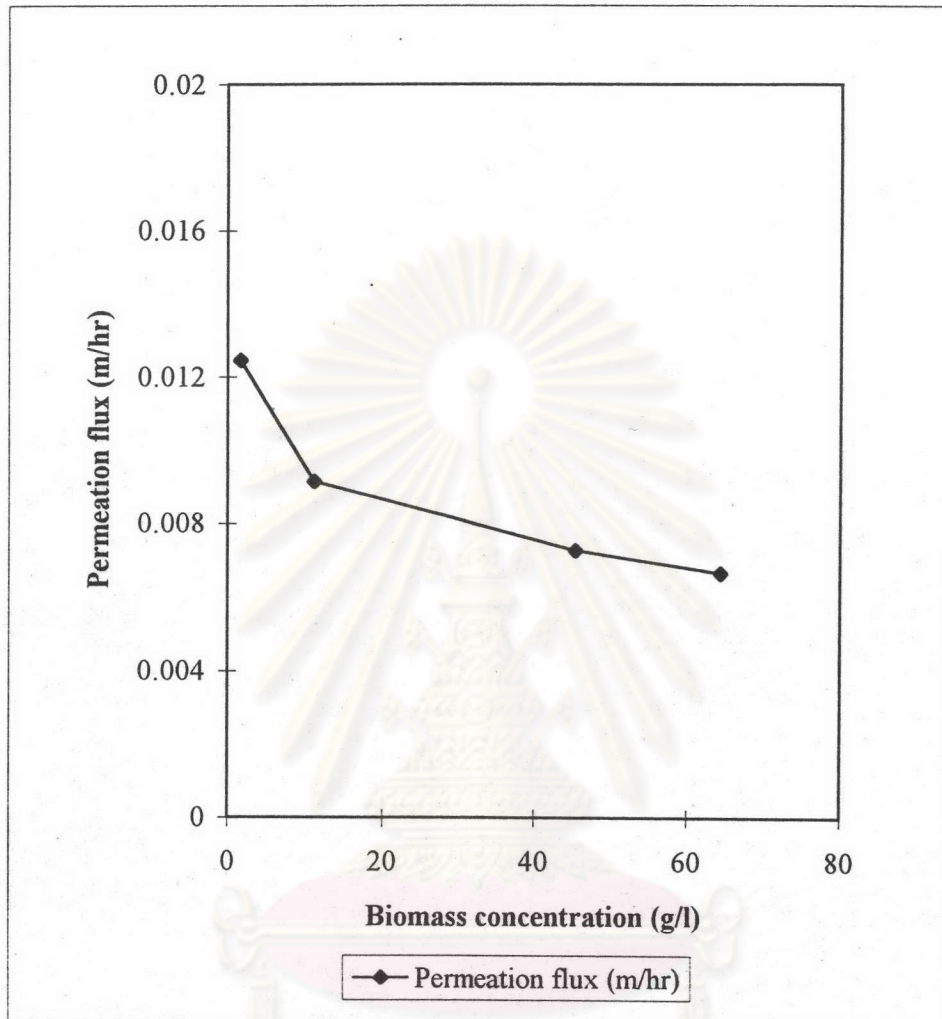


Figure 5.7 Permeation flux as a function of biomass concentration (C_b).

(Muenduen Phisalphone, 1989)

At operating temperature	33°C
applied pressure	0.1 Kg/cm ²
recirculation flow rate	0.4 m ³ /hr.

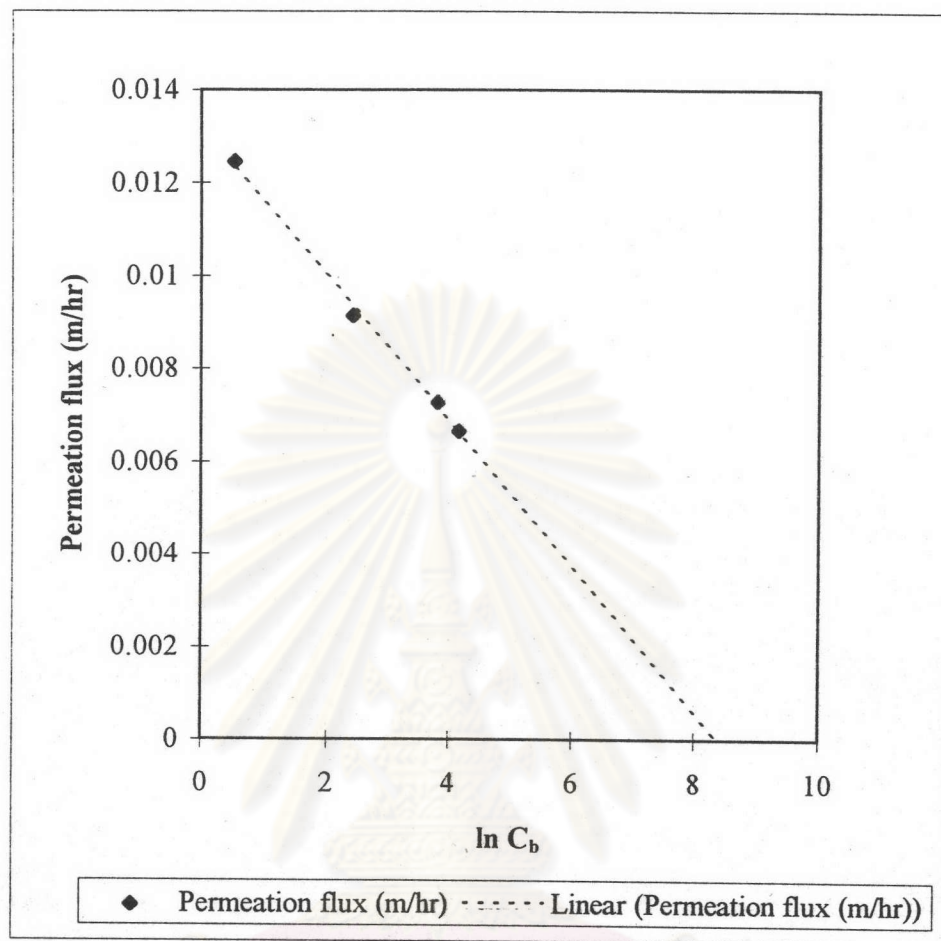


Figure 5.8 Permeation flux as a function of $\ln C_b$. (Muenduen Phisalphone, 1989)

At operating temperature 33°C

applied pressure $0^+ \text{ Kg}_f/\text{cm}^2$

recirculation flow rate $0.4 \text{ m}^3/\text{hr}$

5.2 Effect of Dilution Rate on Biomass Concentration Controlled at 20 g/l

This experiment was carried on three dilution rate variation that were 0.1 hr^{-1} , 0.13 hr^{-1} , and 0.19 hr^{-1} , respectively.

Figure 5.9 shows that solvent productivity increased with dilution, while solvent concentration decreased. The maximum solvent productivity of 1.385 g/l was found at 0.19 hr^{-1} of dilution rate. For a continuous fermentation, residual glucose consumption rate in fermentor was an important parameter because glucose was the energy source of biomass. From Table 5.1, it was found that glucose consumption rate increased rapidly with dilution rate since it was during the growth period. Residual glucose rate was also increased too.

Dilution rate (1/hr)	Glucose feed rate (g/l-hr)	Glucose consumption rate (g/l-hr)	Residual glucose rate (g/l-hr)
0.10	5.0	4.30	0.70
0.13	6.5	4.55	1.95
0.19	9.5	6.08	3.42

Table 5.1 The increasing of residual glucose feed rate at 20 g/l biomass concentration.

Consequently, solvent productivity was slightly increased, while a lot of residual glucose was left in fermentor. In order to improve solvent productivity, biomass concentration should be increased instead of increasing dilution rate.

5.3 Effect of Dilution rate on Biomass Concentration Controlled at 40 g/l

At 40 g/l of biomass concentration, the system was run under the two dilution rate of 0.3 hr^{-1} and 0.5 hr^{-1} .

Figure 5.10 shows the relation between solvent concentration, solvent productivity, and glucose concentration with dilution rate. It was found that dilution rate of 0.5 hr^{-1} achieved the maximum solvent productivity. From reason in section 5.2, it confirmed that the optimum condition at the biomass concentration of 40 g/l was 0.5 hr^{-1} of dilution rate. Both effects had the same trend.

5.4 Effect of Controlled Biomass Concentration on Fermentation performance.

Figures 5.11 and 5.12 show the optimum conditions of each biomass concentration.

The experimental results indicated that specific growth rate depended on dilution rate and did not depend on biomass concentration since the specific growth rate of this system was equal to the multiplication of dilution rate to bleed ratio (B/F). In this experiment the bleed rate was controlled by turbidity controller until the process reached the steady state (about four times of one by dilution rate). Then the bleed rate was constant. Therefore, the specific growth rate depended on dilution rate. The maximum specific growth rate was 0.059 hr^{-1} at the dilution rate of 0.5 hr^{-1} .

The biomass productivity rose with increasing biomass concentration because biomass productivity depended on biomass concentration. In addition, solvent productivity increased with biomass concentration. The maximum solvent productivity at biomass concentration of 80 g/l and dilution rate of 0.5 hr^{-1} was 5.89 g/l-hr.

Unlike solvent productivity, the specific product formation did not depend on biomass concentration. So, the maximum specific product formation was obtained at 20 g/l biomass concentration. We also observed that production yields at various controlled biomass concentrations were nearly constant at 26%. Figure 5.14 shows the relation between CO_2 and H_2 production with biomass concentration. It was found that the production of CO_2 and H_2 at 20 g/l biomass concentration were less than another biomass concentration. It also concluded that biomass, at 20 g/l biomass concentration, consumed glucose to produce solvent more than both of the gases.

From biomass productivity and solvent productivity data, the maximum value was obtained at the dilution rate of 0.5 hr^{-1} and biomass concentration of 80 g/l. So the glucose consumption rate should be maximum too (shown in this figure).

Glucose was mainly used in three ways. First, it was used for cell growth. Second, it was used for producing butanol, acetone, ethanol, acetic acid, and butyric acid, and last, for producing CO_2 and H_2 . Therefore, specific consumption rate (substrate consumption rate per unit mass of the microorganism) should be considered.

It was found that the maximum specific glucose consumption rate was utilized at biomass concentration of 40 g/l. From figure 5.12 and 5.13, they show that at this biomass concentration, most of glucose consumption produced CO₂ and H₂ gases instead of solvent and biomass.

Compositions of CO₂ and H₂ are shown in Figure 5.14, More CO₂ concentration was produced at low biomass concentration which gave high specific solvent productivity. This result was similar to Jones's work that was revealed to cell morphology (22). His study has shown that Clostridium in cigar-shape might be in solventogenesis phase and produced more CO₂ than H₂.



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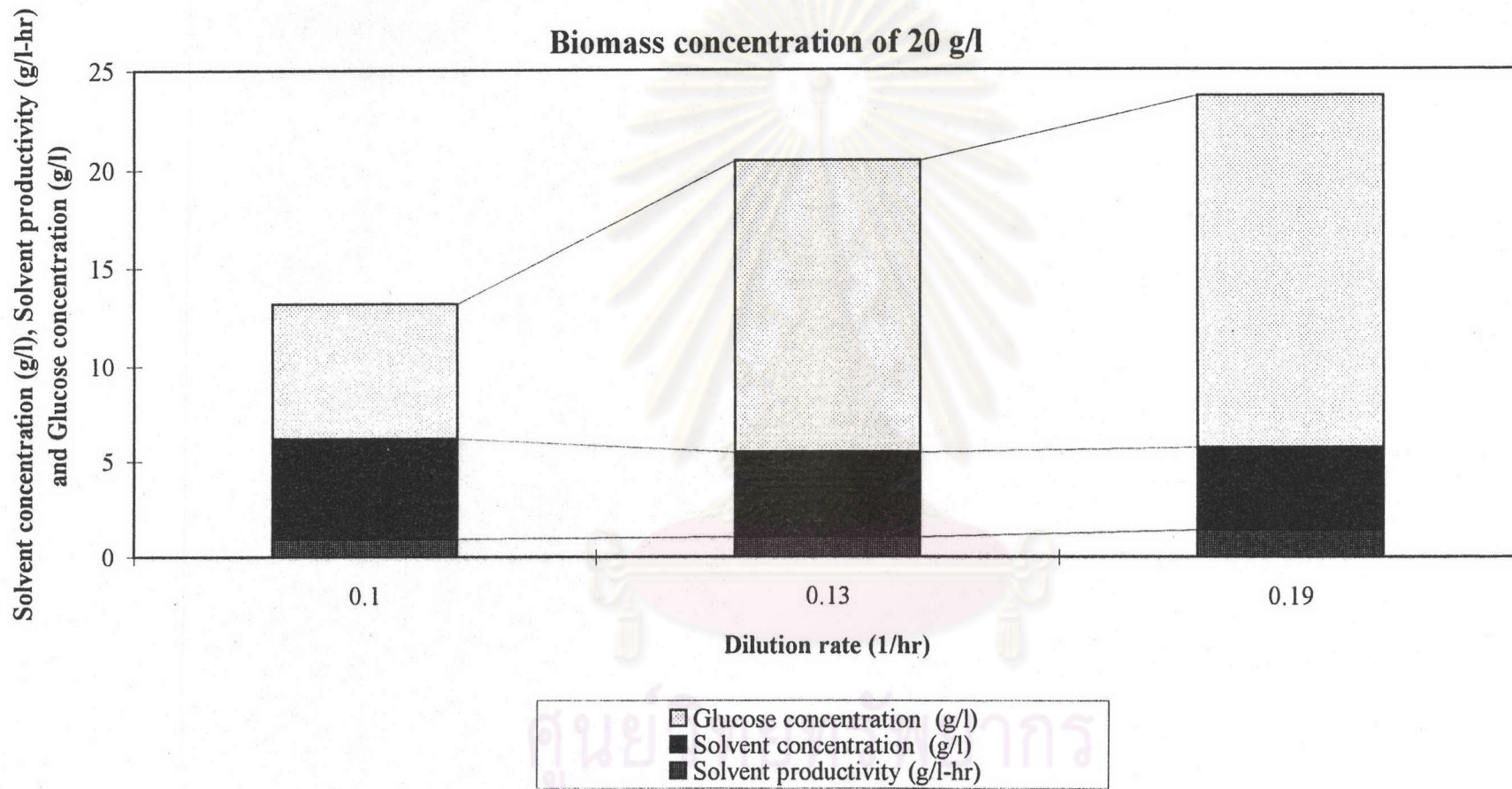


Figure 5.9 Effect of dilution rate on solvent concentration, solvent productivity, and glucose concentration at 20 g/l biomass concentration

Solvent concentration (g/l), Solvent productivity (g/l-hr) and
Glucose concentration (g/l)

Biomass concentration of 40 g/l

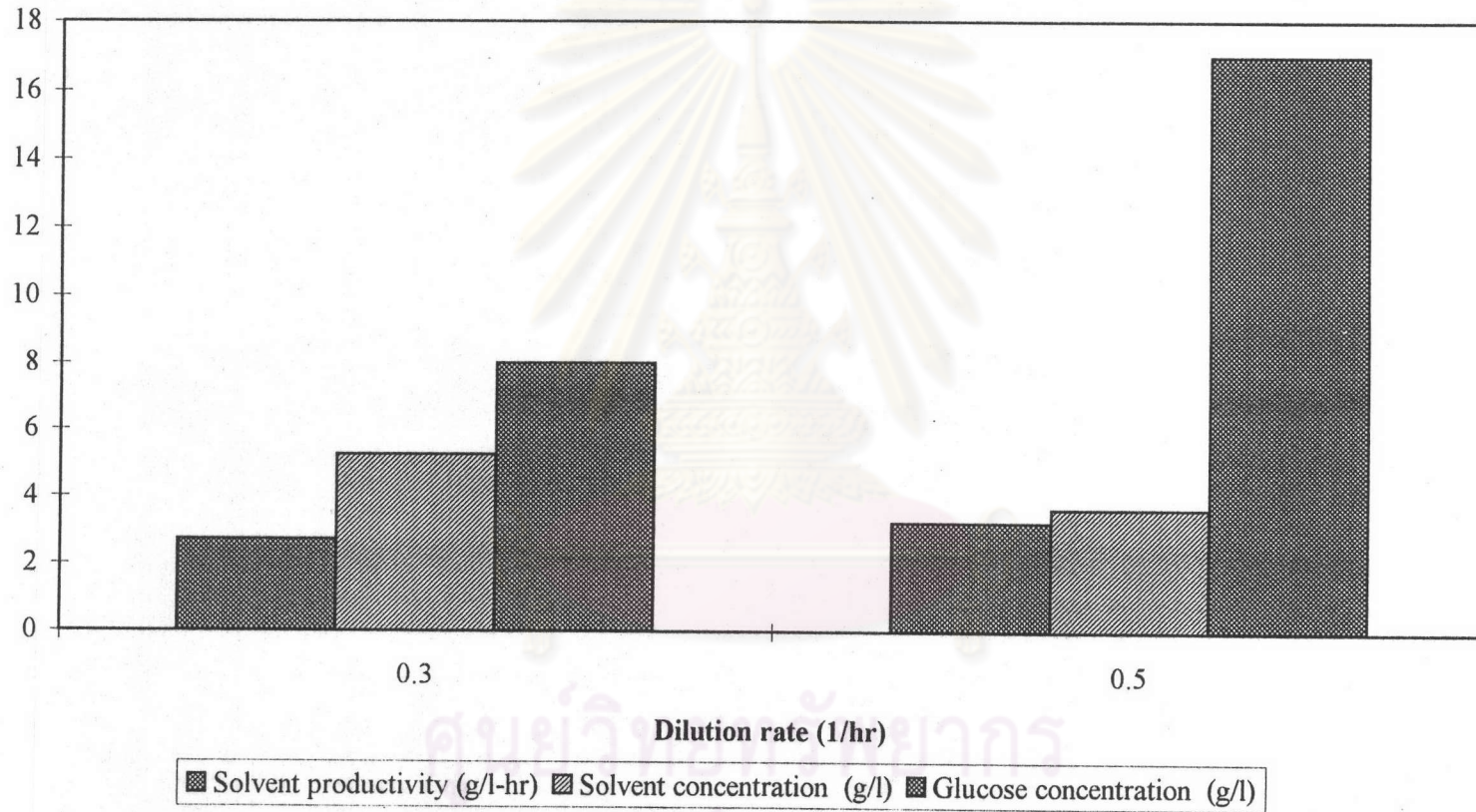


Figure 5.10 Effect of dilution rate on solvent concentration, solvent productivity, and glucose concentration at 40 g/l biomass concentration

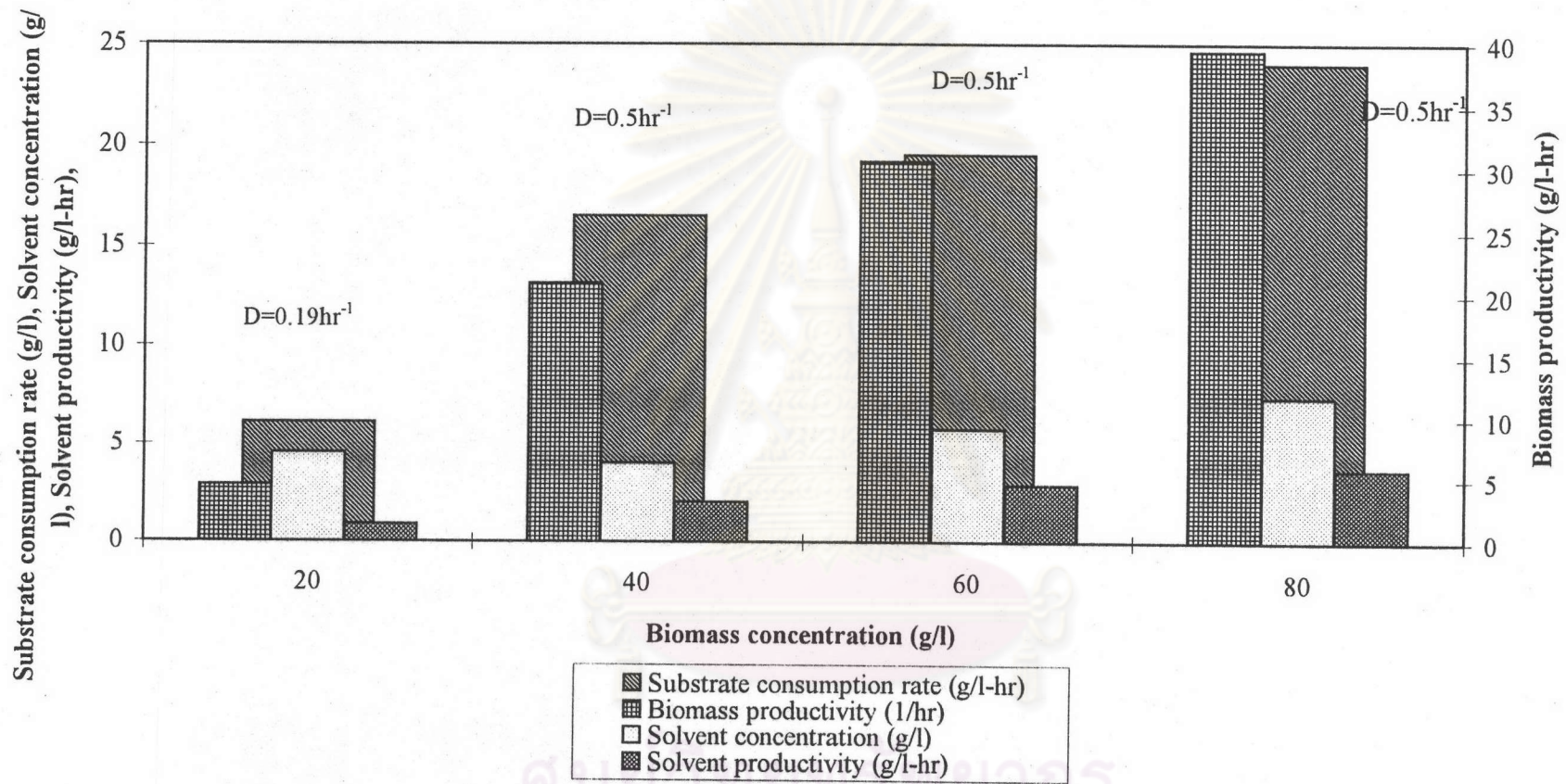


Figure 5.11 Comparison of biomass concentration on substrate consumption rate, solvent concentration, solvent productivity, and biomass productivity

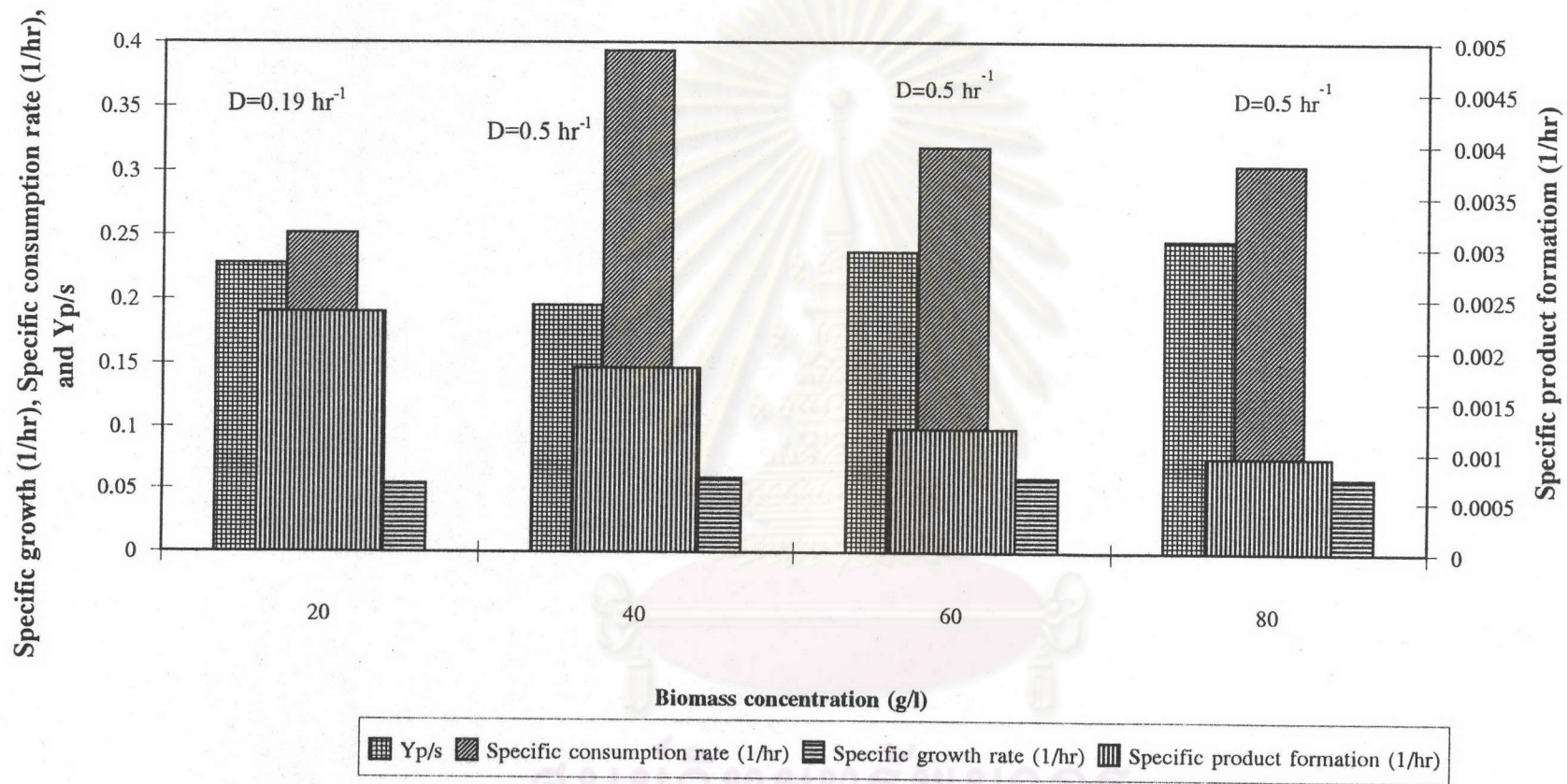


Figure 5.12 Comparison of biomass concentration on specific growth rate, specific consumption rate, specific product formation and Yp/s.

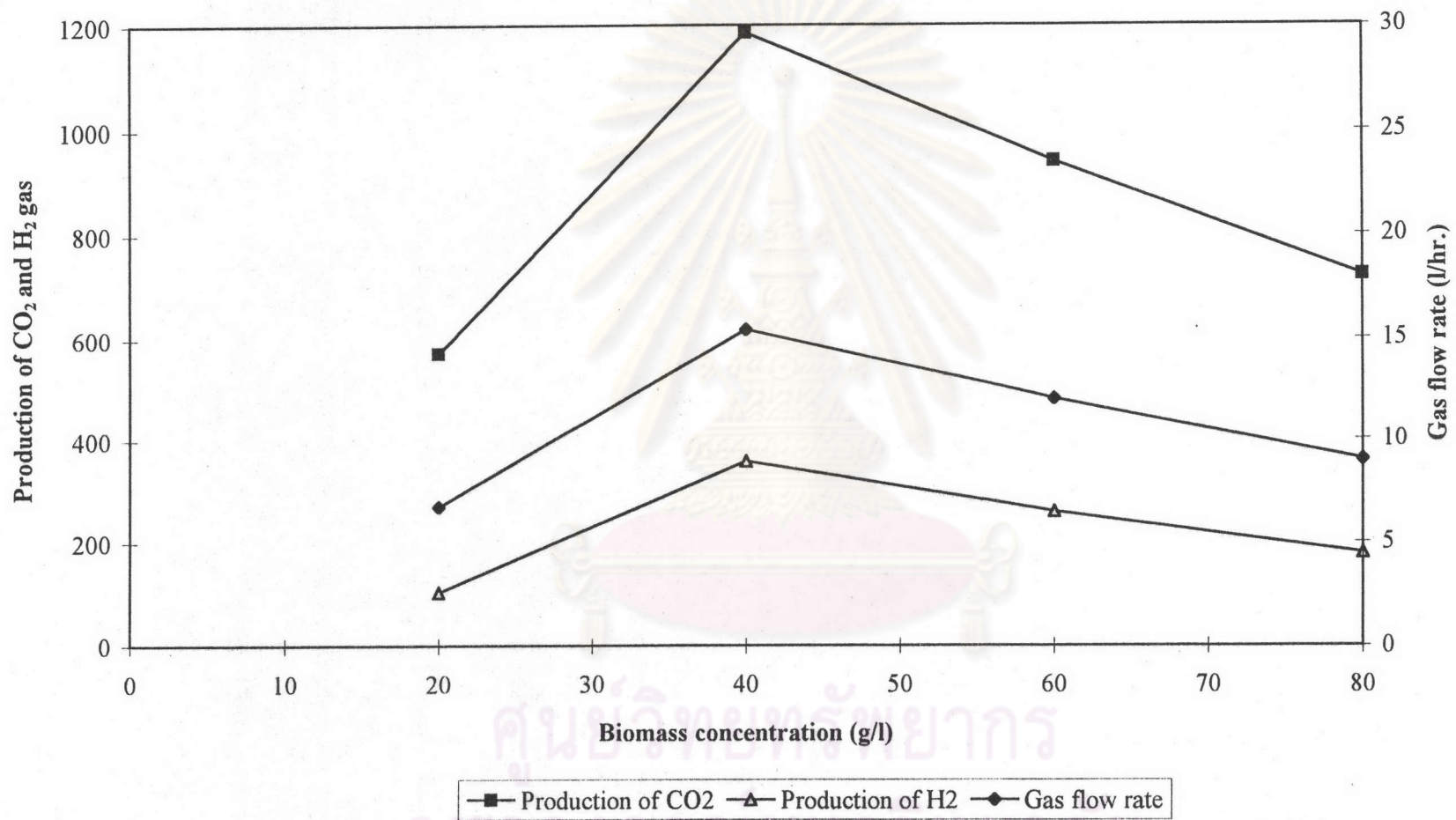


Figure 5.13 Effect of biomass concentration on productivity of CO₂ and H₂

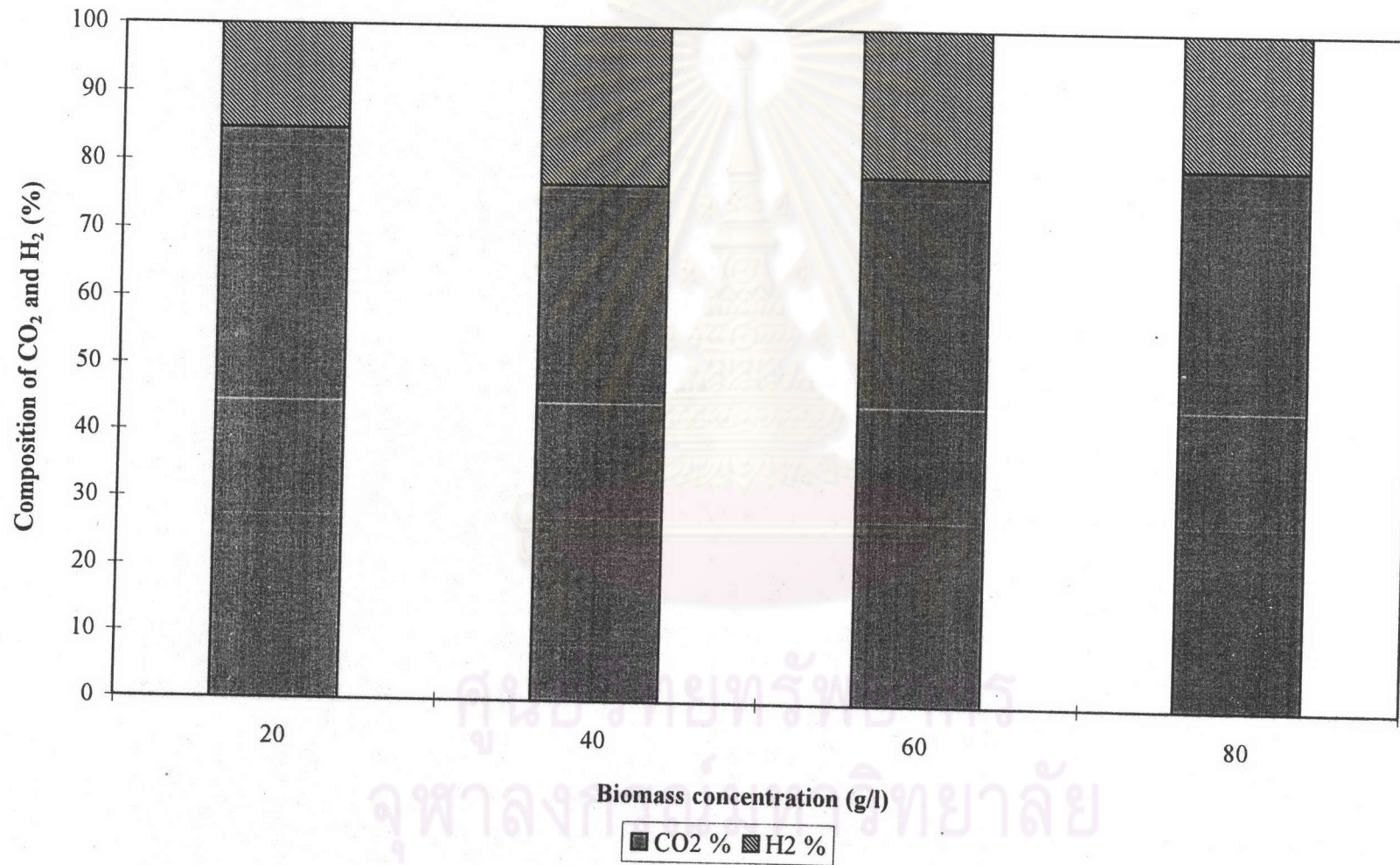


Figure 5.14 Composition of CO₂ and H₂ gas