CHAPTER IV

RESULTS AND DISCUSSION

Almost all of ethanol productions under the use of monoculture system, the organisms of primary interest to industrial fermentation include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Kluyveromyces marxianus*. In order to improve the ethanol fermentation efficiency, the use of a mixed culture of *Saccharomyces cerevisiae* M30 and *Kluyveromyces marxianus* DMKU 3-1042 is applied in the study.

4.1 Batch fermentation of suspended cells: monoculture vs. mixed culture

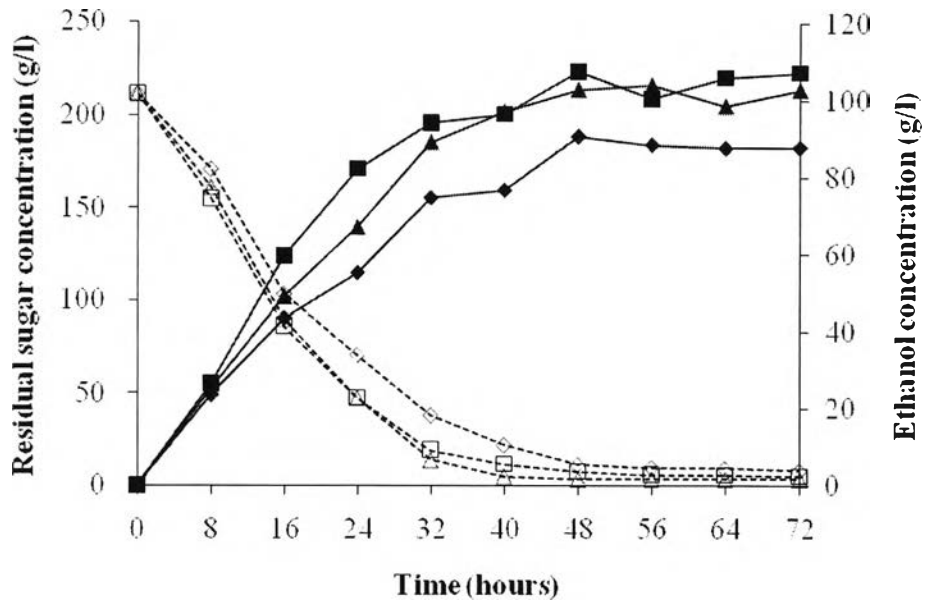
Batch fermentation in shaking-flasks for ethanol production was carried out in duplicate for 72 h at constant temperatures of 33, 37, 40 and 45°C. The effect of temperature on ethanol fermentation in a basal sugar cane juice and cane molasses medium by the monocultures of *S. cerevisiae* M30, *K. marxianus* DMKU 3-1042 and the mixed culture of *S.cerevisiae* M30 and *K.marxianus* DMKU 3-1042, was investigated. Fig. 4.1 to 4.4 demonstrates the experimental results. At the end of the fermentation; the ethanol concentrations from sugar cane juice medium using the mixed cultures at constant temperature of 33, 37, 40 and 45°C were 103.9, 74.9, 66.2 and 62.3 g/l respectively, which were higher or equivalent in comparison to those of *S. cerevisiae* M30 or *K. marxianus* DMKU 3-1042 monocultures. Moreover, the residual sugar concentrations from the systems using the mixed culture were lower than those of the monocultures. The ethanol concentrations by the mixed cultures using cane molasses medium were 87.9, 76.9, 52.0 and 39.7 g/l at constant temperature of 33, 37, 40 and 45°C respectively, which were also higher or at least comparable to those of the monoculture systems. The mixed culture and the monoculture of *K. marxianus* DMKU 3-1042 were found to be capable of producing ethanol than *S. cerevisiae* M30, especially when grew on media containing sugar cane juice. It has been previously demonstrated that the thermotolerant yeast strain

Kluyveromyces marxianus DMKU 3-1042 was an effective strain that could be employed for ethanol production at elevated temperature up to 45 °C when sugar cane juice was used as a raw material (Limtong et al., 2007). The overall fermentation results were summarized in Table 4.2 to 4.5.

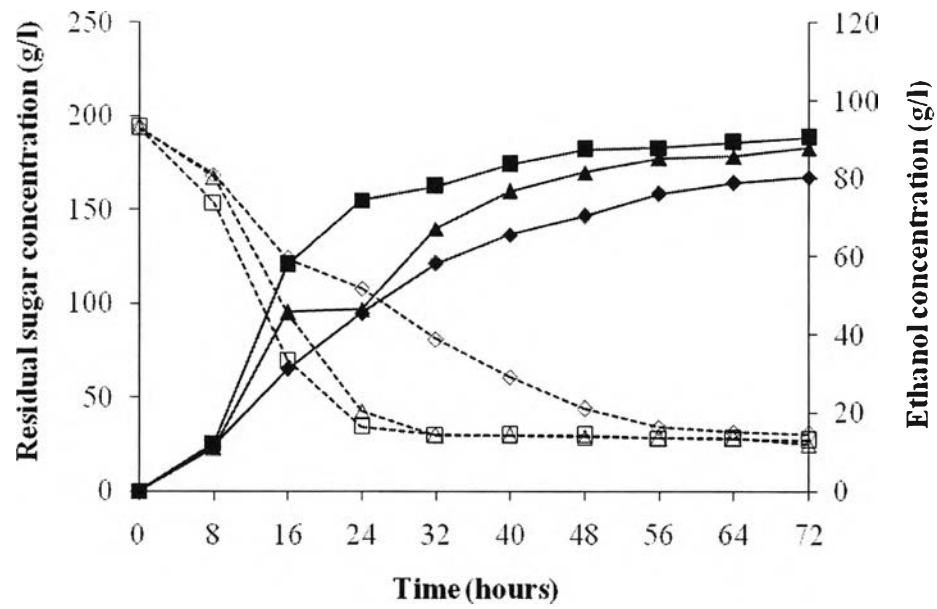
Successful works for ethanol fermentation by mixed cultures have been reported by several investigators. For example, the mixed cultures of *Endomycopsis fibuligera* NRRL and *Zymomonas mobilis* ZM4 could directly and more efficiently fermented cassava starch (22.5% w/v) to ethanol (10.5% v/v) than the monocultures (Vijaya Sarathi Reddy and Basappa, 1996). The mixed culture of *S. cerevisiae* and *K. marxianus* was reported as the effective culture for ethanol fermentation in cheese whey powder solution (Guo et al., 2008). The ethanol concentration of 5.2 ± 1.1% v/v from henequen juice and molasses was obtainable by the use of mixed culture of 75% *S. cerevisiae* and 25% *K. marxianus* (Cáceres-Farfán et al., 2008). Efforts for improved ethanol fermentation by mixed cultures of two microorganisms in a single process have been reported (Fu and Peiris, 2008; Fu et al., 2009; Abate et al., 1996).

Table 4.1 List of samples and labels for this study

Sample's Name	Label
Suspended cells of *S.cerevisiae* M30	SS
Suspended cells of *K.marxianus* DMKU 3-1042	SK
Suspended cells of Mixed culture	SM

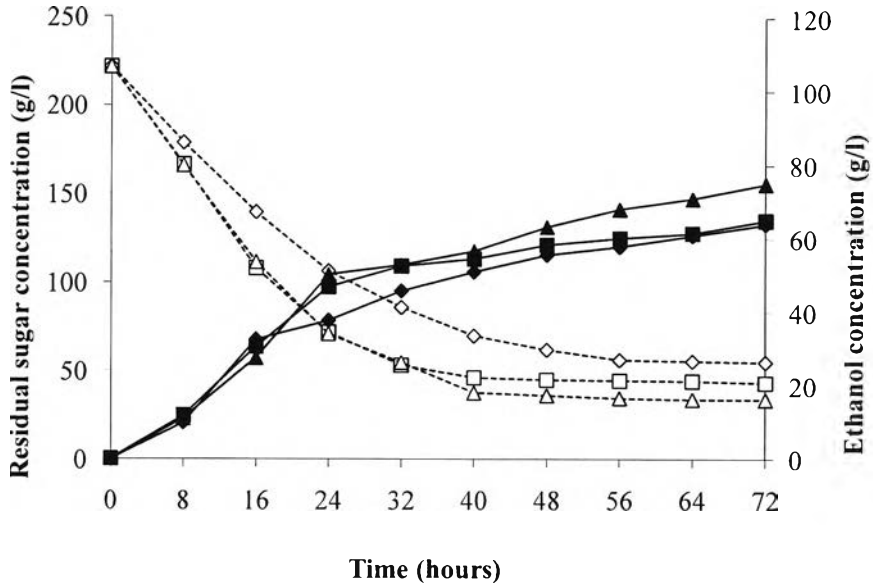


A: Sugar cane juice (33°C)

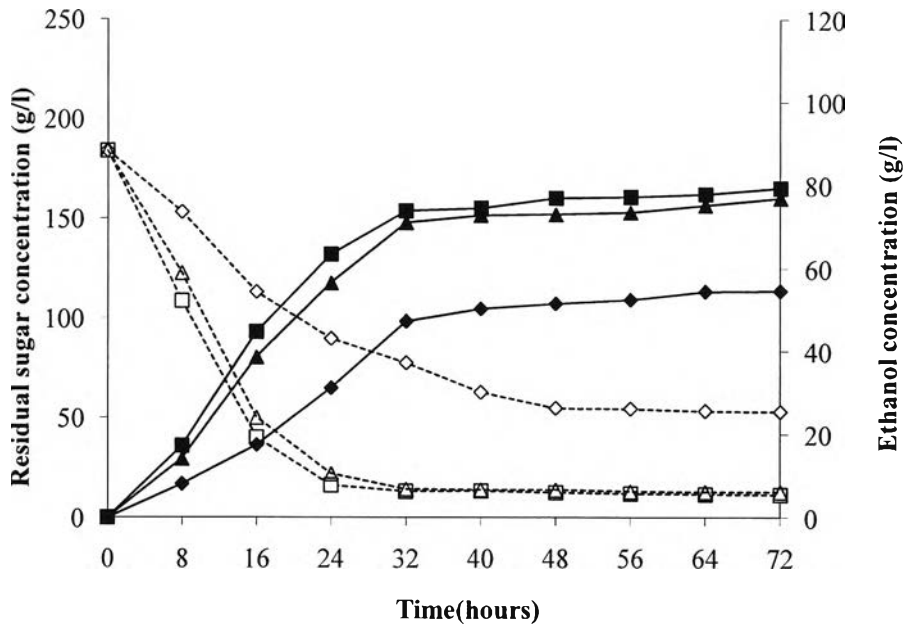


B: Cane molasses (33°C)

Figure 4.1 Ethanol (solid line, —) and residual sugar (dash line, ----) concentration profiles in Sugar cane juice (A) and Cane molasses medium (B) at constant temperature of 33°C; (■, □) = SS; (♦, ◊) = SK; (▲, Δ) = SM.

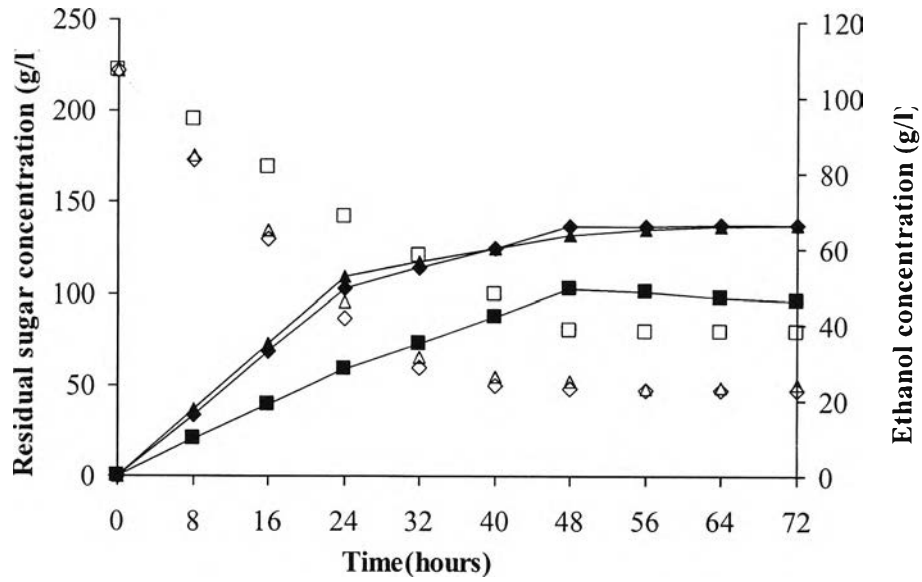


C: Sugar cane juice (37°C)

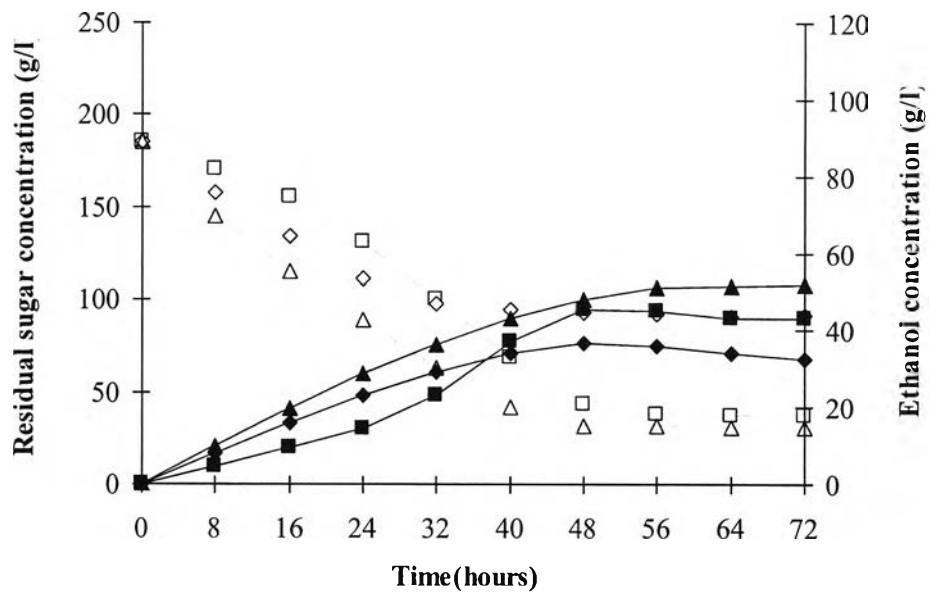


D: Cane molasses (37°C)

Figure 4.2 Ethanol (solid line, —) and residual sugar (dash line, ----) concentration profiles in Sugar cane juice (A) and Cane molasses medium (B) at constant temperature of 37°C; (■, □) = SS; (♦, ◊) = SK; (▲, Δ) = SM.

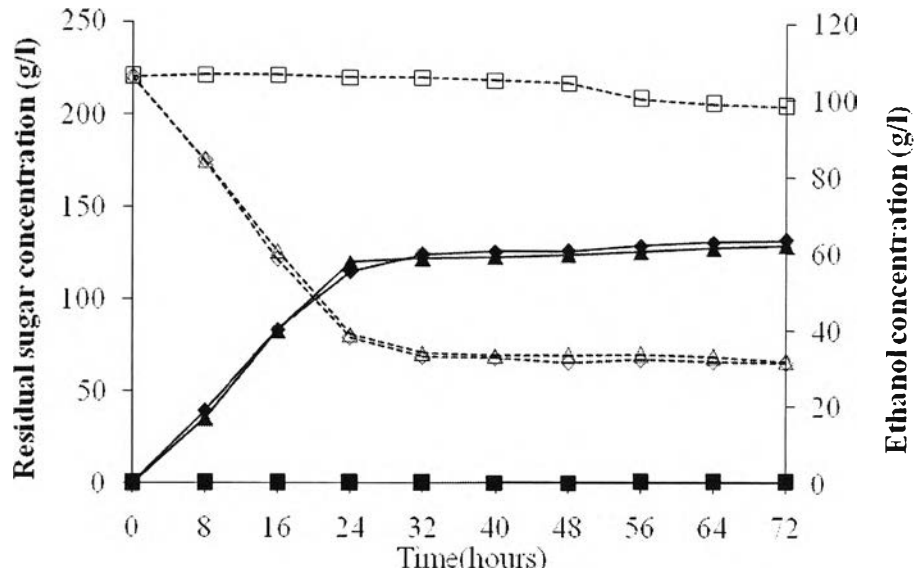


E: Sugar cane juice (40°C)

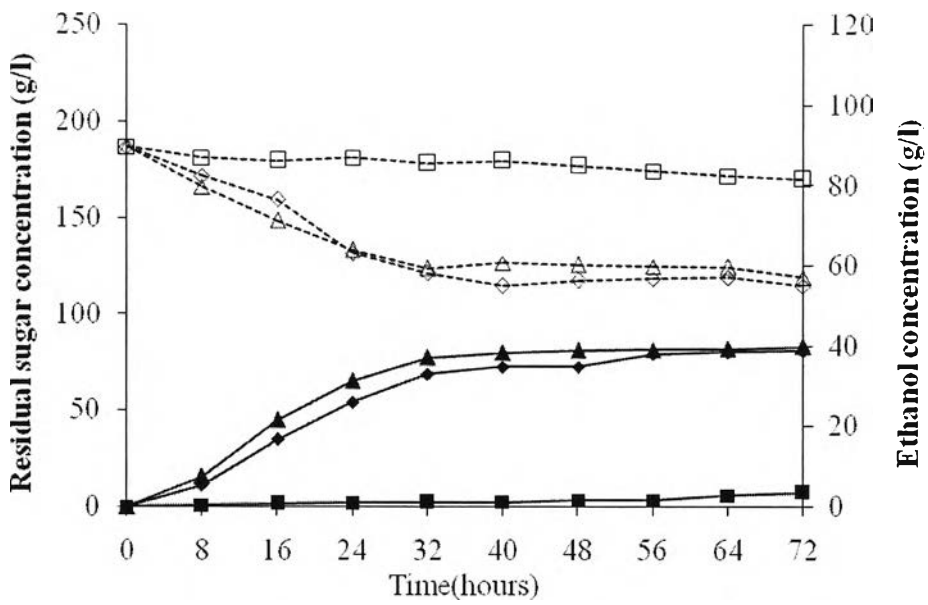


F: Cane molasses (40°C)

Figure 4.3 Ethanol (solid line, —) and residual sugar (dash line, ----) concentration profiles in Sugar cane juice (E) and Cane molasses medium (F) at constant temperature of 40°C; (■, □) = SS; (♦, ◊) = SK; (▲, Δ) = SM.



G: Sugar cane juice (45°C)



H: Cane molasses (45°C)

Figure 4.4 Ethanol (solid line, —) and residual sugar (dash line, ----) concentration profiles in Sugar cane juice (G) and Cane molasses medium (H) at constant temperature of 45°C; (■, □) = SS; (♦, ◊) = SK; (▲, Δ) = SM.

Table 4.2 Batch fermentation of ethanol production at constant temperature of 33°C.

System	Ethanol concentration (g/l)	Residual sugar concentration (g/l)	Free cell concentration (g/l)	$Y_{P/S}$ (%)	Productivity (g/l h)
Sugar cane juice					
SS	107.44	4.96	4.90	51.87	1.49
SK	90.69	8.18	5.50	48.41	1.26
SM	103.90	3.59	5.60	49.67	1.44
Cane molasses					
SS	90.53	27.14	6.04	46.81	1.26
SK	80.29	30.36	4.76	44.13	1.12
SM	87.91	24.91	6.42	45.70	1.22

Table 4.3 Batch fermentation of ethanol production at constant temperature of 37°C.

System	Ethanol concentration (g/l)	Residual sugar concentration (g/l)	Free cell concentration (g/l)	$Y_{P/S}$ (%)	Productivity (g/l h)
Sugar cane juice					
SS	64.89	43.33	3.61	36.00	0.90
SK	63.95	55.14	3.28	38.00	0.89
SM	74.88	33.58	4.02	40.00	1.04
Cane molasses					
SS	79.27	11.44	6.70	46.00	1.10
SK	54.51	52.83	7.33	43.00	0.76
SM	76.89	12.83	6.94	44.00	1.07

Table 4.4 Batch fermentation of ethanol production at constant temperature of 40°C.

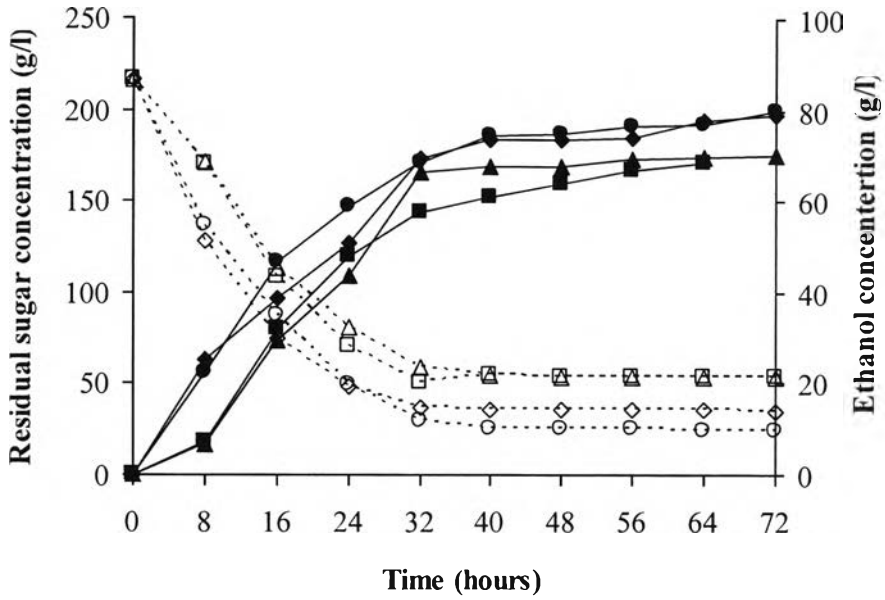
System	Ethanol concentration (g/l)	Residual sugar concentration (g/l)	Free cell concentration (g/l)	$Y_{P/S}$ (%)	Productivity (g/l h)
Sugar cane juice					
SS	46.09	79.15	6.20	38.85	0.64
SK	66.36	47.15	8.78	42.02	0.92
SM	66.17	50.78	8.52	44.11	0.92
Cane molasses					
SS	45.54	37.18	10.61	33.49	0.63
SK	36.83	91.19	9.74	33.86	0.51
SM	51.97	31.09	11.11	37.37	0.72

Table 4.5 Batch fermentation of ethanol production at constant temperature of 45°C.

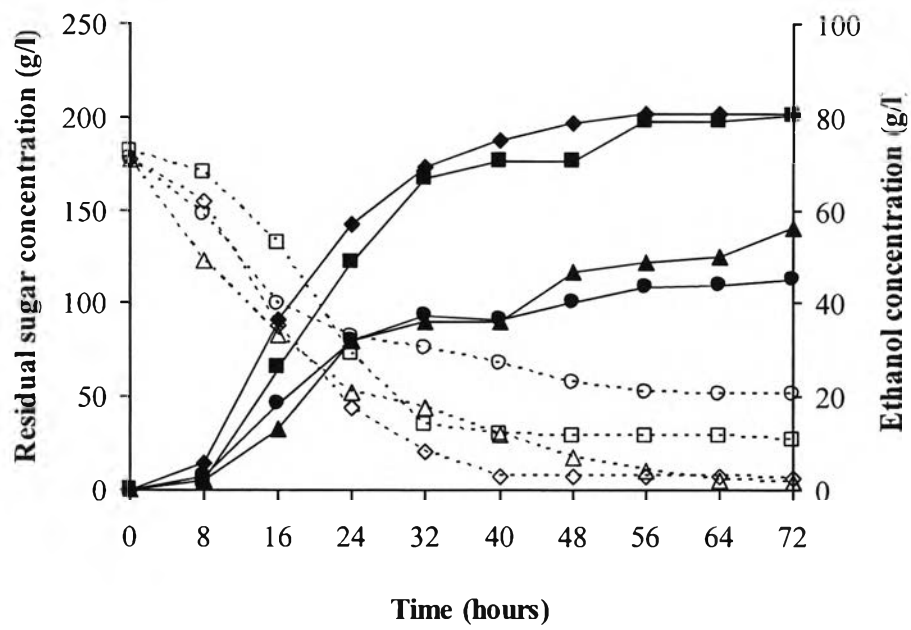
System	Ethanol concentration (g/l)	Residual sugar concentration (g/l)	Free cell concentration (g/l)	$Y_{P/S}$ (%)	Productivity (g/l h)
Sugar cane juice					
SS	0.36	205.16	0.36	1.03	0.01
SK	63.67	66.00	3.11	41.66	0.88
SM	62.34	66.71	2.88	43.22	0.87
Cane molasses					
SS	3.47	169.84	3.40	5.37	0.05
SK	38.67	114.13	4.76	40.80	0.54
SM	39.74	118.48	5.28	46.77	0.55

4.2 Batch fermentation of immobilized cells: monoculture (*K.marxianus* DMKU 3-1042) vs. mixed culture (*S.cerevisiae* M30 and *K.marxianus* DMKU 3-1042).

Ethanol productions at temperature of 37 and 40 °C were performed with different microorganism groups: (1) the monocultures of immobilized *K.marxianus* attachment to thin shell silk cocoons (TSSC); (2) the monocultures of immobilized *K.marxianus* within alginate-loofa (AL); (3) the mixed cultures of immobilized *S.cerevisiae* and *K.marxianus* attachment to TSSC; (4) the mixed cultures of immobilized *S.cerevisiae* and *K.marxianus* within AL. The results obtained from these experiments were shown in Fig.4.5 and 4.6. The higher ethanol productions were observed in the immobilized cells attachment to TSSC carriers compared with those from the immobilized cells within AL. The results of the fermentations are summarized in Table 4.6 and 4.7. The ethanol fermentation by the mixed culture system using thin shell silk cocoon as a carrier was found to be the most effective system. The immobilized mixed culture was found capable of highly efficient ethanol production from both sugar cane juice and cane molasses with the final ethanol concentration of 71.8-80.7 g/l at operating temperature of 37-40°C. In this study, ethanol production using immobilized cell culture was higher in comparison to those of the suspended cultures of the mixed culture or *K. marxianus*. Moreover, the immobilized mixed culture was more effective in the wide range of operations than the immobilized *K. marxianus*. The better activities of the immobilized cells might be attributed to the benefit of the immobilized system that conferred protection to cells when exposed to toxic or inhibitory conditions such as high ethanol concentration. It has been previously demonstrated for the improved ethanol productivity by immobilized *S. cerevisiae* systems (Phisalaphong et al., 2007; Najafpour et al., 2004; Yu et al., 2007) compared with suspended cell cultures.

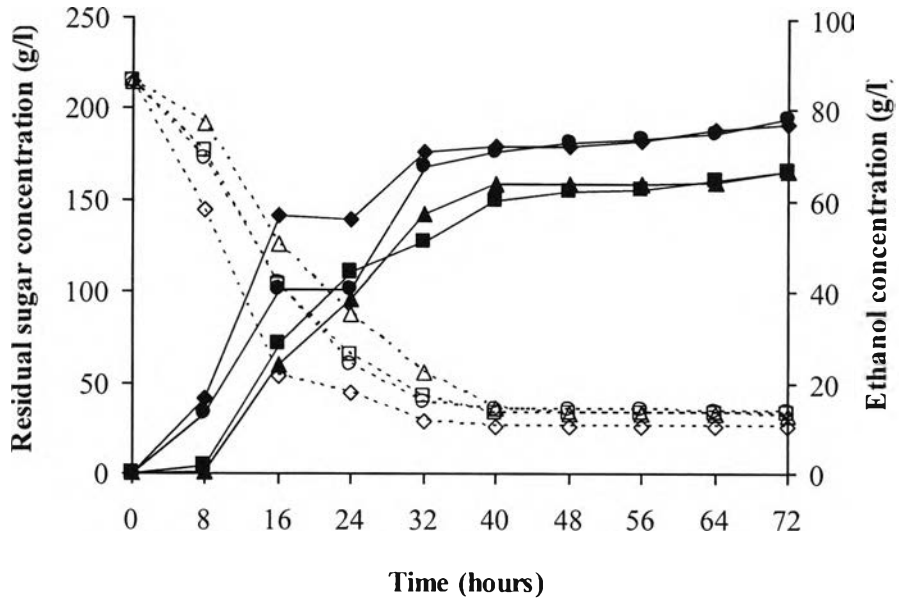


I: Sugar cane juice: Immobilized (37°C)

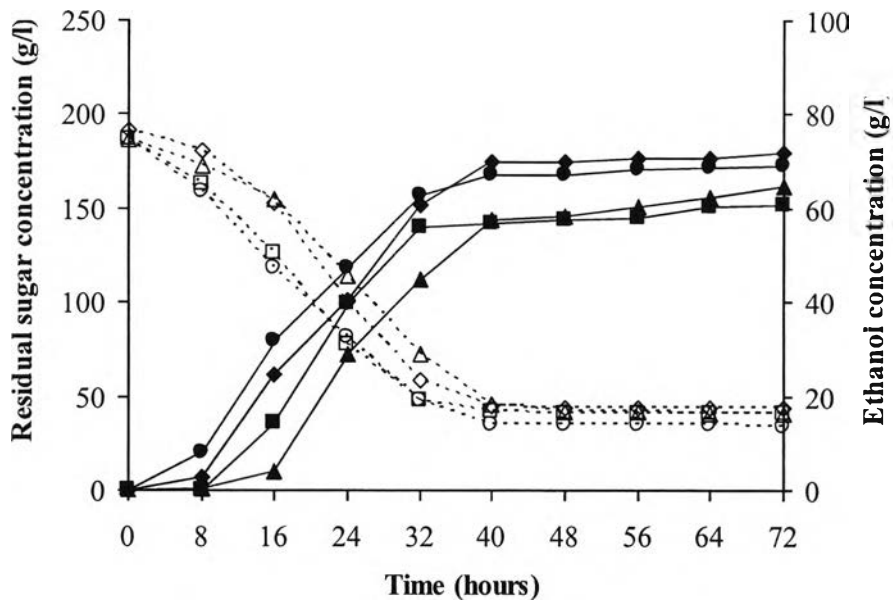


J: Cane molasses: immobilized (37°C)

Figure 4.5 Ethanol (solid line, —) and residual sugar (dash line, ----) concentration profiles in Sugar cane juice (I) and Cane molasses medium (J) at constant temperature of 37°C; (■, □) = Immobilized Mixed culture – AL; (♦, ◊) = Immobilized Mixed culture – TSSC; (▲, Δ) = Immobilized *K.marxianus* – AL; (●, ○) = Immobilized *K.marxianus* – TSSC.



K: Sugar cane juice: Immobilized (40°C)



L: Cane molasses: Immobilized (40°C)

Figure 4.6 Ethanol (solid line, —) and residual sugar (dash line, ----) concentration profiles in Sugar cane juice (K) and Cane molasses medium (L) at constant temperature of 40°C; (■, □) = Immobilized Mixed culture – AL; (♦, ◊) = Immobilized Mixed culture – TSSC; (▲, Δ) = Immobilized *K.marxianus* – AL; (●, ○) = Immobilized *K.marxianus* – TSSC.

Table 4.6 Batch fermentation of ethanol production by immobilized cells at constant temperature of 37°C.

System	37°C					
	Ethanol concentration (g/l)	**Residual sugar concentration (g/l)**	**X_F (g/l)**	**X_I (g/l)**	**Y_I (%)**	**$Y_{P/S}$ (%)**
Sugar cane juice						
Immobilized *K.marxianus*-(AL)	70.04	53.53	2.74	4.8	63.66	39.34
Immobilized *K.marxianus*-(TSSC)	79.79	24.41	2.08	3.9	65.22	40.03
Immobilized Mixed culture-(AL)	70.24	53.27	2.92	6.0	67.26	39.34
Immobilized Mixed culture-(TSSC)	78.91	34.91	0.32	3.3	91.16	40.11
Cane Molasses						
Immobilized *K.marxianus*-(AL)	56.08	3.73	0.43	4.4	91.10	37.27
Immobilized *K.marxianus*-(TSSC)	45.02	50.97	0.49	2.5	83.61	38.30
Immobilized Mixed culture-(AL)	80.35	26.45	1.32	5.9	81.72	37.40
Immobilized Mixed culture-(TSSC)	80.65	6.65	0.01	3.2	99.69	38.30

Table 4.7 Batch fermentation of ethanol production by immobilized cells at constant temperature of 40°C.

System	40°C					
	Ethanol concentration (g/l)	**Residual sugar concentration (g/l)**	**X_F (g/l)**	**X_I (g/l)**	**Y_I (%)**	**$Y_{P/S}$ (%)**
Sugar cane juice						
Immobilized *K.marxianus*-(AL)	66.34	32.24	1.52	3.4	69.11	35.89
Immobilized *K.marxianus*-(TSSC)	78.18	34.08	0.001	2.6	99.96	43.48
Immobilized Mixed culture-(AL)	66.49	32.77	2.10	0.9	30.00	38.73
Immobilized Mixed culture-(TSSC)	76.70	25.40	0.68	1.0	59.52	47.57
Cane Molasses						
Immobilized *K.marxianus*-(AL)	64.67	40.29	1.56	4.2	72.92	42.44
Immobilized *K.marxianus*-(TSSC)	69.11	33.98	0.50	2.5	83.33	39.05
Immobilized Mixed culture-(AL)	60.64	40.29	1.74	5.6	76.29	37.33
Immobilized Mixed culture-(TSSC)	71.84	43.45	0.005	1.8	99.72	46.14

4.3 Continuous ethanol fermentation in packed bed reactor

Continuous fermentation in packed bed reactor has several advantages compared to conventional batch processes mainly due to the higher conversion rate, faster overall fermentation rate, reduced product losses and environmental advantages. Most of these advantages are due to the high cell concentration found in these processes. Such high densities can be reached by immobilization techniques, recovery and recycling of cell biomass (Sánchez and Cardona, 2005). Ethanol fermentation with immobilized cells was generally performed in a packed bed reactor due to simple design and operational control (Valach et al., 2006). The current work was carried out in 1-L packed-bed bioreactor (PBR).

Continuous fermentation in 1-L packed-bed reactor with the mixed culture of *S. cerevisiae* M30 and *K. marxianus* DMKU 3-1042 immobilized on TSSC was investigated. The reactor was fed with cane molasses and sugar cane juice medium at the initial sugar concentration about 220 g/l, initial pH 5. Under the following condition: working volume 0.7 L, at the environmental temperature of initial temperature about 31±1 °C. The dilution rate was tested with was varied from 0.10, 0.21, 0.3 and 0.41 h^{-1}. Prior to inoculation and start up of the fermentation, the column was sterilized by circulation of 70% v/v ethanol for 1 hour and then was kept under UV light overnight. The immobilized cell on TSSC carrier was cultivated with initial sugar concentration about 220 g/l in shaking flask at 200 rpm, 33°C for 24 hour and then the carries were aseptically transferred to the sterilized column. The carrier volume was about 30% (v/v) of the pack bed reactor volume of 1-L. A start-up procedure was required in order to establish a steady state phase. Initially, the fermentation was started by feeding of the prepared medium of sugar cane molasses (220 g/l), through the inlet at the bottom of the column at the dilution rate of 0.10 h^{-1}. The dilution rate was changed after 4 days of 0.10 h^{-1} dilution rate and after 3 days of 0.21, 0.30 and 0.41 h^{-1} dilution rates. After the operation with cane molasses for the dilution rates of 0.10, 0.21, 0.3 and 0.41 h^{-1}, then the substrate has been changed to sugar cane juice medium. The dilution rate was increased after the system reached the steady state, from 0.10 h^{-1} to 0.21 and 0.30 h^{-1}, respectively. After that, the operation

was rolled back to the initial dilution rate (0.10 h^{-1}) for stability checking of the cell activities.

The continuous fermentation system was performed under uncontrolled temperature condition. The environmental temperature was 31±1 °C. Since ethanol fermentation is exothermic reaction, the system generates heat during the operation. Therefore, actual temperatures within the packed bed reactor are rather higher than the environmental temperature. The packed bed temperatures at 5 positions were monitored as shown Figure 4.7.

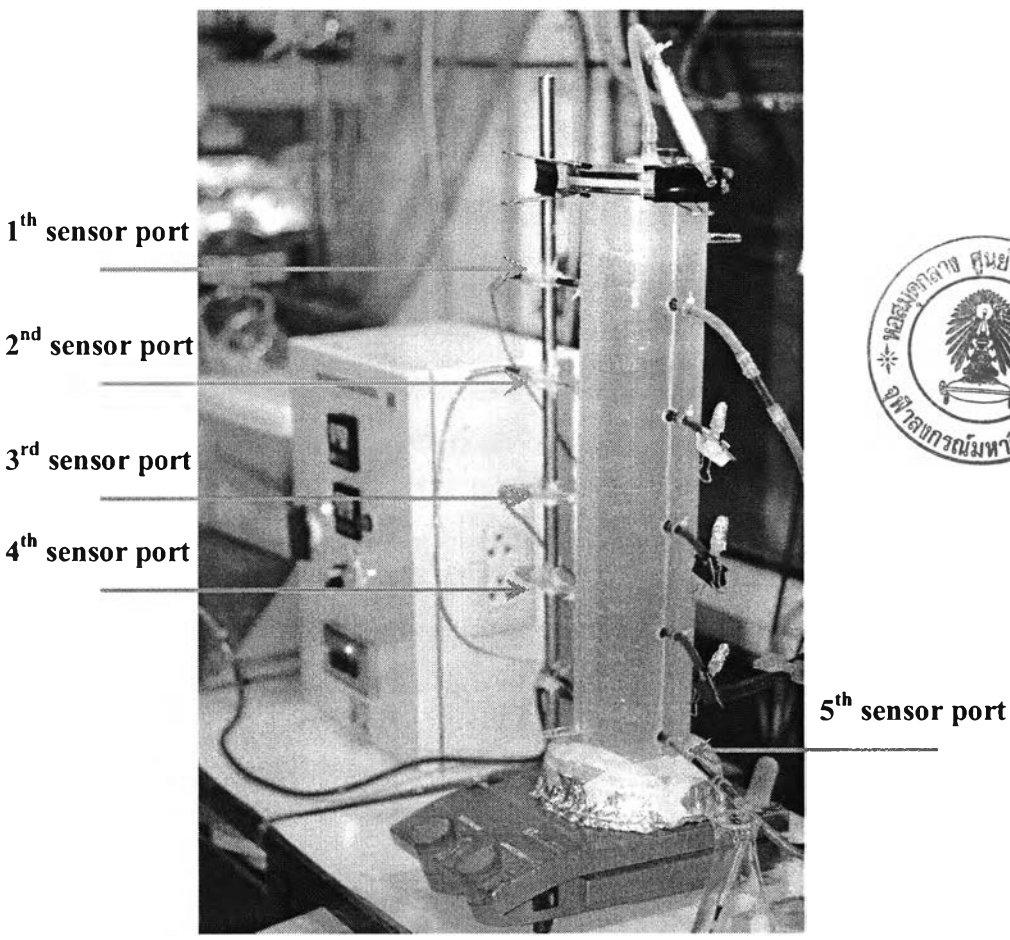


Figure 4.7 Picture of PBR

The ethanol and sugar concentration profiles at various dilution rates using cane molasses and sugar cane juice as feedstock are shown in Figure 4.8. The results for ethanol production, sugar consumption and productivity after the steady state of

various dilution rates of each feedstock are shown in Table 4.9. As expected, the highest steady-state ethanol concentration (71.73 ± 0.56 g/l) and the lowest residual sugar concentration (30.27 ± 1.37 g/l) were obtained at the lowest dilution rate (0.10 h^{-1}) for using cane molasses as feedstock. As the dilution rates were increased to 0.21, 0.30 and 0.41 h^{-1}, after steady state, the ethanol concentrations were at 64.00 ± 0.90, 49.20 ± 1.27 and 45.61 ± 1.10 g/l, respectively and the residual sugar concentrations were at 54.01 ± 2.02, 73.61 ± 2.45 and 85.93 ± 3.42 g/l, respectively. By using sugar cane juice as feedstock, the highest ethanol concentration (86.33 ± 1.34 g/l) and the lowest residual sugar concentration (21.81 ± 3.30 g/l) were obtained at the lowest dilution rate (0.10 h^{-1}). As the dilution rates were increased to 0.21 and 0.30 h^{-1}, after steady state, the ethanol concentrations were at 70.88 ± 0.31 and 56.47 ± 1.25 g/l, respectively and the residual sugar concentrations were at 40.82 ± 1.17 and 81.98 ± 4.51 g/l, respectively. This result indicated that the outlet ethanol concentration decreased when the dilution rate increased due to the decrease of the residence time.

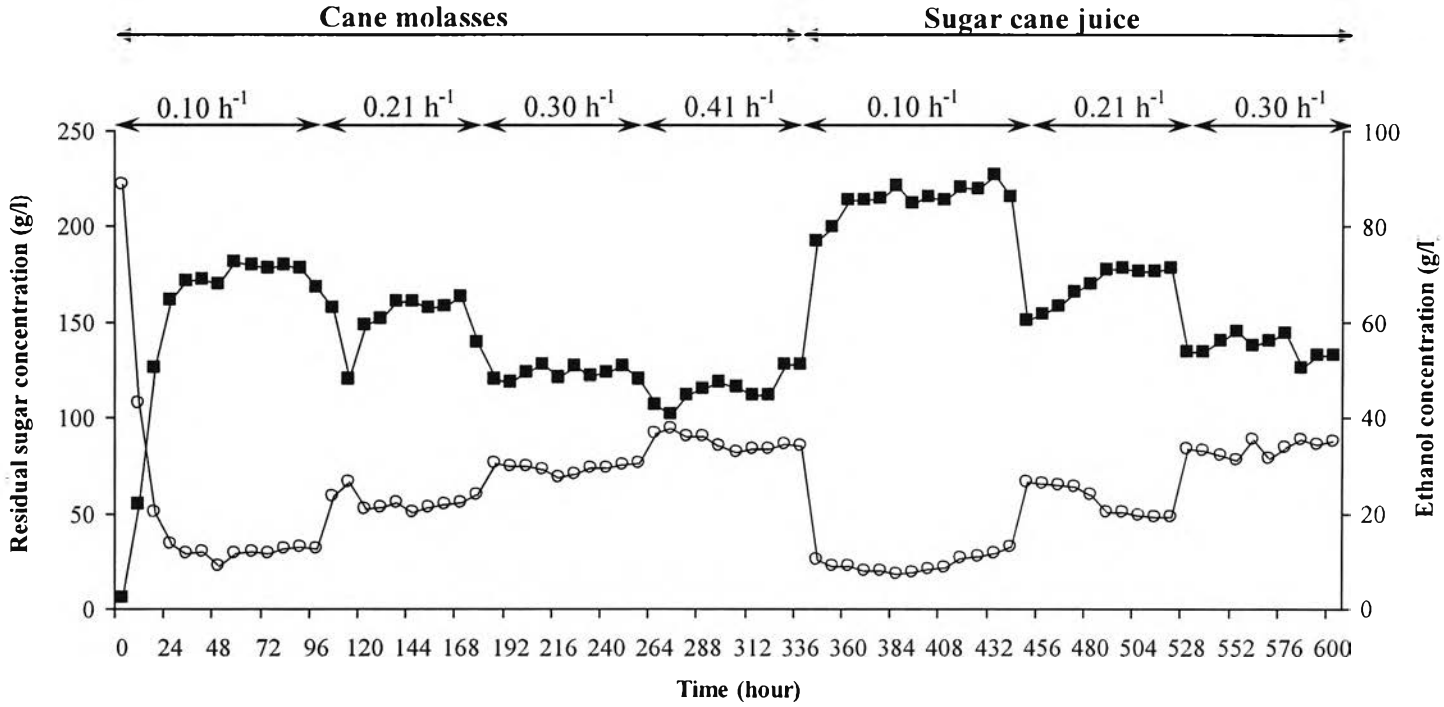


Figure 4.8 Continuous fermentation in a PBR at the dilution rate of 0.10, 0.21, 0.30 and 0.41 h^{-1}; ○, Residual sugar concentration and ■, Ethanol concentration

Table 4.8 Continuous fermentation on free cell (effluent) concentration in a PBR

Time (h)	Dilution rate (h^{-1})	Free cell (g/l)
0	0.10	0.00
24	0.10	0.40
48	0.10	1.10
72	0.10	1.40
96	0.10	1.40
120	0.21	1.40
144	0.21	2.00
168	0.21	2.15
192	0.30	2.20
216	0.30	2.00
240	0.30	2.00
264	0.41	4.20
288	0.41	5.00
312	0.41	6.20
336	0.41	6.00
360	0.10	3.20
384	0.10	2.20
408	0.10	2.20
432	0.10	2.80
456	0.21	2.60
480	0.21	3.20
504	0.21	2.80
528	0.30	2.20
552	0.30	2.80
576	0.30	3.00
600	0.30	4.00

Table 4.9 Effect of dilution rate on continuous ethanol production and ethanol productivity, average at steady state

Dilution rate (h^{-1})	Ethanol concentration (g/l)	Residual sugar Concentration (g/l)	$Y_{P/S}$ (%)	Productivity (g/l h)
Cane molasses				
0.10	71.73 (± 0.56)	30.27 (± 1.37)	37.41	7.17 (± 0.06)
0.21	64.00 (± 0.90)	54.01 (± 2.02)	38.10	12.80 (± 0.18)
0.30	49.20 (± 1.27)	73.61 (± 2.45)	33.16	14.76 (± 0.38)
0.41	45.61 (± 1.10)	85.93 (± 3.42)	33.52	18.24 (± 0.44)
Sugar cane juice				
0.10	86.33 (± 1.34)	21.81 (± 3.30)	43.00	8.63 (± 0.13)
0.21	70.88 (± 0.31)	40.82 (± 1.17)	40.97	14.18 (± 0.06)
0.30	56.47 (± 1.25)	81.98 (± 4.51)	40.36	16.94 (± 0.38)

The effect of dilution rate on ethanol productivity was illustrated in Figure 4.9. It was indicated that the ethanol productivity increased as the dilution rate was increased. The productivity increased from 8.63 ± 0.13 g/l h at the lowest dilution rate (0.10 h^{-1}) to 16.94 ± 0.38 g/l h at a dilution rate of 0.30 h^{-1} for feedstock of sugar cane juice medium, which were comparatively higher than those using feedstock of cane molasses medium. By using the molasses medium, the productivities of 7.17 ± 0.06 g/l h to 14.76 ± 0.38 g/l h were obtained at the dilution rates of 0.10-0.30 h^{-1}. The result after 25 days of operation demonstrates high-performance consistency and stability of the immobilized mixed culture in TSSC carrier.

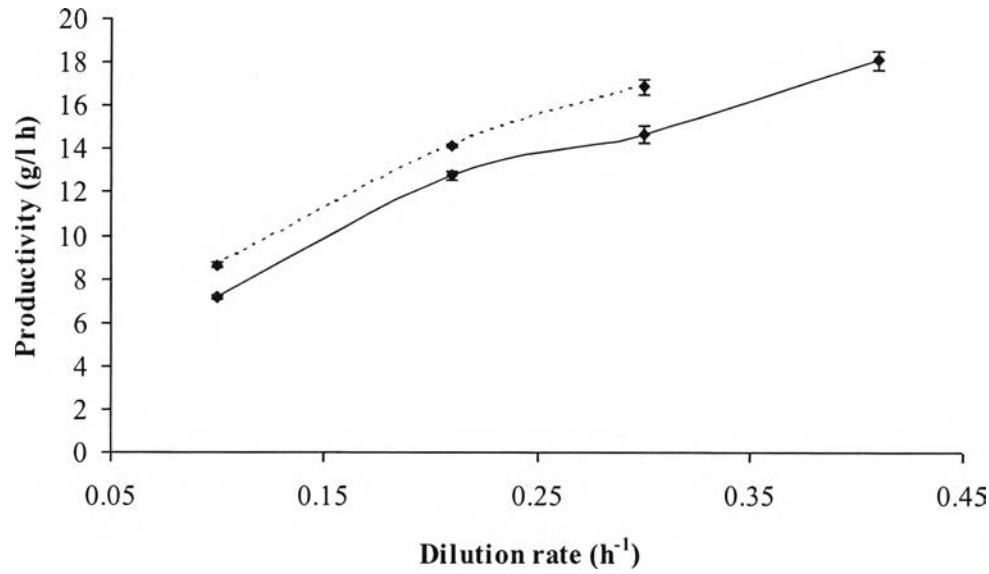


Figure 4.9 Effect of dilution rate on ethanol productivity; (—♦—), Cane molasses and (----♦----), Sugar cane juice

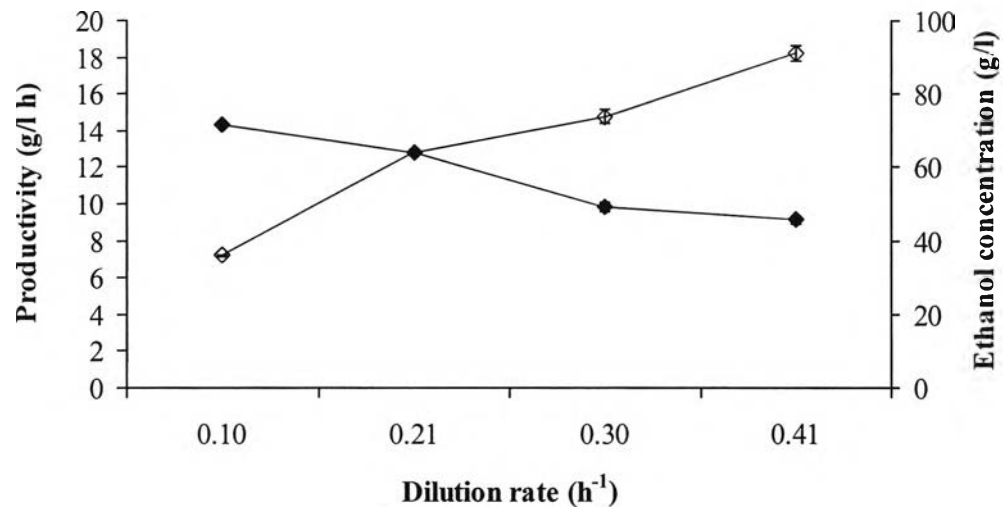


Figure 4.10 The ethanol productivities of ethanol fermentation at steady state for the cane molasses medium; (—◇—), productivity and (—♦—), ethanol concentration

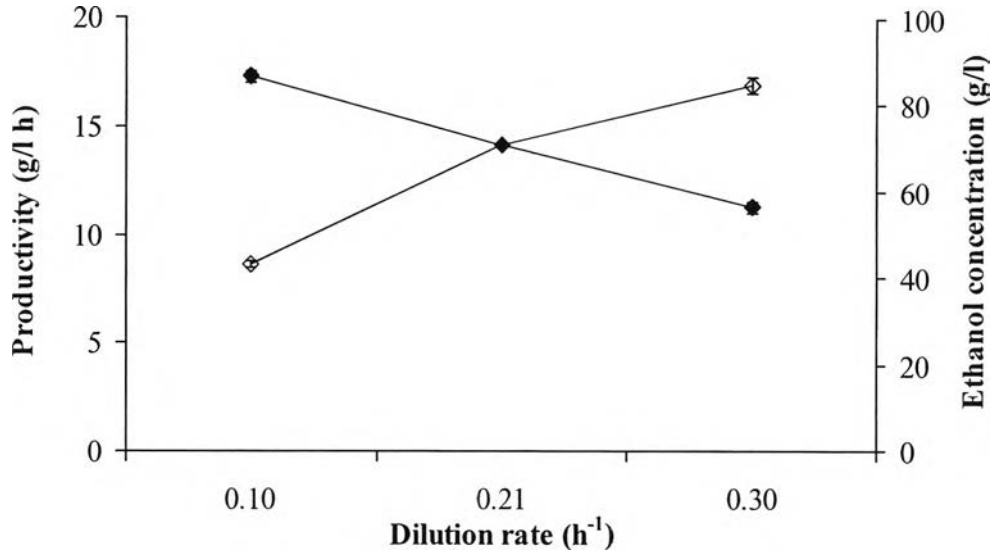
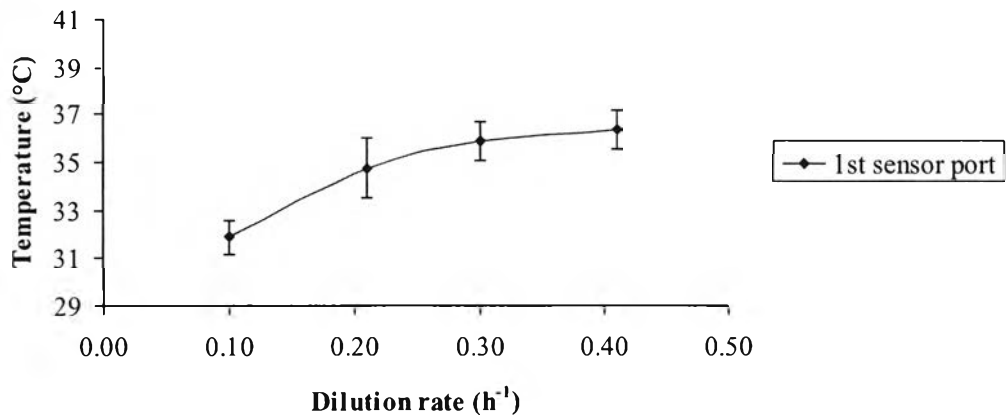
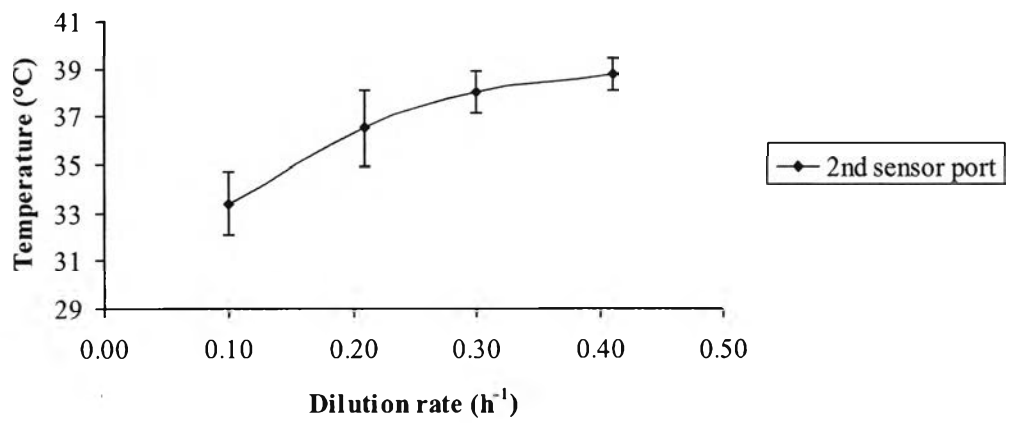


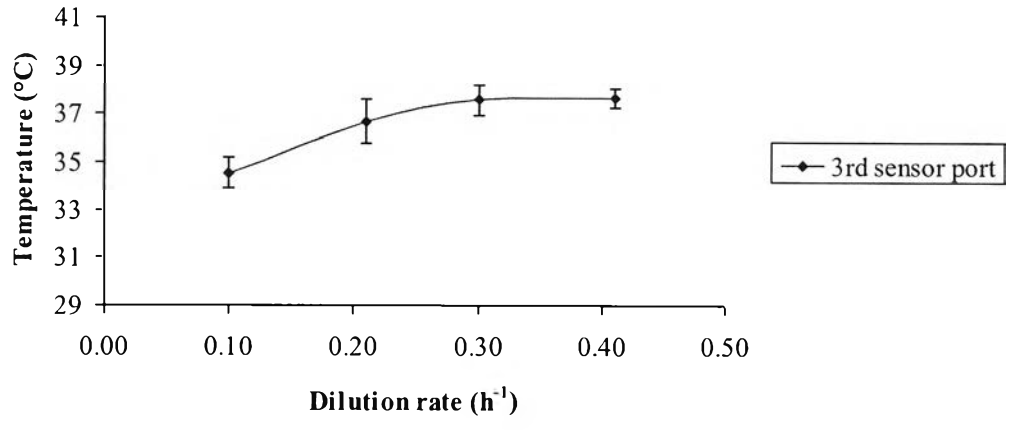
Figure 4.11 The ethanol productivities of ethanol fermentation at steady state for the sugar cane juice medium; (—◇—), productivity and (—♦—), ethanol concentration

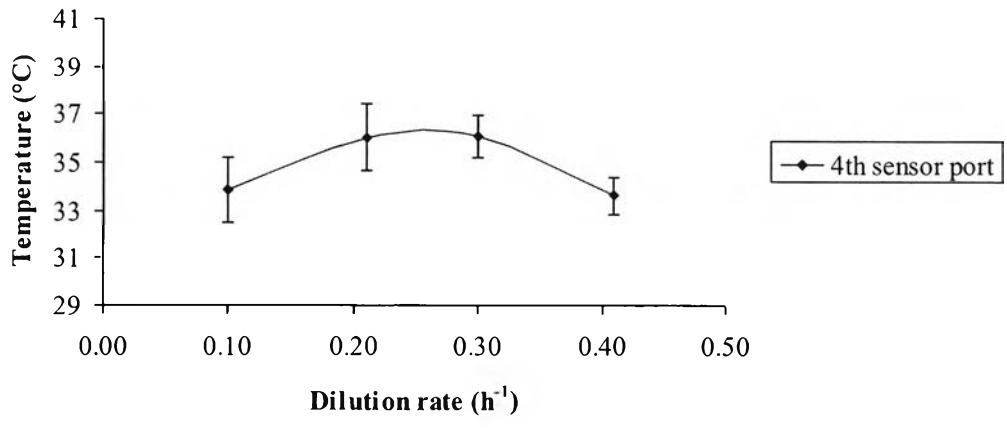
Temperature profiles of the packed bed reactor during the continuous fermentation were monitored by the temperature sensors placed in the center at 5 different positions of the column (Figure 4.12 and Figure 4.13). The average environmental temperature was 31±1 °C. The temperature in the center of the column was rather higher than the outer part due to the metabolic activities of microorganisms that produced heat during the fermentation. The highest temperature in the PBR was detected at the 2nd position. The bed temperature increased with the dilution rate. By using cane molasses medium, the maximum temperature of 38.76 ± 0.68 °C was remarked at the 2nd position during the operation at the highest dilution rate (0.41 h^{-1}). As the dilution rates were increased from 0.10 to 0.21, 0.30 and 0.40 h^{-1}, at the 2nd sensor port, the temperatures changed from 33.40 ± 1.30 to 36.54 ± 1.59, 38.03 ± 0.89 and 38.76 ± 0.08 °C, respectively.

By using sugar cane juice medium, it was found that the average temperature at 0.30 h^{-1} dilution rate at the 1st, 2nd, 3rd, 4th and 5th sensor port were 38.19 ± 0.48, 39.36 ± 0.51, 38.46 ± 0.34, 35.82 ± 0.78 and 31.30 ± 0.50°C, respectively. The average temperature in each position is comparatively higher than the values obtained when cane molasses medium was used as the substrate, according to the more effective of ethanol production in cane juice medium. The results revealed that the mixed culture system of *S. cerevisiae* M30 and *K. marxianus* DMKU 3-1042 in TSSC carrier is an excellent system in terms of its growth and fermentation properties at high temperature range under different feedstock (cane molasses or sugar cane juice). The temperature profiles at the 2nd sensor port were found corresponding to the ethanol productivity as shown in Figure 4.14 and 4.15.






Temperature (°C)
41
39
37
35
33
31
29
0.00
0.10
0.20
0.30
0.40
0.50
Dilution rate (h-1)
3rd sensor port


Temperature (°C)
41
39
37
35
33
31
29
0.00
0.10
0.20
0.30
0.40
0.50
Dilution rate (h-1)
4th sensor port

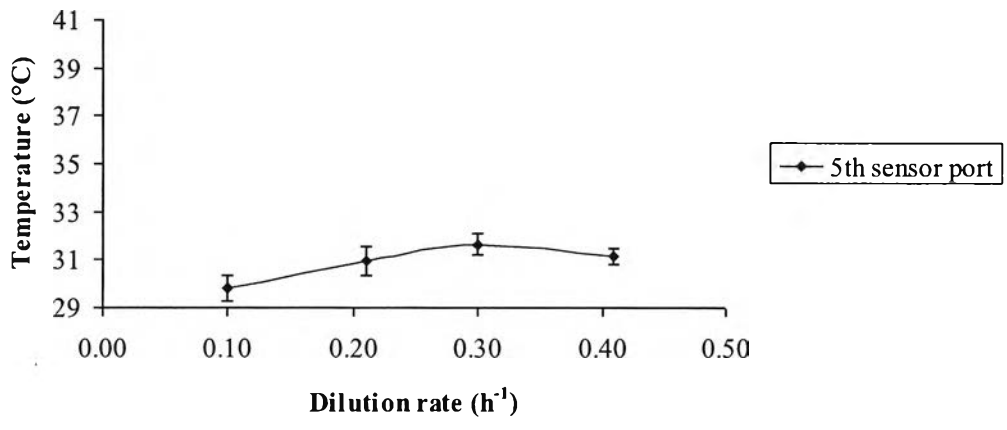
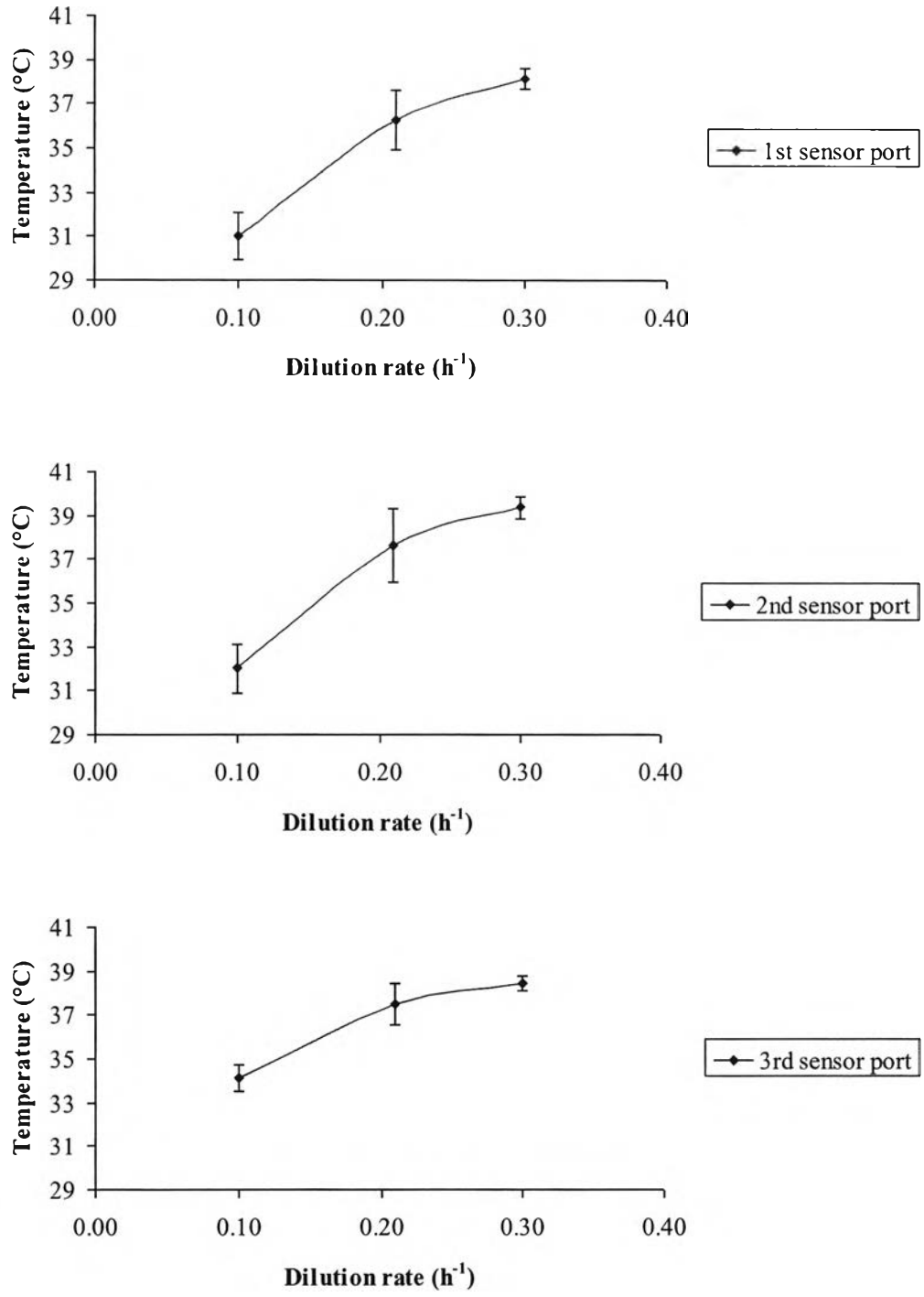

Temperature (°C)
41
39
37
35
33
31
29
0.00
0.10
0.20
0.30
0.40
0.50
Dilution rate (h-1)
5th sensor port

Figure 4.12 Temperature profile of the system at the positions within the column of 5 positions as shown in the Figure 4.7 for cane molasses



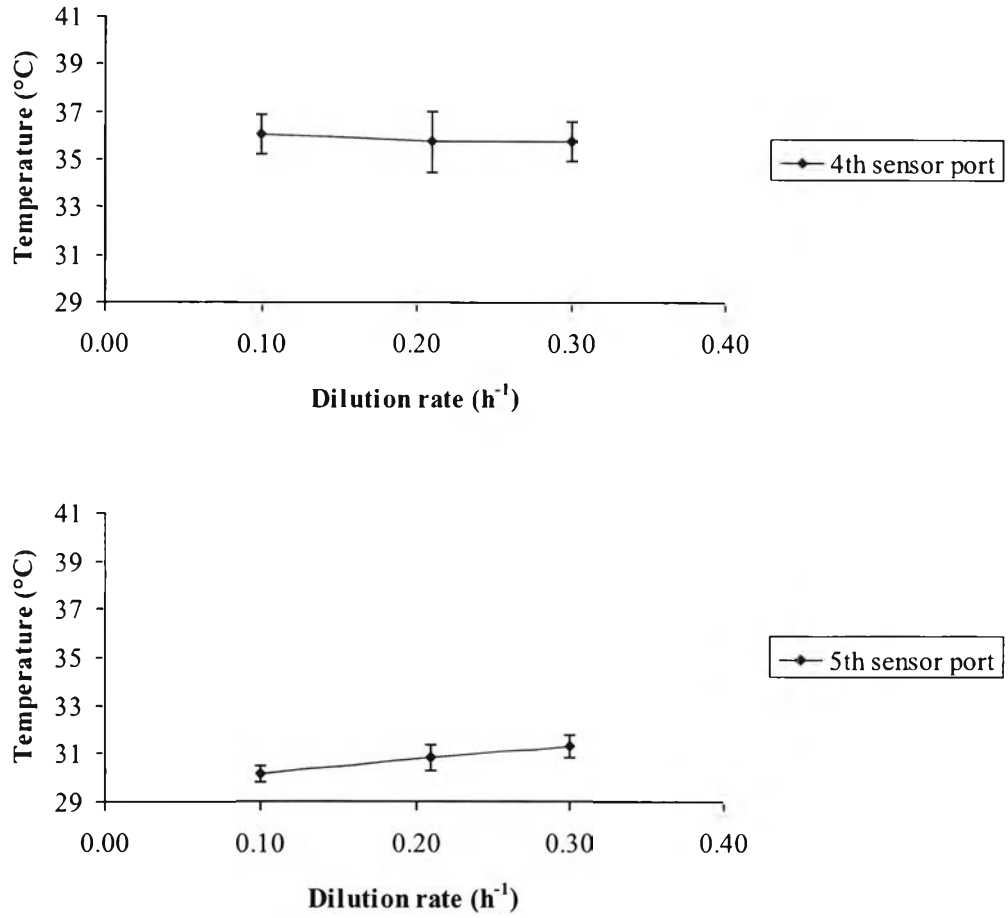


Figure 4.13 Temperature profile of the system at the positions within the column of 5 positions as shown in the Figure 4.7 for sugar cane juice

Table 4.10 Temperature profiles of the system within the column at 1st - 5th sensor ports of the PBR as shown in the Figure 4.7

Dilution rate (h^{-1})	1st sensor port (°C)	2nd sensor port (°C)	3rd sensor port (°C)	4th sensor port (°C)	5th sensor port (°C)
			Cane molasses		
0.10	31.87 (± 0.71)	33.40 (± 1.30)	34.55 (± 0.64)	33.85 (± 1.34)	29.84 (± 0.54)
0.21	34.76 (± 1.26)	36.54 (± 1.59)	36.71 (± 0.93)	36.04 (± 1.36)	30.93 (± 0.61)
0.30	35.86 (± 0.81)	38.03 (± 0.89)	37.60 (± 0.62)	36.07 (± 0.90)	31.64 (± 0.43)
0.41	36.36 (± 0.81)	38.76 (± 0.68)	37.69 (± 0.41)	33.62 (± 0.75)	31.14 (± 0.34)
			Sugar cane juice		
0.10	31.02 (± 1.09)	32.01 (± 1.23)	34.14 (± 0.59)	36.06 (± 0.82)	30.15 (± 0.35)
0.21	36.28 (± 1.34)	37.64 (± 1.70)	37.50 (± 0.97)	35.79 (± 1.24)	30.82 (± 0.52)
0.30	38.19 (± 0.48)	39.36 (± 0.51)	38.46 (± 0.34)	35.82 (± 0.79)	31.30 (± 0.50)

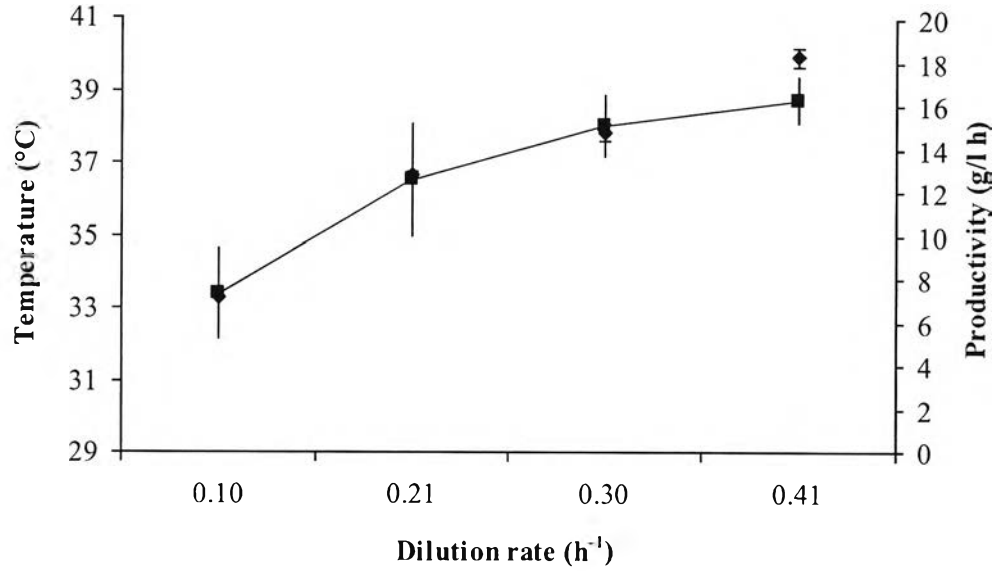


Figure 4.14 Effect of dilution rate on temperature profile of the system and productivity at the 2nd sensor port for feedstock as cane molasses medium; (—■—), Temperature; (----♦----), Productivity

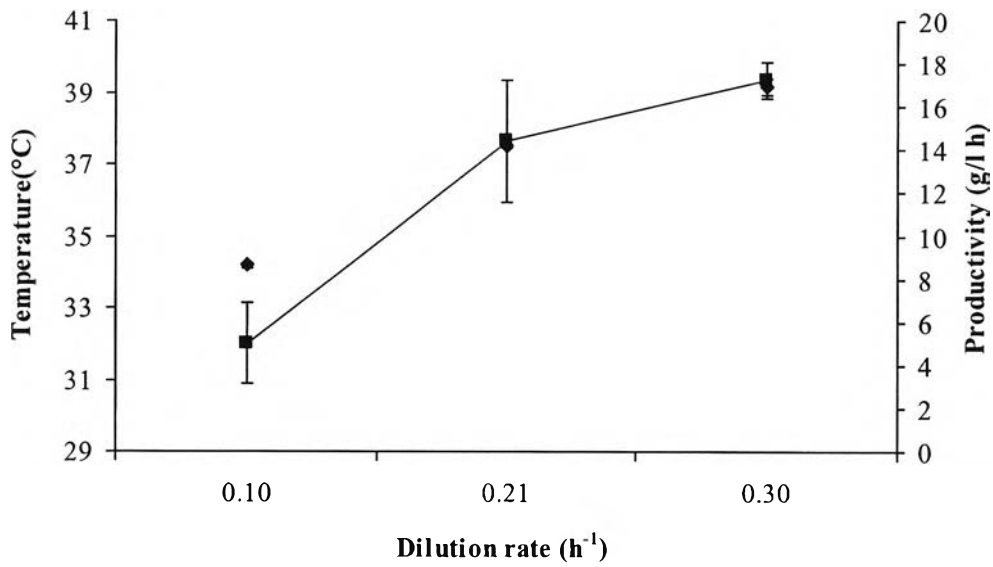


Figure 4.15 Effect of dilution rate on temperature profile of the system and productivity at the 2nd sensor port for feedstock as sugar cane juice medium; (—■—), Temperature; (----♦----), Productivity

At the end of the fermentation, the concentrations of free cells in the effluent, in the reactor and immobilized cells in the reactor were determined (Table 4.11). The immobilized cell concentration increased from 5.83 g/l at the initial period to 32.26 g/l at the end of the operation with 5.70 g/l free cell in the reactor and 2.53 g/l free cell in the effluent. The overall immobilized yield was 79.67%. This demonstrated that yeast cells could grow and regenerate inside the TSSC carrier in the PBR. The immobilized mixed cell culture system was found effective with high stability throughout the long period of continuous fermentation.

Table 4.11 Yeast cell concentrations at the end of continuous fermentation

Cell concentrations	**(g/l)**
Immobilized cell – at the beginning of fermentation	5.83
Immobilized cell – at the end of the fermentation	32.26
Free cell in reactor	5.70
Free cell in effluent	2.53
Immobilized yield (%)	79.67

In commercial ethanol production facilities, productivity is important as well as efficiency of substrate conversion and product concentration, especially since a major expense of the process was the cost of the fermentable raw material and product separation process. Although our system was primarily designed for efficient substrate conversion to ethanol, the productivities reported in this experiment were competitive with previous reports for a comparable system. Najafpour et al. (2004) on the ethanol fermentation in immobilized cell reactor resulted in the ethanol productivity of 2.8 g/l h with the initial sugar concentration and dilution rate of 50 g/l and 0.17 h^{-1}, respectively. The work of Göksungur et al. (2001) studied the production of ethanol from beet molasses by Ca-alginate immobilized yeast cells in a PBR and reported that the ethanol concentration and productivity were 46.18 g/l and 10.16 g/l h, respectively with the initial sugar concentration and dilution rate of 109 g/l and 0.22 h^{-1}, respectively.

A scanning electron microscope (SEM) was used to compare the images of the TSSC carriers before ethanol fermentation and at the end of fermentation. Figure 4.16 to Figure 4.21 represent the images of carrier from the beginning of the fermentation to the end of fermentation. The amount of cells inside and outside the carriers from time to time was increased. For long term performance, the free cell leakage occurred, which could be observed from the free cells in the reactor and the free cells in the effluent. Figure 4.22 to Figure 4.25 show the image of suspended cell culture in the reactor and in the effluent.

The result in the PBR demonstrated that the mixed culture in TSSC carrier was favorable for high ethanol production and had good stability under a wide range of operating temperatures with feedstock of cane molasses and sugar cane juice as the carbon source in continuous fermentation. With favorable mechanical and biocompatibility properties and proper porous structure, it resulted in a stable operation and high density of biomass. The mixed culture with TSSC carrier has a good potential of reusability to continuously produce ethanol at high temperature (35 – 40 °C).

Figure 4.16 TSSC outer surface before fermentation

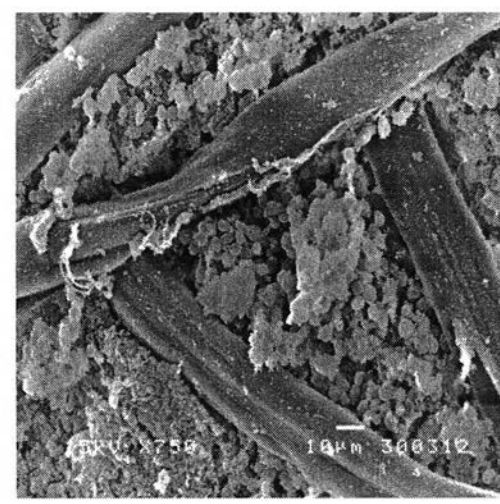


Figure 4.17 TSSC outer surface at the end of continuous fermentation

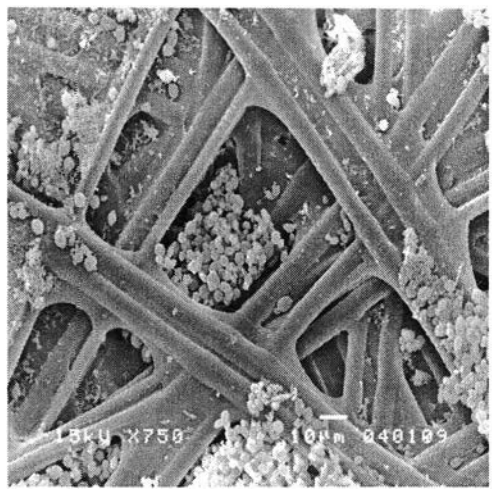


Figure 4.18 TSSC inner surface before fermentation

Figure 4.19 TSSC inner surface at the end of continuous fermentation

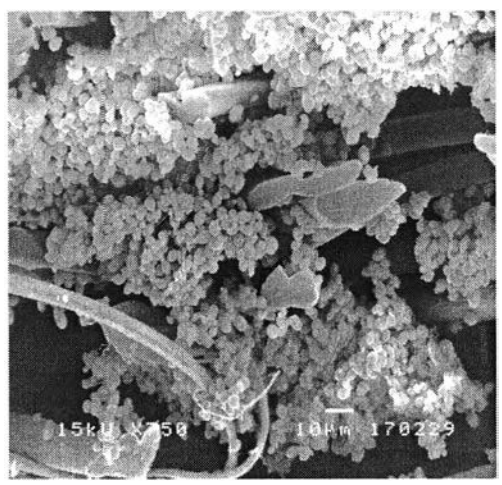


Figure 4.20 TSSC cross section before fermentation

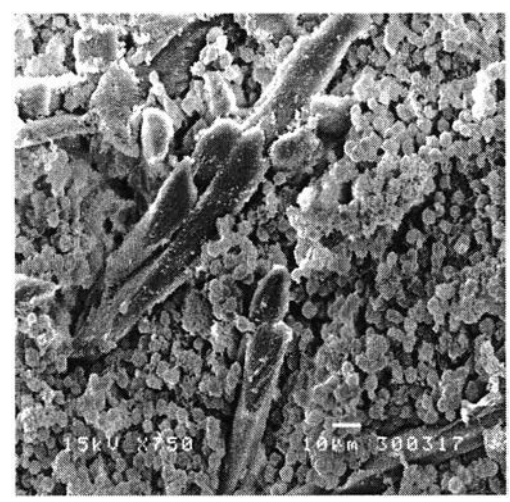


Figure 4.21 TSSC cross section at the end of continuous fermentation

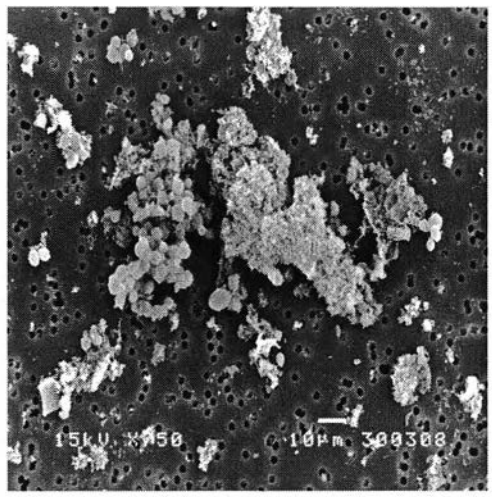


Figure 4.22 Free cell in reactor (x 750)

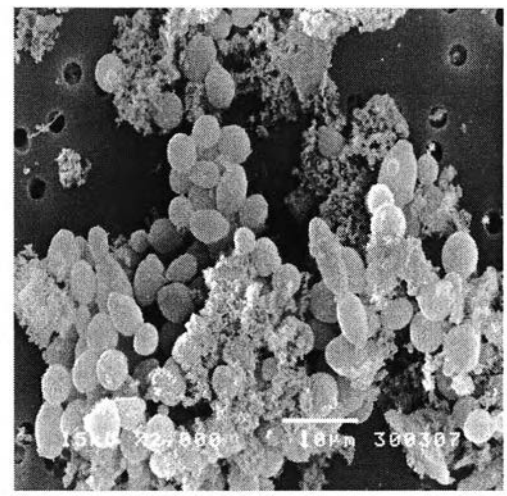


Figure 4.23 Free cell in reactor (x 2,000)

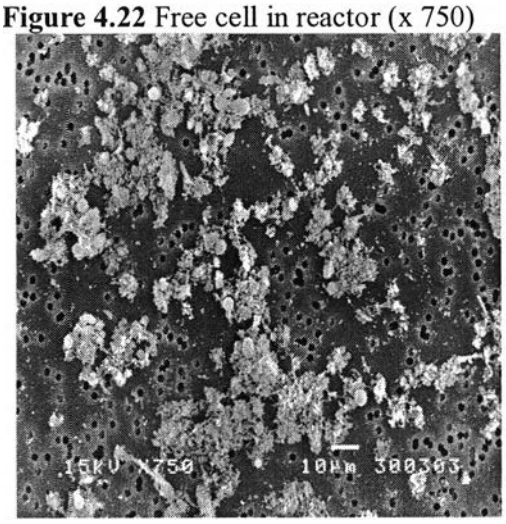


Figure 4.24 Free cell in the effluent (x 750)

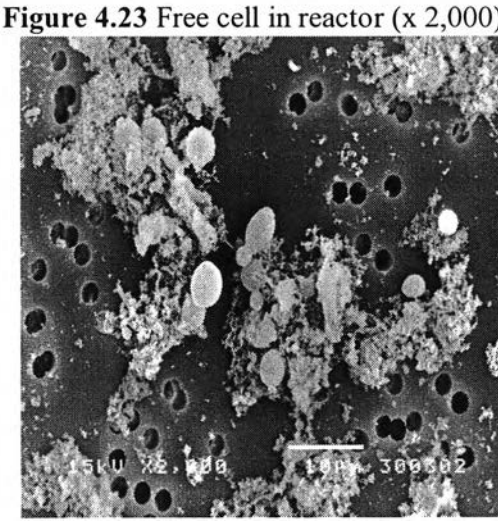


Figure 4.25 Free cell in the effluent (x 2,000)