CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Selection of suitable digestion method

4.1.1 Commercial enzyme addition strategy

Dried longan flesh with low sugar level was digested by three commercial enzymes, namely, α-amylase (A), carbohydrase (VP) and endo-xylanase (WX) with corresponding specific enzyme activities of 100 kNU (kilo – Novo α-amylase unit), 25 FBG (fungal beta-glucanase unit) and 500 FXU (fungal xylanase unit), respectively (equivalent to the mass fraction of 0.17 g enzyme/g dried longan flesh powder) as well as 200 kNU, 50 FBG and 1000 FXU, respectively (equivalent to the mass fraction of 0.33 g enzyme/g dried longan flesh powder) without/with implementation of pressurized steamer. The specific sugars consumption/production levels were calculated in the upcoming sections.

4.1.1.1 Specific sucrose consumption

The digestion of low sugar dried longan flesh with various digestion methods resulted in the decreasing of sucrose level in comparison to the control. The digestion reaction from enzyme to sucrose, which was a disaccharide, could result in monosaccharides such glucose and fructose (Horton *et al*., 1996). Ratanapanon (2004) also described the application of enzymes in carbohydrase group which constituted sucrase enzyme also yielded glucose and fructose.

From Table 4.1, the results were compared to the control. The digestion by the combination of three commercial enzymes using the mass fraction of 0.17 without/with a pressurized steamer gave maximum decreasing values of specific sucrose consumption statistically ($p \leq 0.05$) (2.43 ± 0.13 and 2.64 ± 0.02 g specific sugar consumption/g enzyme/l, respectively).

The digestion by only endo – xylanase using the mass fraction of 0.33 g/g without/with a pressurized steamer gave minimum decreasing values of specific sucrose consumption statistically ($p \leq 0.05$) (0.88 ± 0.02 and 1.09 ± 0.01 g/g/l, respectively) as shown in Fig. 4.1.

These results were similar to Phromkunthong *et al*. (2001) who investigated the effect of commercial enzyme on the transformation of disaccharide to monosaccharide in herbivorous fish and hybrid catfish commercial feeds. The enzyme was added into each experiment except in the control where no enzyme was added. The results portrayed that the enzyme added experiment had more increasing of monosaccharide sugar levels than the control statistically ($p \leq 0.05$)

Table 4.1 Specific sucrose consumption (g/g/l) after digested by three commercial enzymes using the mass fraction of 0.17 and 0.33 g/g without/with a pressurized steamer

Type of Enzyme	Specific sucrose production (g sugar/g enzyme/l mixture)															
	No claving								Claving							
	0.17 g/g				0.33 g/g				0.17 g/g				0.33 g/g			
1.No enzyme	0.31 ± 0.07	a	I		0.32 ± 0.08	a	I		0.34 ± 0.03	a	I		0.33 ± 0.04	a	I	
2.A, VP &WX	2.43 ± 0.13	b	I		1.22 ± 0.04	b	II		2.64 ± 0.02	b	I		1.27 ± 0.02	b	II	
3.A & VP	2.02 ± 0.22	b,c,d	I		1.10 ± 0.06	b,c	II		2.49 ± 0.07	b,c	I		1.29 ± 0.05	b	II	
4.VP & WX	2.07 ± 0.32	b,c,d	I		1.09 ± 0.10	b,c,d,e	II		2.33 ± 0.07	c,d	I		1.26 ± 0.05	b,c	II	
5.A & WX	2.06 ± 0.10	b,c	I		1.09 ± 0.09	b,c,d	II		2.32 ± 0.10	c,d,e	I		1.24 ± 0.01	b	II	
6.Only VP	1.88 ± 0.13	c,d	I		0.95 ± 0.08	c,d,e	II		2.14 ± 0.07	d,e	I		1.16 ± 0.02	c,d	II	
7.Only A	1.76 ± 0.10	c,d	I		0.91 ± 0.05	d,e	II		2.13 ± 0.04	e	I		1.10 ± 0.02	d,e	II	
8.Only WX	1.61 ± 0.09	d	I		0.88 ± 0.02	e	II		2.10 ± 0.05	e	I		1.09 ± 0.01	e	II	

The number with the same Roman numeral (I – II) and alphabet (a – e) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

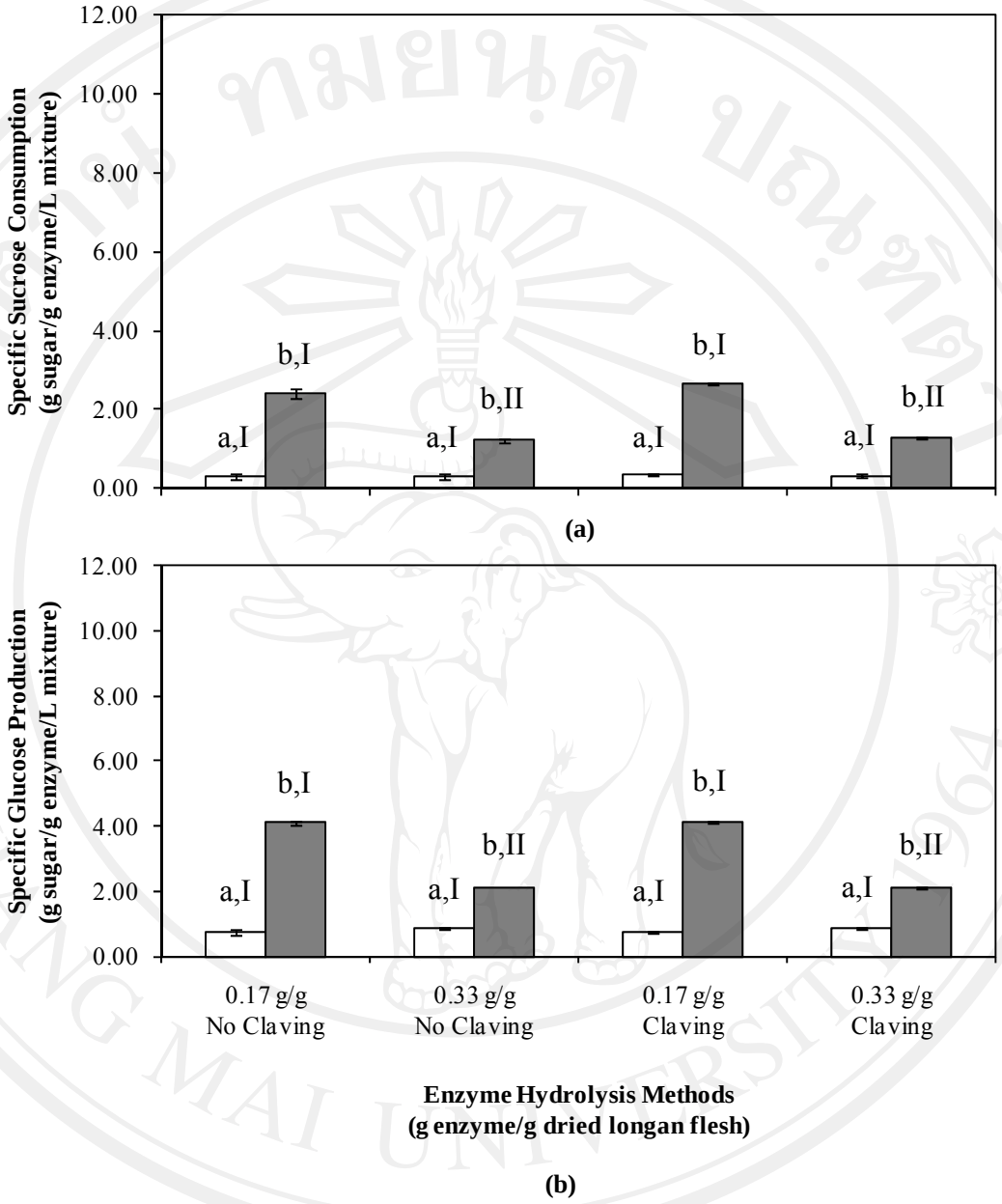


Figure 4.1 Specific (a) sucrose consumption (b) glucose production after digestion by three commercial enzymes (shaded) and control (unshaded) using the mass fraction of 0.17 g/g and 0.33 g/g without/with a pressurized steamer. The numbers with the same Roman numeral (I – II) and alphabet (a – b) indicated no significant difference ($p > 0.05$) which were explained in Table 4.1 – 4.4.

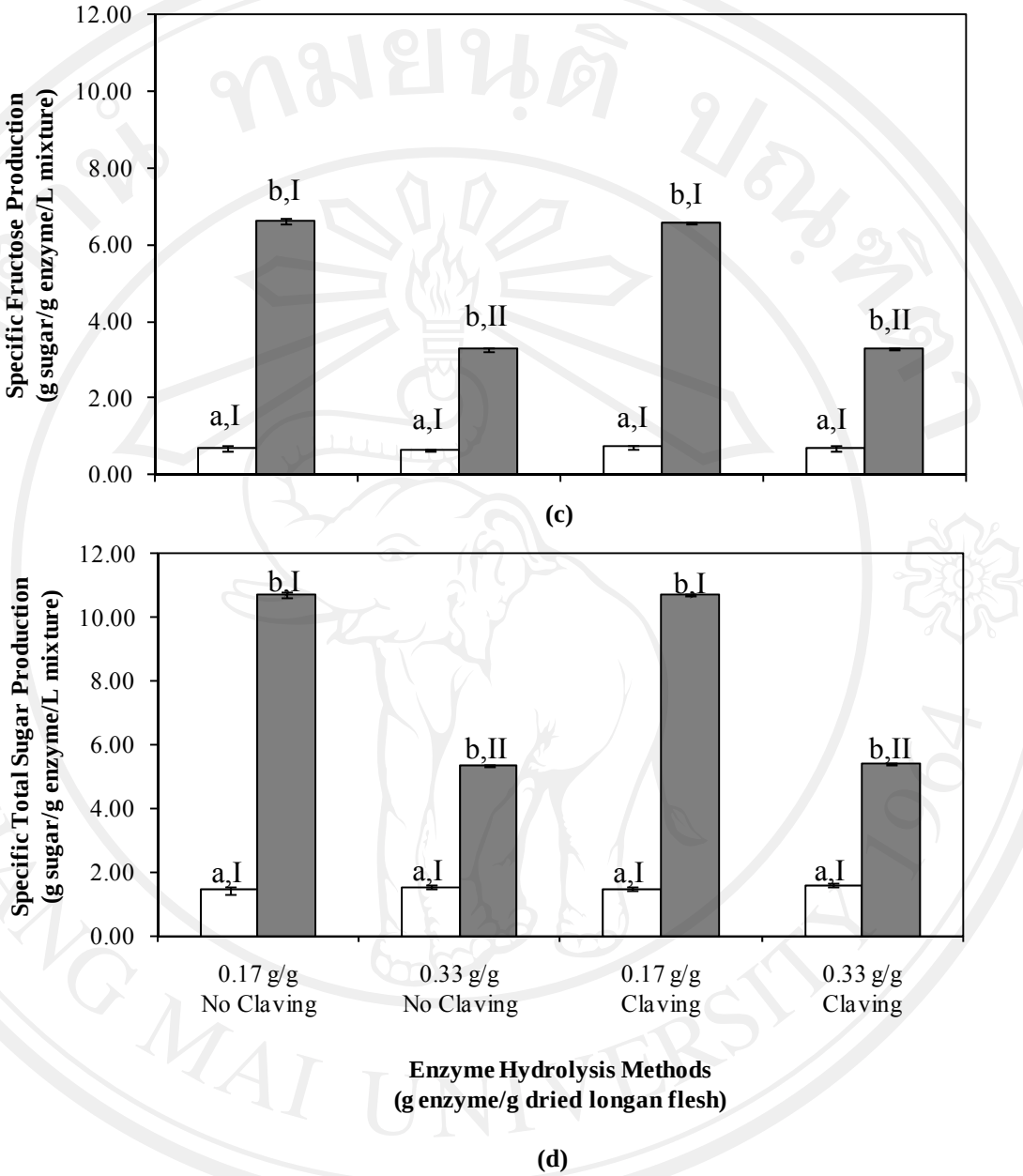


Figure 4.1 (cont.) Specific (c) fructose production (d) total sugar production (g/g/l) after digested by three commercial enzymes (shaded) and control (unshaded) using the mass fraction of 0.17 g/g and 0.33 g/g without/with a pressurized steamer. The numbers with the same Roman numeral (I – II) and alphabet (a – b) indicated no significant difference ($p > 0.05$) which were explained in Table 4.1 – 4.4.

4.1.1.2 Specific glucose production

From Table 4.2, the results were compared to the control. The digestion by the combination of three commercial enzymes using the mass fraction of 0.17 without/with a pressurized steamer gave the highest values of specific glucose production statistically ($p \leq 0.05$) which were 4.09 ± 0.06 and 4.11 ± 0.02 g specific glucose production/g enzyme/l, respectively.

The digestion by only endo – xylanase using the mass fraction of 0.33 g/g without/with a pressurized steamer gave the lowest values of specific glucose production statistically ($p \leq 0.05$) (1.42 ± 0.02 and 1.56 ± 0.01 g/g/l, respectively) as shown in Fig. 4.1.

Table 4.2 Specific glucose production (g/g/l) after digested by three commercial enzymes using the mass fraction of 0.17 and 0.33 g/g without/with a pressurized steamer

Type of Enzyme	Specific glucose production (g sugar/g enzyme/l mixture)															
	No claving								Claving							
	0.17 g/g				0.33 g/g				0.17 g/g				0.33 g/g			
1.No enzyme	0.73 ± 0.09	a	I		0.87 ± 0.04	a	I		0.75 ± 0.04	a	I		0.88 ± 0.02	a	I	
2.A, VP & WX	4.09 ± 0.06	b	I		2.08 ± 0.02	b	II		4.11 ± 0.02	b	I		2.09 ± 0.02	b	II	
3.A & VP	3.67 ± 0.27	b,c	I		1.92 ± 0.04	c	II		3.99 ± 0.07	b,c	I		2.08 ± 0.05	b,c	II	
4.VP & WX	3.18 ± 0.26	c,e	I		1.68 ± 0.10	c,d	II		3.91 ± 0.05	c	I		1.99 ± 0.02	c	II	
5.A & WX	2.08 ± 0.20	d	I		1.30 ± 0.10	e	II		3.92 ± 0.03	c	I		1.98 ± 0.05	b,c	II	
6.Only VP	2.93 ± 0.08	e	I		1.48 ± 0.04	d,e	II		3.05 ± 0.04	d,e	I		1.57 ± 0.01	d	II	
7.Only A	2.76 ± 0.08	e	I		1.42 ± 0.04	e	II		3.02 ± 0.03	d	I		1.57 ± 0.01	d	II	
8.Only WX	2.80 ± 0.14	e	I		1.42 ± 0.02	e	II		2.94 ± 0.02	e	I		1.56 ± 0.01	d	II	

The number with the same Roman numeral (I – II) and alphabet (a – e) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

4.1.1.3 Specific fructose production

From Table 4.3, the results were compared to the control. The digestion by the combination of three commercial enzymes using the mass fraction of 0.17 without/with a pressurized steamer gave the highest values of specific fructose production statistically ($p \leq 0.05$) which were 6.62 ± 0.07 and 6.59 ± 0.03 g specific fructose production/g enzyme/l, respectively.

The digestion by only endo – xylanase using the mass fraction of 0.33 g/g without/with a pressurized steamer gave the lowest values of specific fructose production statistically ($p \leq 0.05$) which were 1.42 ± 0.03 and 1.58 ± 0.02 g/g/l, respectively (Fig. 4.1).

As portrayed in Fig. 4.1, the specific fructose production was higher than the specific glucose production statistically ($p \leq 0.05$). However Yoon *et al*. (2005) found that the fructose level was 2 folds lower than glucose level after carrot digestion using commercial carbohydrase group enzymes. This was compared to the current study where α-amylase, carbohydrase and endo-xylanase were used. The contradicting results were probably caused by different types of enzyme and raw materials being used.

Table 4.3 Specific fructose production (g/g/l) after digestion by three commercial enzymes using the mass fraction of 0.17 and 0.33 g/g without/with a pressurized steamer

Type of Enzyme	Specific fructose production (g sugar/g enzyme/l mixture)															
	No claving								Claving							
	0.17 g/g				0.33 g/g				0.17 g/g				0.33 g/g			
1.No enzyme	0.70 ± 0.06	a	I		0.65 ± 0.04	a	I		0.72 ± 0.03	a	I		0.70 ± 0.05	a	I	
2.A, VP & WX	6.62 ± 0.07	b	I		3.27 ± 0.03	b	II		6.59 ± 0.03	b	I		3.29 ± 0.02	b	II	
3.A & VP	5.44 ± 0.07	c	I		2.78 ± 0.07	c	II		5.59 ± 0.04	c	I		3.01 ± 0.03	c	II	
4.VP & WX	5.07 ± 0.05	d	I		2.70 ± 0.10	c	II		5.36 ± 0.08	d	I		2.88 ± 0.02	c	II	
5.A & WX	5.07 ± 0.08	d	I		2.62 ± 0.04	c	II		5.34 ± 0.11	d	I		2.70 ± 0.03	c	II	
6.Only VP	2.88 ± 0.06	e	I		1.51 ± 0.02	d	II		2.94 ± 0.02	e	I		1.62 ± 0.02	d	II	
7.Only A	2.82 ± 0.07	e	I		1.42 ± 0.04	d,e	II		2.74 ± 0.03	e	I		1.59 ± 0.02	d,e	II	
8.Only WX	2.79 ± 0.11	e	I		1.42 ± 0.03	e	II		2.69 ± 0.02	e	I		1.58 ± 0.02	e	II	

The number with the same Roman numeral (I – II) and alphabet (a – e) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

4.1.1.4 Specific total sugars production

The specific glucose and fructose production were compared to the control (Table 4.4). The digestion by the combination of three commercial enzymes using the mass fraction of 0.17 without/with a pressurized steamer gave the highest values of specific total sugars consumption statistically ($p \leq 0.05$) which were 10.72 ± 0.10 and 10.70 ± 0.04 g specific total sugars production/g enzyme/l, respectively.

The digestion by only endo – xylanase using the mass fraction of 0.33 g/g without/with a pressurized steamer gave the lowest values of specific total sugars consumption statistically ($p \leq 0.05$) which were 2.84 ± 0.04 and 3.14 ± 0.02 g/g/l, respectively (Fig. 4.1).

The results were similar to those of Phromkunthong *et al*. (2001) who stated that disaccharides in several plant raw materials such residues of soybean, coconut and cassava could be converted to monosaccharides by the enzymes in carbohydrase and endo – xylanase groups. Prajummuang and Leelawacharamas (2003) also reported that α-amylase enzyme could also digest flour into sugars.

Table 4.4 Specific total sugars production (g/g/l) after digestion by three commercial enzymes using the mass fraction of 0.17 and 0.33 g/g without/with a pressurized steamer

Type of Enzyme	Specific total sugar production (g sugar/g enzyme/l mixture)															
	No claving								Claving							
	0.17 g/g				0.33 g/g				0.17 g/g				0.33 g/g			
1.No enzyme	1.43 ± 0.11	a	I		1.52 ± 0.06	A	I		1.47 ± 0.05	a	I		1.58 ± 0.06	a	I	
2.A, VP & WX	10.72 ± 0.10	b	I		5.35 ± 0.04	B	II		10.70 ± 0.04	b	I		5.38 ± 0.02	b	II	
3.A & VP	9.11 ± 0.27	c	I		4.70 ± 0.09	C	II		9.58 ± 0.08	c	I		5.09 ± 0.06	c	II	
4.VP & WX	8.25 ± 0.27	c	I		4.39 ± 0.14	C	II		9.28 ± 0.09	c	I		4.87 ± 0.03	c	II	
5.A & WX	7.15 ± 0.21	d	I		3.92 ± 0.11	D	II		9.26 ± 0.11	d	I		4.68 ± 0.05	d	II	
6.Only VP	5.81 ± 0.10	e	I		3.00 ± 0.5	E	II		5.99 ± 0.05	e	I		3.19 ± 0.02	e	II	
7.Only A	5.57 ± 0.11	e	I		2.84 ± 0.06	e,f	II		5.76 ± 0.04	e	I		3.16 ± 0.02	e,f	II	
8.Only WX	5.59 ± 0.18	e	I		2.84 ± 0.04	f	II		5.63 ± 0.03	e	I		3.14 ± 0.02	f	II	

The number with the same Roman numeral (I – II) and alphabet (a – f) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

4.1.1.5 Economical specific sugars production

The investigation of commercial viability for specific sugar production (μg specific sugar production/baht/l) revealed that the digestion by three commercial enzymes using the mass fraction of 0.17 g/g without a pressurized steamer was the most economical statistically ($p \leq 0.05$) (1,097 ± 10 μg/baht/l). The economical specific sugar production in this case was higher than the situation where the pressurized steamer was employed by 3.3 folds.

On the other hand, the digestion by only α-amylase, only carbohydrase and only endo – xylanase using the mass fraction of 0.33 g/g without/with a pressurized steamer were the least economical statistically ($p \leq 0.05$) which were 98 ± 1, 97 ± 1 and 97 ± 1 μg/baht/l, respectively as shown in Fig. 4.2 and Table 4.5.

Table 4.5 Economical specific sugar production (μg/baht/l) after digestion by three commercial enzymes using the mass fraction of 0.17 and 0.33 g/g without/with a pressurized steamer

Type of Enzyme	Specific sugar production on commercial aspect (μg sugar/baht/l mixture) after addition of enzyme															
	No claving						Claving									
	0.17 g/g				0.33 g/g				0.17 g/g				0.33 g/g			
1.No enzyme	146 ± 11	a	I		156 ± 6	a	I		45 ± 2	a	II		49 ± 2	a	II	
2.A, VP & WX	1,097 ± 10	b	I		548 ± 4	b	II		329 ± 1	b	III		166 ± 1	b	IV	
3.A & VP	932 ± 28	c	I		481 ± 9	c	II		295 ± 2	c	III		157 ± 2	c	IV	
4.VP & WX	844 ± 28	c	I		449 ± 14	c	II		286 ± 3	d	III		150 ± 1	d	IV	
5.A & WX	731 ± 22	d	I		401 ± 11	d	II		285 ± 4	c,d	III		144 ± 2	e	IV	
6.Only VP	595 ± 10	e	I		307 ± 5	e	II		184 ± 1	e	III		98 ± 1	f	IV	
7.Only A	570 ± 11	e	I		291 ± 6	e,f	II		177 ± 1	f	III		97 ± 1	f	IV	
8.Only WX	572 ± 18	e	I		290 ± 4	f	II		173 ± 1	g	III		97 ± 1	f	IV	

The number with the same Roman numeral (I – IV) and alphabet (a – f) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row different rows of the same column, respectively.

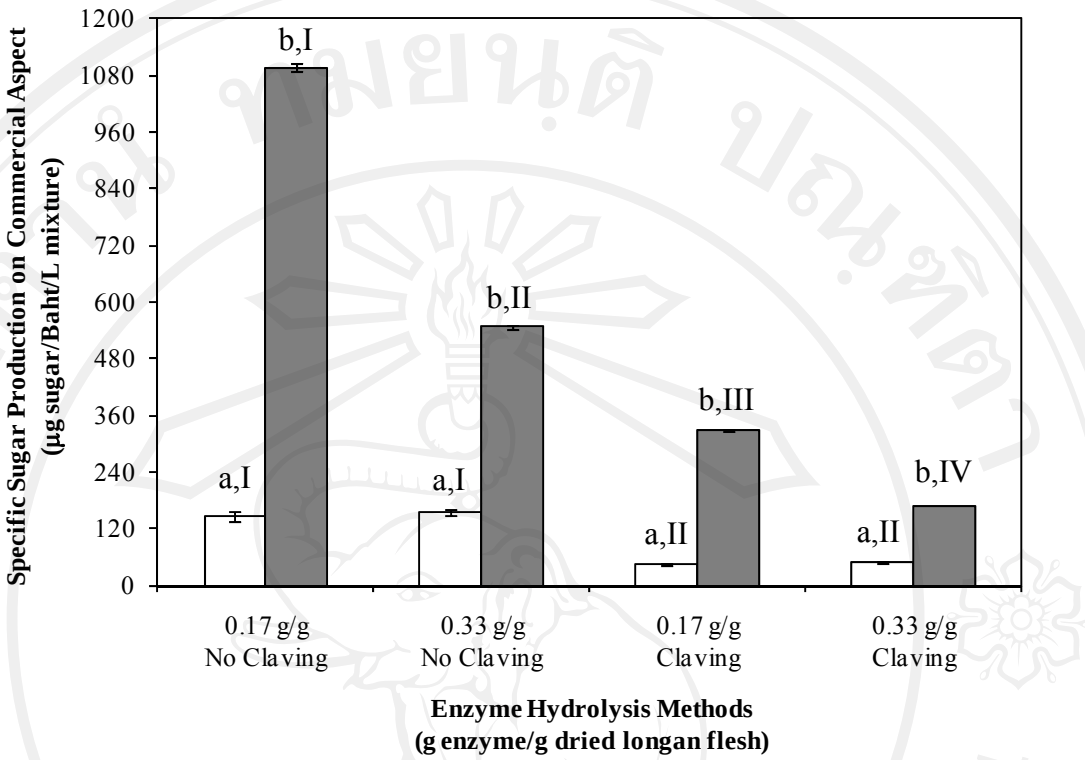


Figure 4.2 Economical specific sugar production (µg/baht/l) after digestion by three commercial enzymes using the mass fraction of 0.17 and 0.33 g/g without/with a pressurized steamer.

4.1.2 Effects of digestion mixture ratio

Dried longan flesh with low sugar level was digested by the mixture of acetic acid and sodium hydroxide solutions. Four levels of acetic acid solution concentration (0.5, 1.0, 1.5 and 2.0%) were mixed with 0.1%(w/v) sodium hydroxide solution with the volume ratio of 2:1, 1:1 and 1:2. Four ratio of dried longan flesh powder to digestive solution of 1:10, 2:10, 3:10 and 5:10 were subsequently investigated. The specific sugar consumption/production and specific sugar production on the economical aspect was considered as following;

4.1.2.1 Specific sucrose consumption

The digestion of low sugar dried longan flesh with various digestion method resulted in the decreasing of sucrose level in comparison to the control. The enzymatic digestion of sucrose, which was a disaccharide, resulted in monosaccharides such glucose and fructose (Horton *et al*., 1996). Ratanapanone (2004) described the application of enzymes in carbohydrase group which constituted sucrase enzyme that yielded glucose and fructose.

From Fig. 4.3 and Table 4.6, the digestion condition with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 2:10(w/v) using 2%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:1 ratio resulted in the maximum decrease of specific sucrose consumption level statistically ($p \leq 0.05$) of 2.75 ± 0.01 g/g/l.

This was compared to the digestion condition with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio of 5:10(w/v) using 0.5%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 2:1 ratio resulted in the minimum decrease of specific sucrose consumption level statistically ($p \leq 0.05$) of 2.43 ± 0.01 g/g/l.

Table 4.6 Specific sucrose consumption (g/g/l) for four dried longan flesh : buffer ratios

Concentration of acetic acid	acetic acid : sodium hydroxide ratio		
	1:2	1:1	2:1
Dried longan flesh : buffer ratio at 1:10			
0.5%	2.54 ± 0.01 a I	2.59 ± 0.01 a II	2.49 ± 0.01 a III
1.0%	2.58 ± 0.02 b I	2.63 ± 0.01 b II	2.54 ± 0.02 b III
1.5%	2.62 ± 0.01 c I	2.66 ± 0.01 b II	2.56 ± 0.02 b,c III
2.0%	2.65 ± 0.01 d I	2.71 ± 0.01 c II	2.59 ± 0.02 c III
Dried longan flesh : buffer ratio at 2:10			
0.5%	2.58 ± 0.01 a I	2.64 ± 0.01 a I	2.54 ± 0.01 a I
1.0%	2.63 ± 0.02 b I	2.69 ± 0.01 b II	2.59 ± 0.02 b I
1.5%	2.66 ± 0.02 c I	2.71 ± 0.01 b II	2.62 ± 0.02 b,c I
2.0%	2.69 ± 0.01 d I	2.75 ± 0.01 c II	2.66 ± 0.02 c I
Dried longan flesh : buffer ratio at 3:10			
0.5%	2.54 ± 0.02 a I	2.59 ± 0.01 a II	2.49 ± 0.01 a III
1.0%	2.57 ± 0.02 b I	2.63 ± 0.01 b II	2.53 ± 0.02 a,b III
1.5%	2.62 ± 0.02 c I	2.66 ± 0.01 b II	2.55 ± 0.02 b,c III
2.0%	2.65 ± 0.02 d I	2.71 ± 0.01 c II	2.60 ± 0.02 c III
Dried longan flesh : buffer ratio at 5:10			
0.5%	2.47 ± 0.01 a I	2.50 ± 0.01 a II	2.43 ± 0.01 a III
1.0%	2.52 ± 0.01 b I	2.56 ± 0.01 b II	2.48 ± 0.02 a,b III
1.5%	2.56 ± 0.01 c I	2.59 ± 0.01 b II	2.52 ± 0.02 b,c III
2.0%	2.62 ± 0.01 d I	2.65 ± 0.01 c II	2.57 ± 0.02 c III

The number with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

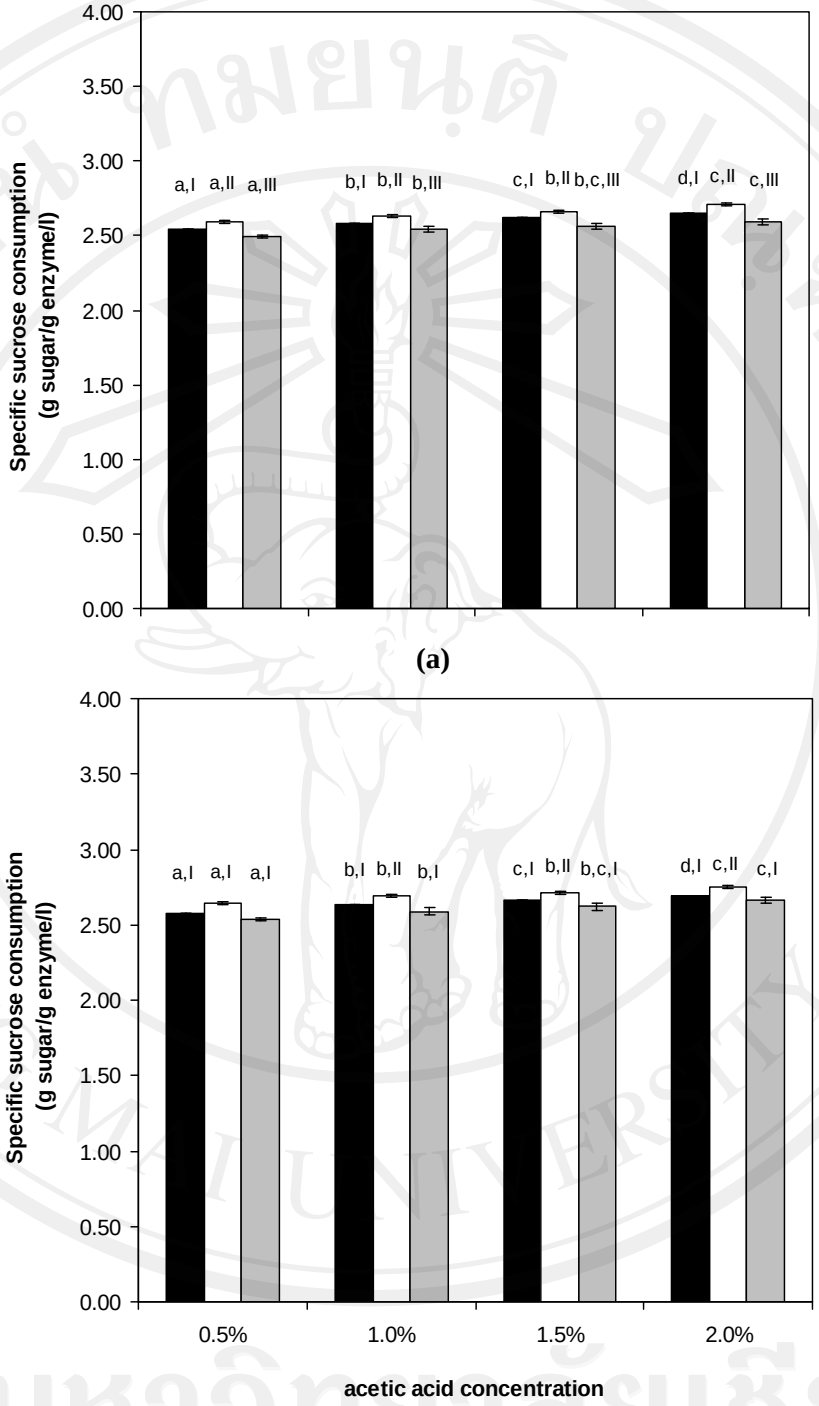
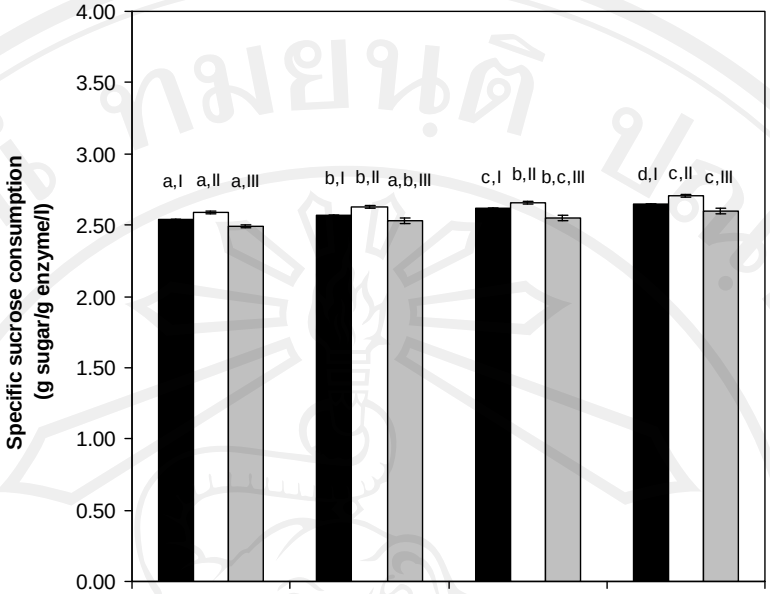
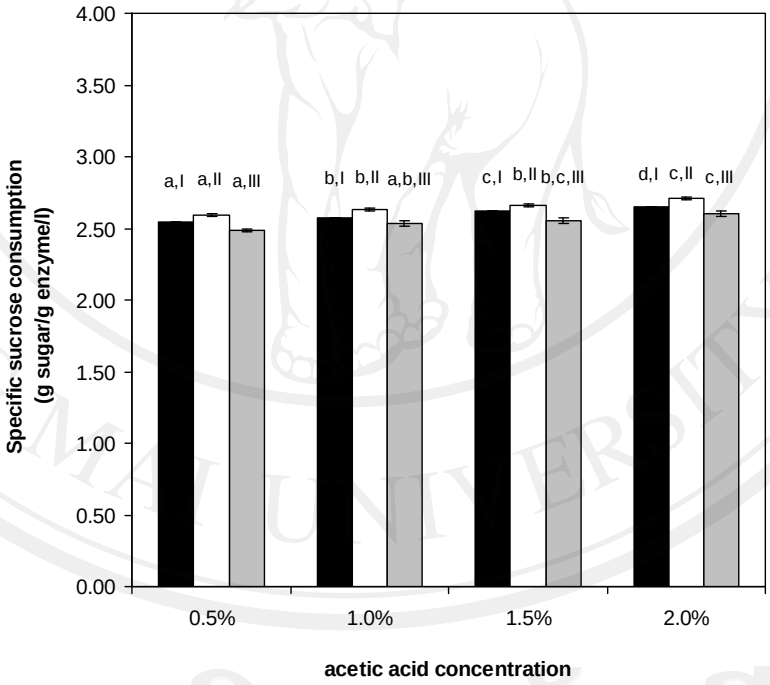


Figure 4.3 Specific sucrose consumption (g/g/l) for the digestion with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 1:10 (a) and 2:10 (b) with the digestive solution consisting of 0.5, 1.0, 1.5 and 2.0% acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:2 (black shaded), 1:1 (unshaded) and 2:1 (grey shaded) ratio. The numbers with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) which were explained in Table 4.6.



(c)



(d)

Figure 4.3 (cont.) Specific sucrose consumption (g/g/l) for the digestion with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 3:10 (c) and 5:10 (d) with the digestive solution consisting of 0.5, 1.0, 1.5 and 2.0% acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:2 (black shaded), 1:1 (unshaded) and 2:1 (grey shaded) ratio. The numbers with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) which were explained in Table 4.6.

4.1.2.2 Specific glucose production

From Fig. 4.4 and Table 4.7, the digestion condition with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 2:10 and 3:10(w/v) using 2%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:1 ratio resulted in the similar maximum increase of specific glucose production level statistically ($p \leq 0.05$) of 4.44 ± 0.03 g/g/l.

This was compared to the digestion condition with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio of 5:10(w/v) using 0.5%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 2:1 ratio which resulted in the minimum increase of specific glucose production level statistically ($p \leq 0.05$) of 4.12 ± 0.01 g/g/l.

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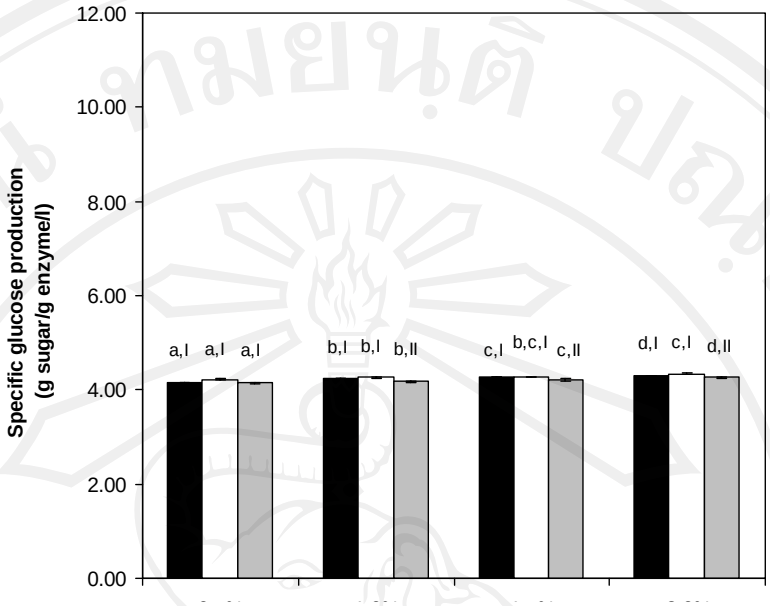
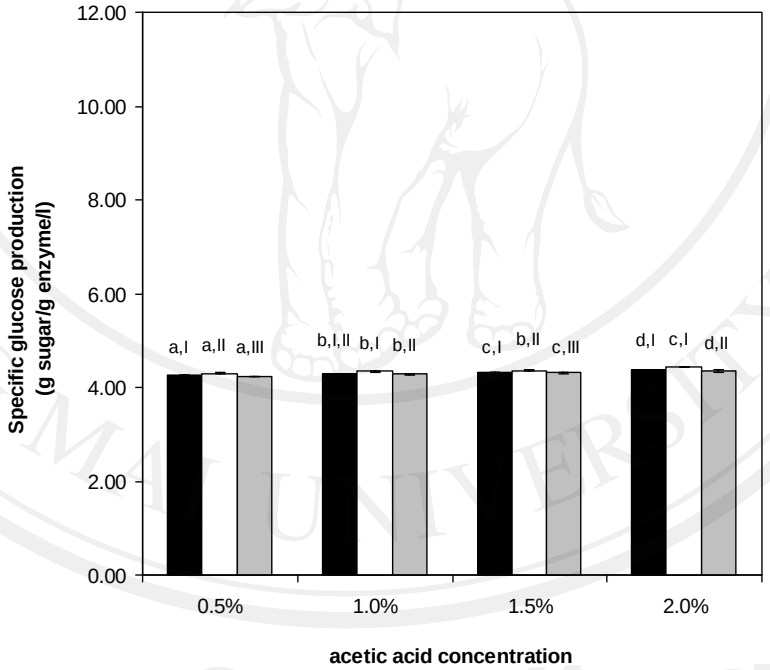
Table 4.7 Specific glucose production (g/g/l) for four dried longan flesh : buffer ratios

Concentration of acetic acid	acetic acid : sodium hydroxide ratio											
	1:2				1:1				2:1			
Dried longan flesh : buffer ratio at 1:10												
0.5%	4.17 ± 0.01	a	I		4.22 ± 0.01	a	I		4.15 ± 0.01	a	I	
1.0%	4.23 ± 0.01	b	I		4.26 ± 0.01	b	I		4.18 ± 0.01	b	II	
1.5%	4.26 ± 0.01	c	I		4.27 ± 0.01	b,c	I		4.21 ± 0.01	c	II	
2.0%	4.31 ± 0.01	d	I		4.34 ± 0.03	c	I		4.26 ± 0.02	d	II	
Dried longan flesh : buffer ratio at 2:10												
0.5%	4.26 ± 0.01	a	I		4.30 ± 0.01	a	II		4.23 ± 0.02	a	III	
1.0%	4.30 ± 0.01	b	I,II		4.34 ± 0.01	b	I		4.28 ± 0.01	b	II	
1.5%	4.33 ± 0.02	c	I		4.36 ± 0.01	b	II		4.31 ± 0.01	c	III	
2.0%	4.38 ± 0.01	d	I		4.44 ± 0.03	c	I		4.35 ± 0.01	d	II	
Dried longan flesh : buffer ratio at 3:10												
0.5%	4.22 ± 0.01	a	I		4.26 ± 0.01	a	II		4.19 ± 0.01	a	III	
1.0%	4.26 ± 0.01	b	I		4.30 ± 0.01	b	II		4.24 ± 0.01	b	I	
1.5%	4.28 ± 0.01	b	I		4.34 ± 0.01	c	II		4.28 ± 0.01	c	I	
2.0%	4.34 ± 0.01	c	I		4.44 ± 0.03	d	II		4.33 ± 0.01	d	I	
Dried longan flesh : buffer ratio at 5:10												
0.5%	4.15 ± 0.02	a	I		4.19 ± 0.01	a	II		4.12 ± 0.01	a	III	
1.0%	4.19 ± 0.02	b	I		4.23 ± 0.01	b	II		4.16 ± 0.02	b	III	
1.5%	4.21 ± 0.02	b	I		4.24 ± 0.01	b	II		4.18 ± 0.02	b	III	
2.0%	4.25 ± 0.03	c	I		4.30 ± 0.01	c	II		4.23 ± 0.02	c	I	

The number with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

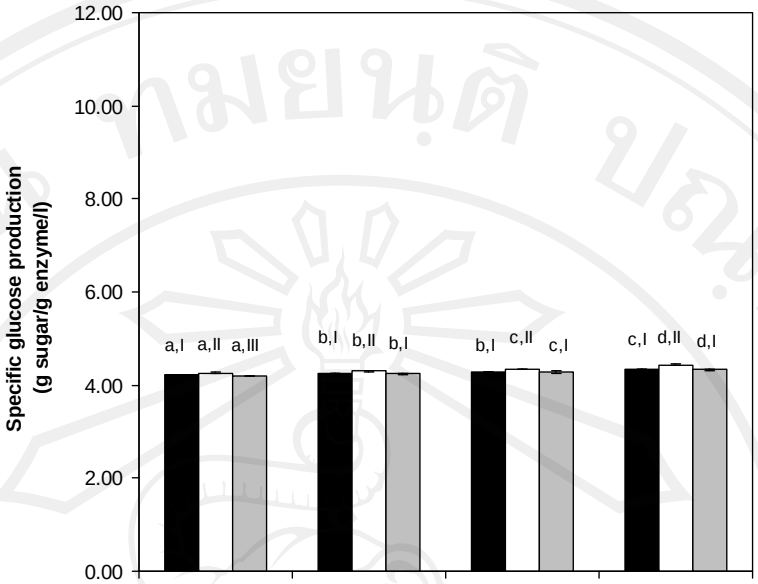


(a)

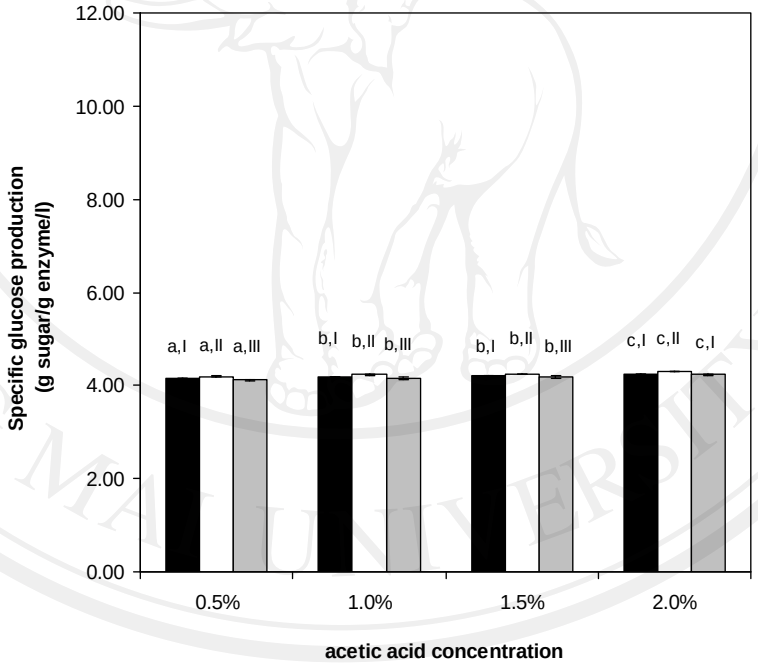


(b)

Figure 4.4 Specific glucose production (g/g/l) for the digestion with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 1:10 (a) and 2:10 (b) with the digestive solution consisting of 0.5, 1.0, 1.5 and 2.0% acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:2 (black shaded), 1:1 (unshaded) and 2:1 (grey shaded) ratio. The numbers with the same Roman numeral (I – II) and alphabet (a – d) indicated no significant difference ($p > 0.05$) which were explained in Table 4.7.



(c)



(d)

Figure 4.4 (cont.) Specific glucose production (g/g/l) for the digestion with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 3:10 (c) and 5:10 (d) with the digestive solution consisting of 0.5, 1.0, 1.5 and 2.0% acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:2 (black shaded), 1:1 (unshaded) and 2:1 (grey shaded) ratio. The numbers with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) which were explained in Table 4.7.

4.1.2.3 Specific fructose production

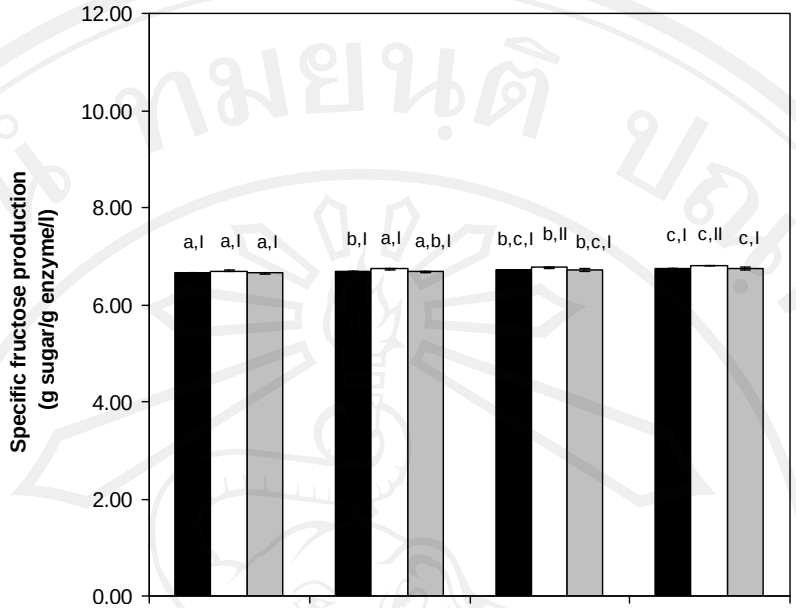
From Fig. 4.5 and Table 4.8, the digestion condition with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 2:10(w/v) using 2%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:1 ratio resulted in the similar maximum increase of specific glucose production level statistically ($p \leq 0.05$) of 6.88 ± 0.02 g/g/l.

This was compared to the digestion condition with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio of 5:10(w/v) using 0.5%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 2:1 ratio which resulted in the minimum increase of specific glucose production level statistically ($p \leq 0.05$) of 6.62 ± 0.02.

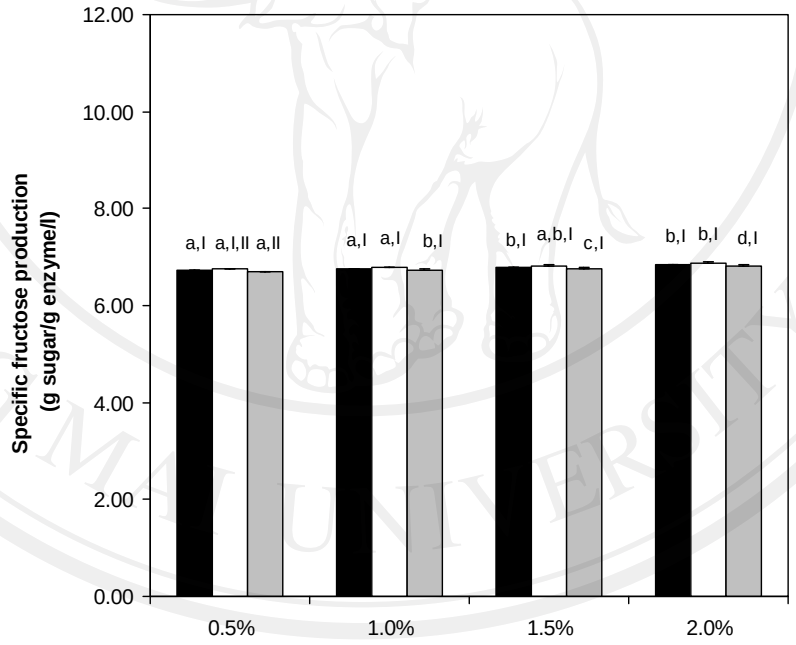
Table 4.8 Specific fructose production (g/g/l) for four dried longan flesh : buffer ratios

Concentration of acetic acid	acetic acid : sodium hydroxide ratio											
	1:2				1:1				2:1			
Dried longan flesh : buffer ratio at 1:10												
0.5%	6.66 ± 0.02	a	I		6.70 ± 0.04	a	I		6.65 ± 0.04	a	I	
1.0%	6.70 ± 0.03	b	I		6.73 ± 0.02	a	I		6.68 ± 0.03	a,b	I	
1.5%	6.72 ± 0.02	b,c	I		6.76 ± 0.03	b	II		6.71 ± 0.03	b,c	I	
2.0%	6.75 ± 0.02	c	I		6.80 ± 0.02	c	II		6.74 ± 0.04	c	I	
Dried longan flesh : buffer ratio at 2:10												
0.5%	6.72 ± 0.02	a	I		6.76 ± 0.04	a	I,II		6.70 ± 0.04	a	II	
1.0%	6.75 ± 0.02	a	I		6.79 ± 0.03	a	I		6.74 ± 0.03	b	I	
1.5%	6.79 ± 0.01	b	I		6.83 ± 0.03	a,b	I		6.77 ± 0.03	c	I	
2.0%	6.84 ± 0.02	b	I		6.88 ± 0.02	b	I		6.83 ± 0.04	d	I	
Dried longan flesh : buffer ratio at 3:10												
0.5%	6.69 ± 0.02	a	I		6.72 ± 0.03	a	II		6.66 ± 0.04	a	III	
1.0%	6.72 ± 0.02	b	I		6.75 ± 0.03	b	II		6.69 ± 0.04	b	I	
1.5%	6.73 ± 0.02	b	I		6.77 ± 0.04	c	II		6.71 ± 0.03	c	I	
2.0%	6.77 ± 0.02	c	I		6.81 ± 0.04	d	II		6.75 ± 0.05	d	I	
Dried longan flesh : buffer ratio at 5:10												
0.5%	6.64 ± 0.01	a	I		6.66 ± 0.05	a	II		6.62 ± 0.02	a	III	
1.0%	6.69 ± 0.01	b	I		6.70 ± 0.04	b	I		6.65 ± 0.03	b	II	
1.5%	6.70 ± 0.01	b	I,II		6.72 ± 0.04	c	I		6.68 ± 0.04	c	II	
2.0%	6.74 ± 0.02	b	I		6.77 ± 0.03	d	II		6.73 ± 0.05	d	I	

The number with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

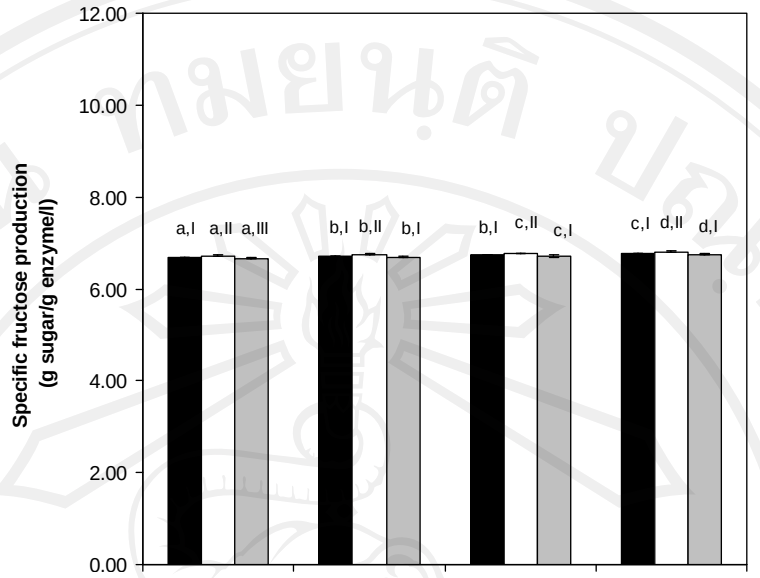


(a)



(b)

Figure 4.5 Specific fructose production (g/g/l) for the digestion with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 1:10 (a) and 2:10 (b) with the digestive solution consisting of 0.5, 1.0, 1.5 and 2.0% acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:2 (black shaded), 1:1 (unshaded) and 2:1 (grey shaded) ratio. The numbers with the same Roman numeral (I – II) and alphabet (a – c) indicated no significant difference ($p > 0.05$) which were explained in Table 4.8.



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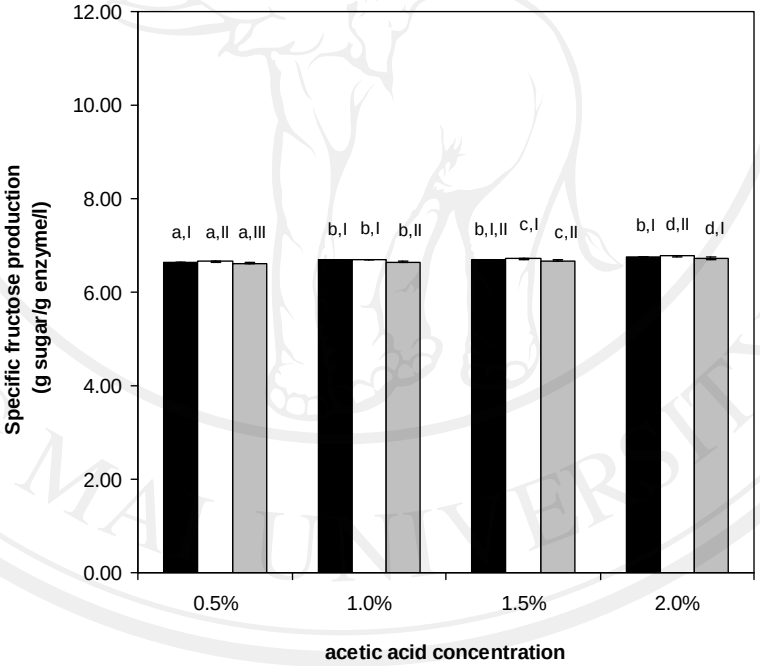


(d)

Figure 4.5 (cont.) Specific fructose production (g/g/l) for the digestion with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 3:10 (c) and 5:10 (d) with the digestive solution consisting of 0.5, 1.0, 1.5 and 2.0% acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:2 (black shaded), 1:1 (unshaded) and 2:1 (grey shaded) ratio. The numbers with the same Roman numeral (I – II) and alphabet (a – d) indicated no significant difference ($p > 0.05$) which were explained in Table 4.8.

4.1.2.4 Specific total sugars production

From Fig. 4.6 and Table 4.9, the digestion condition with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 2:10(w/v) using 2%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:1 ratio resulted in the similar maximum increase of specific glucose production level statistically ($p \leq 0.05$) of 11.33 ± 0.01 g/g/l.

This was compared to the digestion condition with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio of 5:10(w/v) using 0.5%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 2:1 ratio which resulted in the minimum increase of specific glucose production level statistically ($p \leq 0.05$) of 10.74 ± 0.01.

These results were similar to Grohmann *et al*. (2009) who investigated of enzymatic hydrolysis of polysaccharides in orange peel by commercial cellulase and pectinase enzymes. The high yield of saccharification could be maintained even at substrate concentrations as high as 22 – 23%. Thus, the application of dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 2:10(w/v) (20%) should be able to provide the highest values of specific total sugars production as described here.

Similar results were also observed by Aguilar *et al*. (2002) who used acids as catalysts for sugarcane bagasse hydrolysis as these could break down heterocyclic ether bonds between sugar monomers in the polymeric chains forming by hemicellulose and cellulose.

Gupta *et al*. (2009) investigated the hydrolysis and fermentation of bayahonda Blanca (spanish) for the production of ethanol by *S. cerevisiae* and *Pichia stipitis*. The release of sugar increased with the elevation in acid concentration but the digestion efficiency would decline if too high acid concentration was implemented. Consequently, the digestion condition with 2%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution with the ratio of 1:1 resulted in the

maximum increase of specific total sugars production level statistically ($p \leq 0.05$). The proposed methodology in this study was also supported by Yoon *et al*. (2005) who used the similar mixture of digestion solvent to digest a soluble-fibre hydrolysate from carrot pomace.

Table 4.9 Specific total sugars production (g/g/l) for four dried longan flesh : buffer ratios

Concentration of acetic acid	acetic acid : sodium hydroxide ratio		
	1:2	**1:1**	**2:1**
Dried longan flesh : buffer ratio at 1:10			
0.5%	10.83 ± 0.01 a I	10.92 ± 0.02 a II	10.80 ± 0.02 a III
1.0%	10.93 ± 0.01 b I	10.99 ± 0.01 b II	10.86 ± 0.01 b III
1.5%	10.98 ± 0.01 c I	11.03 ± 0.01 c II	10.93 ± 0.01 c III
2.0%	11.06 ± 0.01 d I	11.15 ± 0.01 d II	11.01 ± 0.01 d III
Dried longan flesh : buffer ratio at 2:10			
0.5%	10.98 ± 0.01 a I	11.06 ± 0.02 a II	10.93 ± 0.01 a III
1.0%	11.05 ± 0.01 b I	11.13 ± 0.01 b II	11.01 ± 0.01 b III
1.5%	11.12 ± 0.01 c I	11.19 ± 0.01 c II	11.09 ± 0.01 c III
2.0%	11.22 ± 0.01 d I	11.33 ± 0.01 d II	11.19 ± 0.02 d III
Dried longan flesh : buffer ratio at 3:10			
0.5%	10.91 ± 0.01 a I	10.99 ± 0.01 a II	10.86 ± 0.02 a III
1.0%	10.98 ± 0.01 b I	11.06 ± 0.01 b II	10.93 ± 0.02 b III
1.5%	11.01 ± 0.01 c I	11.11 ± 0.02 c II	10.99 ± 0.02 c III
2.0%	11.12 ± 0.01 d I	11.25 ± 0.01 d II	11.07 ± 0.02 d III
Dried longan flesh : buffer ratio at 5:10			
0.5%	10.80 ± 0.01 a I	10.85 ± 0.02 a II	10.74 ± 0.01 a III
1.0%	10.88 ± 0.01 b I	10.93 ± 0.02 b II	10.81 ± 0.01 b III
1.5%	10.91 ± 0.01 c I	10.96 ± 0.02 c II	10.85 ± 0.01 c III
2.0%	10.99 ± 0.01 d I	11.07 ± 0.01 d II	10.97 ± 0.02 d III

The number with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

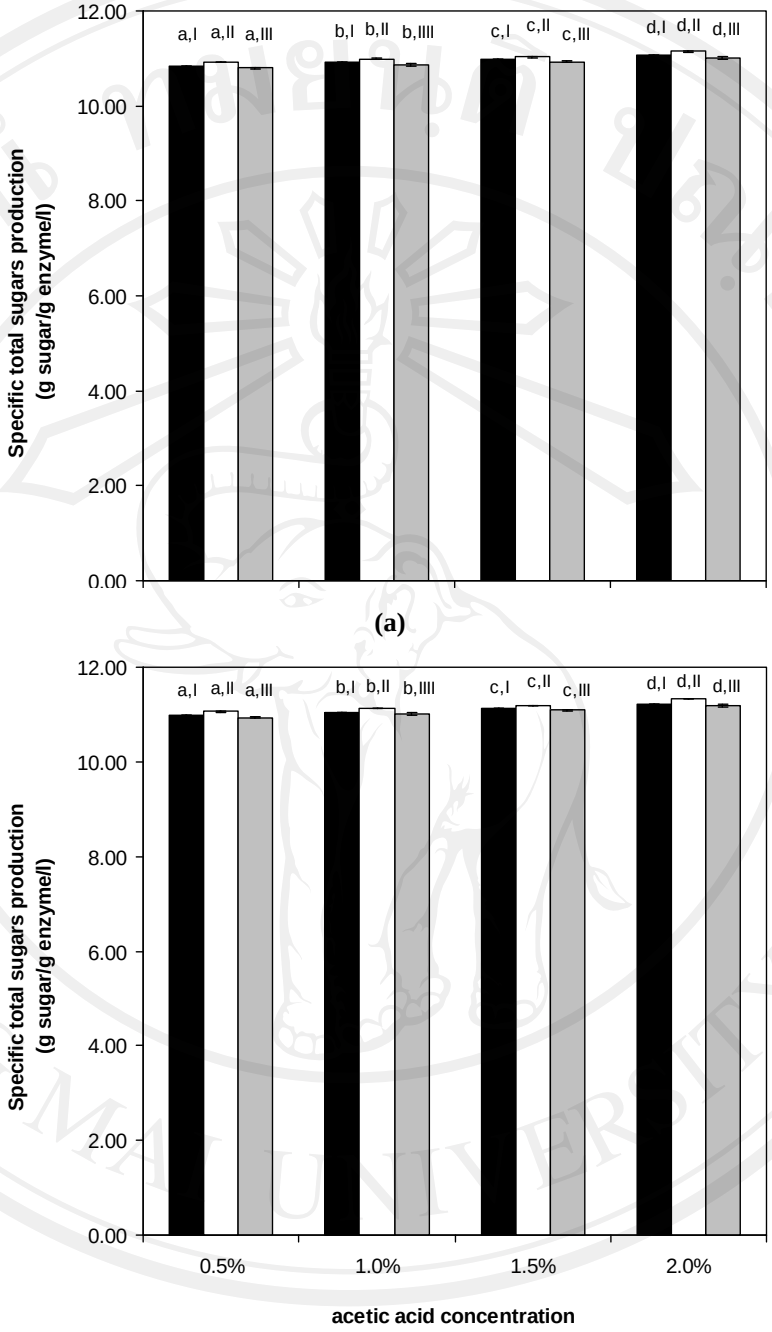
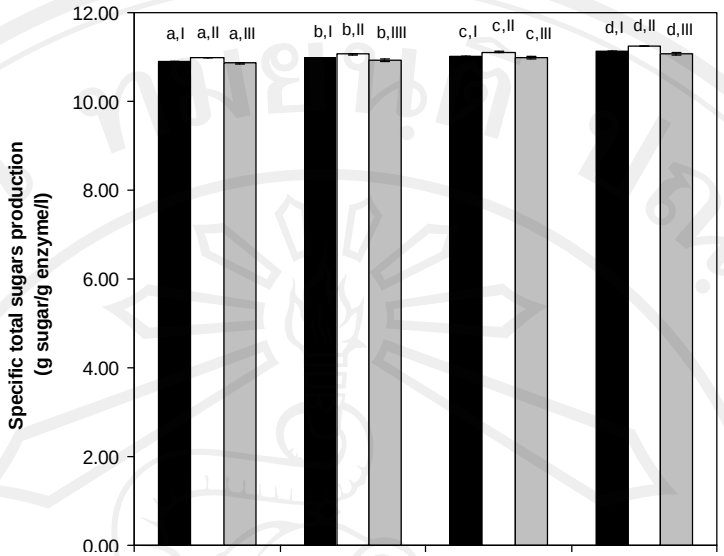


Figure 4.6 Specific total sugars production (g/g/l) for the digestion with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 1:10 (a) and 2:10 (b) with the digestive solution consisting of 0.5, 1.0, 1.5 and 2.0% acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:2 (black shaded), 1:1 (unshaded) and 2:1 (grey shaded) ratio. The numbers with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) which were explained in Table 4.9.



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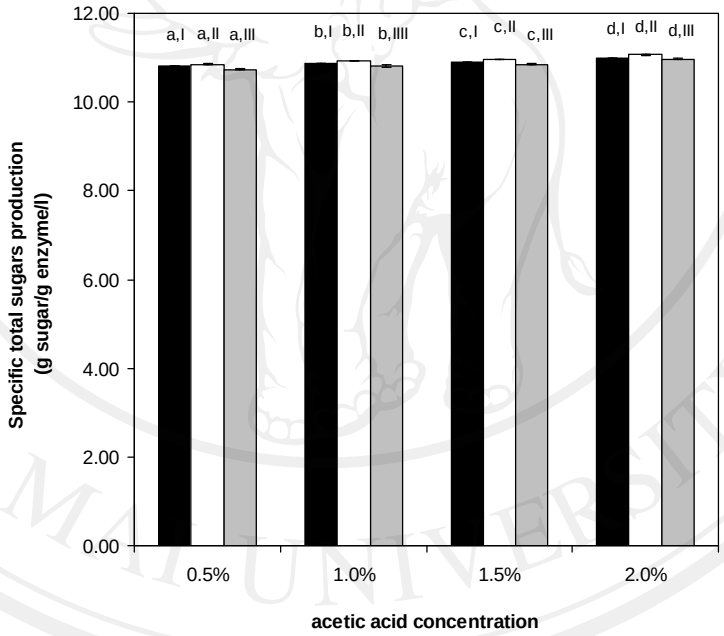


(d)

Figure 4.6 (cont.) Specific total sugars production (g/g/l) for the digestion with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 3:10 (c) and 5:10 (d) with the digestive solution consisting of 0.5, 1.0, 1.5 and 2.0% acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:2 (black shaded), 1:1 (unshaded) and 2:1 (grey shaded) ratio. The numbers with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) which were explained in Table 4.9.

4.1.2.5 Economical specific sugars production

For the commercial viability for specific sugar production level (μg specific sugar production/baht/l), the digestion condition with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 2:10(w/v) using 2%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:1 ratio resulted in the most commercial viable ($p \leq 0.05$) (1,159 ± 47 μg/baht/l) in comparison with the other conditions.

In the other hand, the digestion condition with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio of 5:10(w/v) using 0.5%(w/v) acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 2:1 ratio was the least economical statistically ($p \leq 0.05$) which was 1,098 ± 22 μg/baht/l as shown in Fig. 4.7 and Table 4.10.

Table 4.10 Economical specific sugar production (μg/baht/l) for four dried longan flesh : buffer ratios

Concentration of acetic acid	acetic acid : sodium hydroxide ratio														
	1:2					1:1					2:1				
Dried longan flesh : buffer ratio at 1:10															
0.5%	1,108.28	±	0.14	a	I	1,116.66	±	0.52	a	II	1,104.92	±	0.34	a	III
1.0%	1,118.31	±	0.25	b	I	1,123.86	±	0.05	b	II	1,110.56	±	0.60	b	III
1.5%	1,123.56	±	0.05	c	I	1,128.18	±	0.24	c	II	1,117.67	±	0.80	c	III
2.0%	1,131.55	±	0.14	d	I	1,140.20	±	1.22	d	II	1,126.34	±	0.82	d	III
Dried longan flesh : buffer ratio at 2:10															
0.5%	1,123.29	±	0.30	a	I	1,131.48	±	1.71	a	II	1,118.43	±	0.00	a	III
1.0%	1,129.92	±	0.10	b	I	1,138.19	±	0.76	b	II	1,126.52	±	0.03	b	III
1.5%	1,137.21	±	0.32	c	I	1,144.53	±	1.13	c	II	1,134.06	±	0.18	c	III
2.0%	1,147.87	±	0.58	d	I	1,158.68	±	0.47	d	II	1,144.22	±	0.04	d	III
Dried longan flesh : buffer ratio at 3:10															
0.5%	1,115.74	±	0.28	a	I	1,124.00	±	0.04	a	II	1,110.58	±	0.41	a	III
1.0%	1,123.37	±	0.12	b	I	1,131.06	±	0.59	b	II	1,118.18	±	0.14	b	III
1.5%	1,126.22	±	0.37	c	I	1,136.65	±	0.46	c	II	1,124.47	±	0.46	c	III
2.0%	1,137.21	±	0.46	d	I	1,151.15	±	1.57	d	II	1,132.86	±	0.16	d	III
Dried longan flesh : buffer ratio at 5:10															
0.5%	1,104.39	±	0.31	a	I	1,109.47	±	0.09	a	II	1,098.20	±	0.06	a	III
1.0%	1,112.99	±	0.15	b	I	1,118.43	±	0.57	b	II	1,105.83	±	0.02	b	III
1.5%	1,116.10	±	0.35	c	I	1,120.71	±	0.49	c	II	1,110.24	±	0.04	c	III
2.0%	1,123.98	±	0.49	d	I	1,132.12	±	1.48	d	II	1,121.55	±	0.02	d	III

The number with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

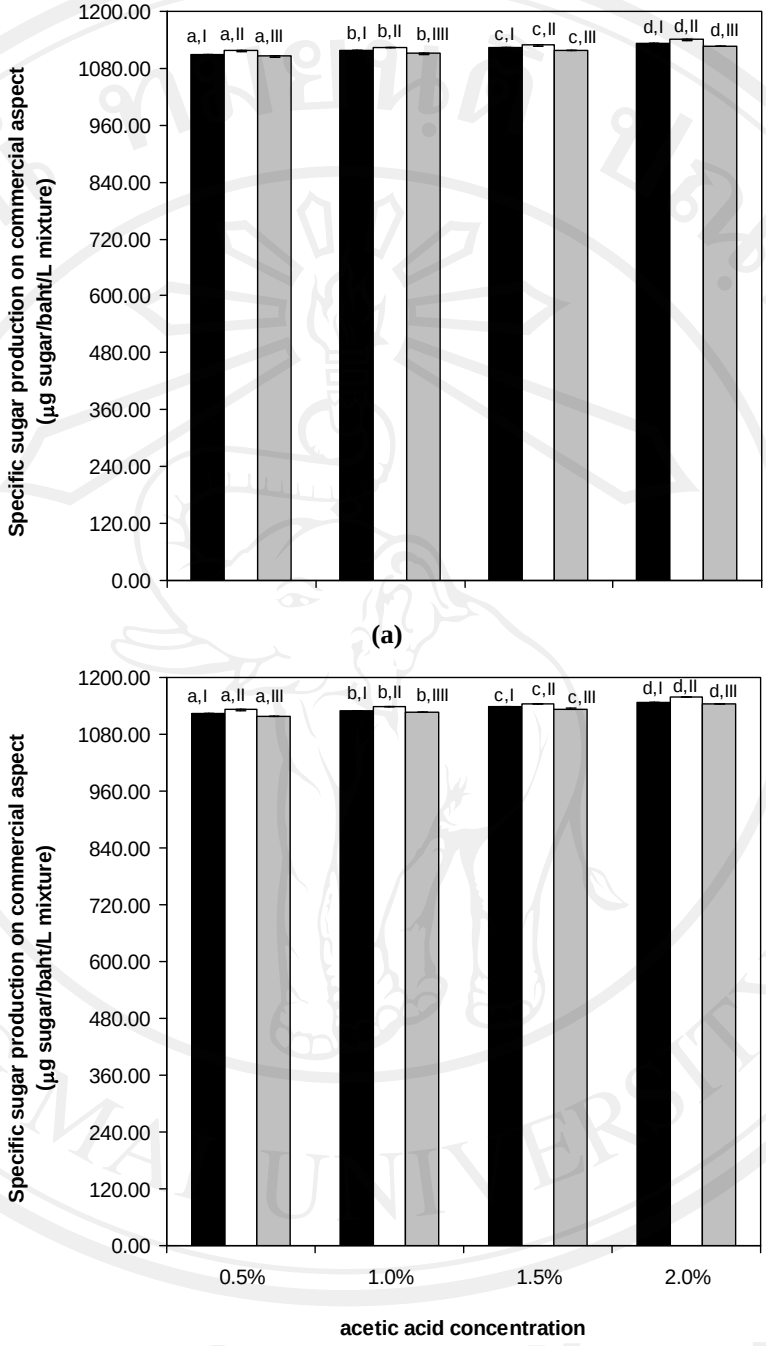
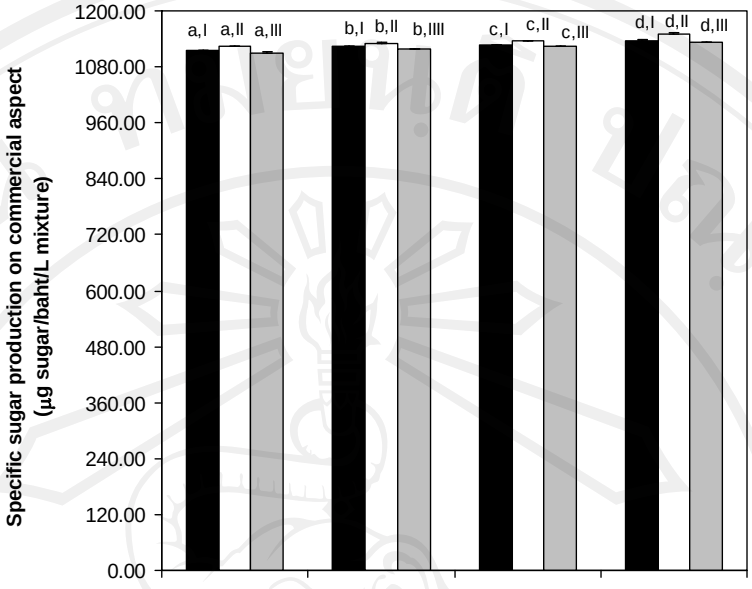


Figure 4.7 Economical specific sugar production (µg/baht/l) for the digestion with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 1:10 (a) and 2:10 (b) with the digestive solution consisting of 0.5, 1.0, 1.5 and 2.0% acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:2 (black shaded), 1:1 (unshaded) and 2:1 (grey shaded) ratio. The numbers with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) which were explained in Table 4.10.



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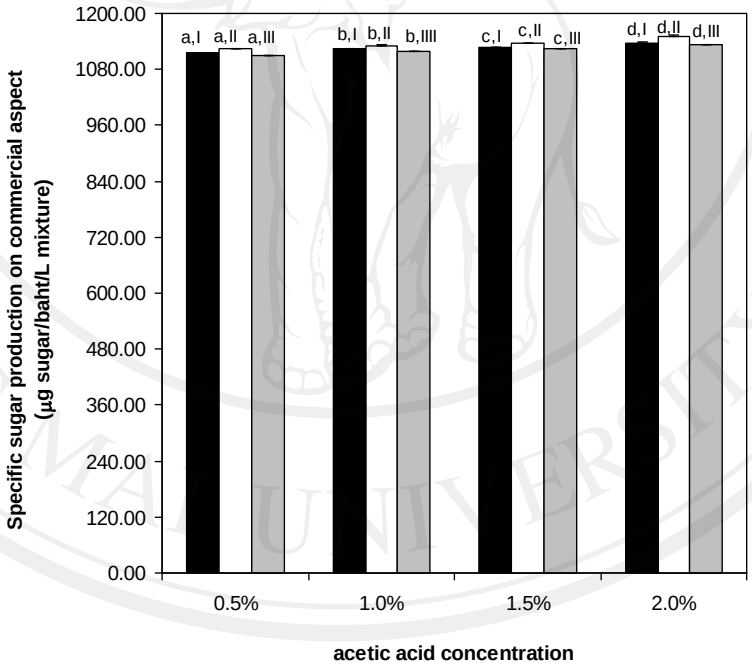


(d)

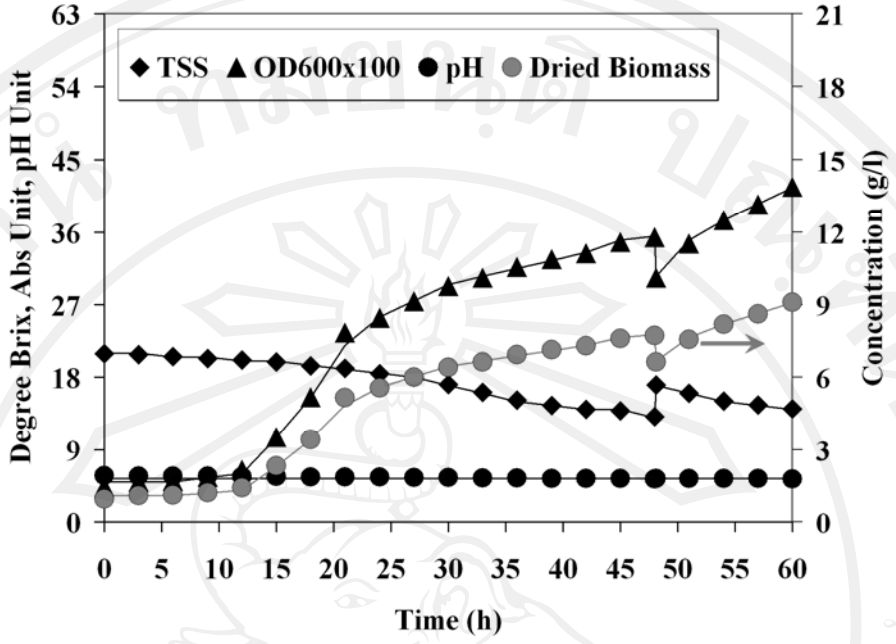
Figure 4.7 (cont.) Economical specific sugar production (µg/baht/l) for the digestion with the dried longan flesh powder to acetic acid solution/sodium hydroxide solution ratio at 3:10 (c) and 5:10 (d) with the digestive solution consisting of 0.5, 1.0, 1.5 and 2.0% acetic acid solution and 0.1%(w/v) sodium hydroxide solution at 1:2 (black shaded), 1:1 (unshaded) and 2:1 (grey shaded) ratio. The numbers with the same Roman numeral (I – III) and alphabet (a – d) indicated no significant difference ($p > 0.05$) which were explained in Table 4.10.

4.2 Determination of growth kinetics

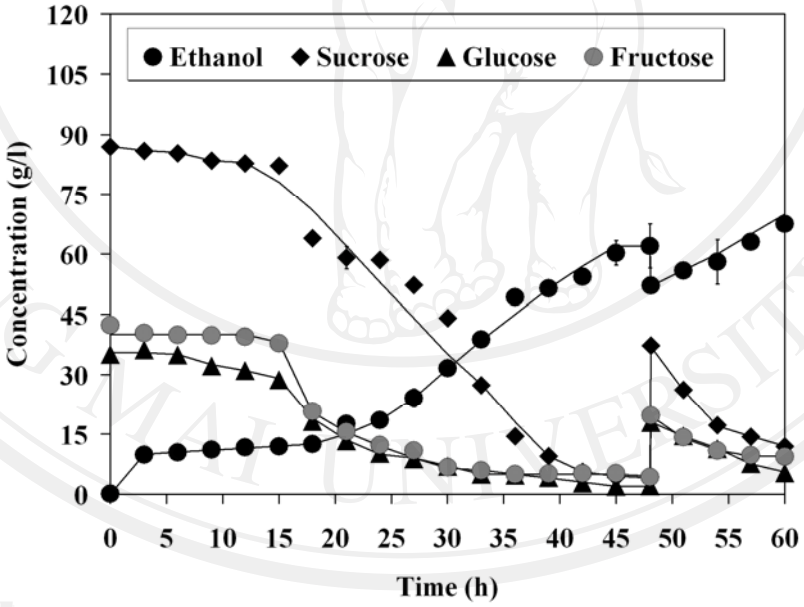
This study investigated the fed batch cultivation of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 by starting with 5,000 ml batch cultivation for 48 h using dried longan extract (DLE) medium as a carbon source. The cultivation culture was then divided into two portions of 1,000 ml. The addition of 500 ml DLE medium was then followed to the first portion while the equivalent volume of digested dried longan flesh hydrolysate (DDLFH) medium (from section 4.1) was carried out to the latter. The cultivation was then allowed to proceed for the next 12 h at 30^{o}C. The detailed kinetic profiles of the microbial growth with addition of DLE and DDLFH medium are shown in Fig. 4.8 – 4.13, respectively. Each figure is divided into two parts, namely; part (a) describes the kinetic profiles of TSS, pH level, OD600 and dried biomass concentration which could be related to OD600 by third order polynomial equations as previously mentioned in previous section; part (b) portrays the kinetic profiles of substrates such as sucrose, glucose and fructose concentrations, as well as the product or ethanol concentration. These profiles were further analyzed to obtain ethanol yield (Yp/s) which described the ratio of the produced ethanol concentration over the consumed sucrose, glucose and fructose concentrations as shown in Table 4.20 – 4.22.

The detailed analysis of each cultivation profile with hypothesis testing for DLE and DDLFH feeding is tabulated in Table 4.11 – 4.19. The first nine tables (Table 4.11 – 4.19) portray the statistical comparison of TSS, pH level, OD600 and dried biomass concentration data extracted from Fig. 4.8(a) – 4.13(a) which include the analyses of differences between the final and initial levels, average and maximum rates. Similar analyses and comparisons were also carried out for sugars and ethanol concentrations. Table 4.11 – 4.19 presents these information in terms of differences, average as well as maximum rates.

The kinetic profiles describing the microbial growth of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 had similar trends as shown in Fig. 4.8(a) – 4.13(a). In term of pH level and TSS decreasing, there was negligible change with a slight continuous decreasing trend with cultivation period. The profiles of dried biomass concentration and OD600 for the cultivation of all strains were similar in shape and trend.

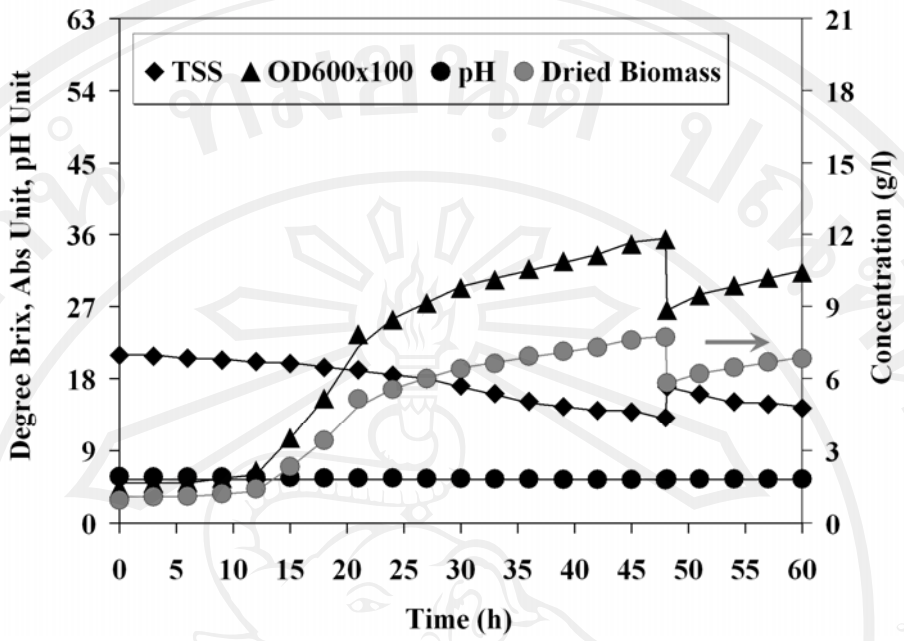


(a)

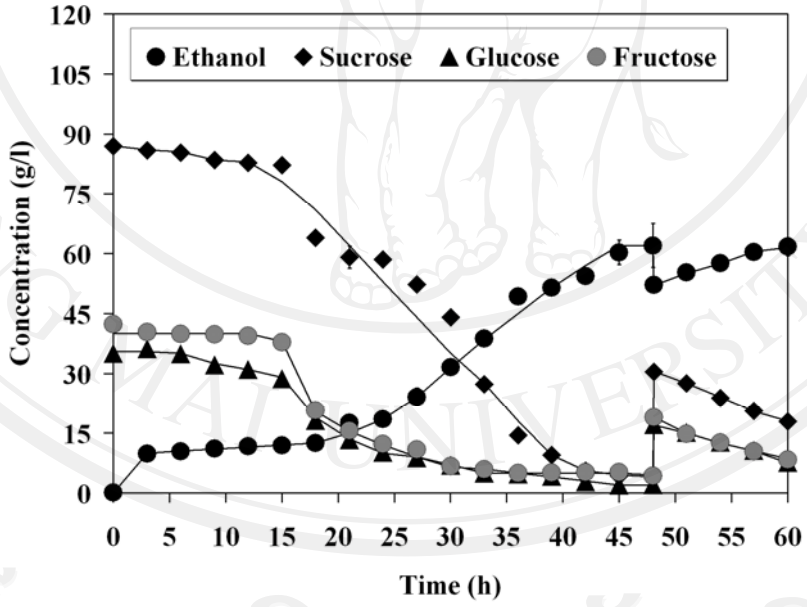


(b)

Figure 4.8 Growth kinetics of *S. cerevisiae* TISTR 5606 in fed batch system which utilized DLE media in batch stage for 48 h before feeding of DLE media for the next 24 h during 60 h cultivation period in a static condition at 30°C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g/l); (b) concentration profiles of ethanol, sucrose, glucose and fructose concentrations.



(a)



(b)

Figure 4.9 Growth kinetics of *S. cerevisiae* TISTR 5606 in fed batch system which utilized DLE media in batch stage for 48 h before feeding of DDLFH media for the next 24 h during 60 h cultivation period in a static condition at 30°C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g/l); (b) concentration profiles of ethanol, sucrose, glucose and fructose concentrations.

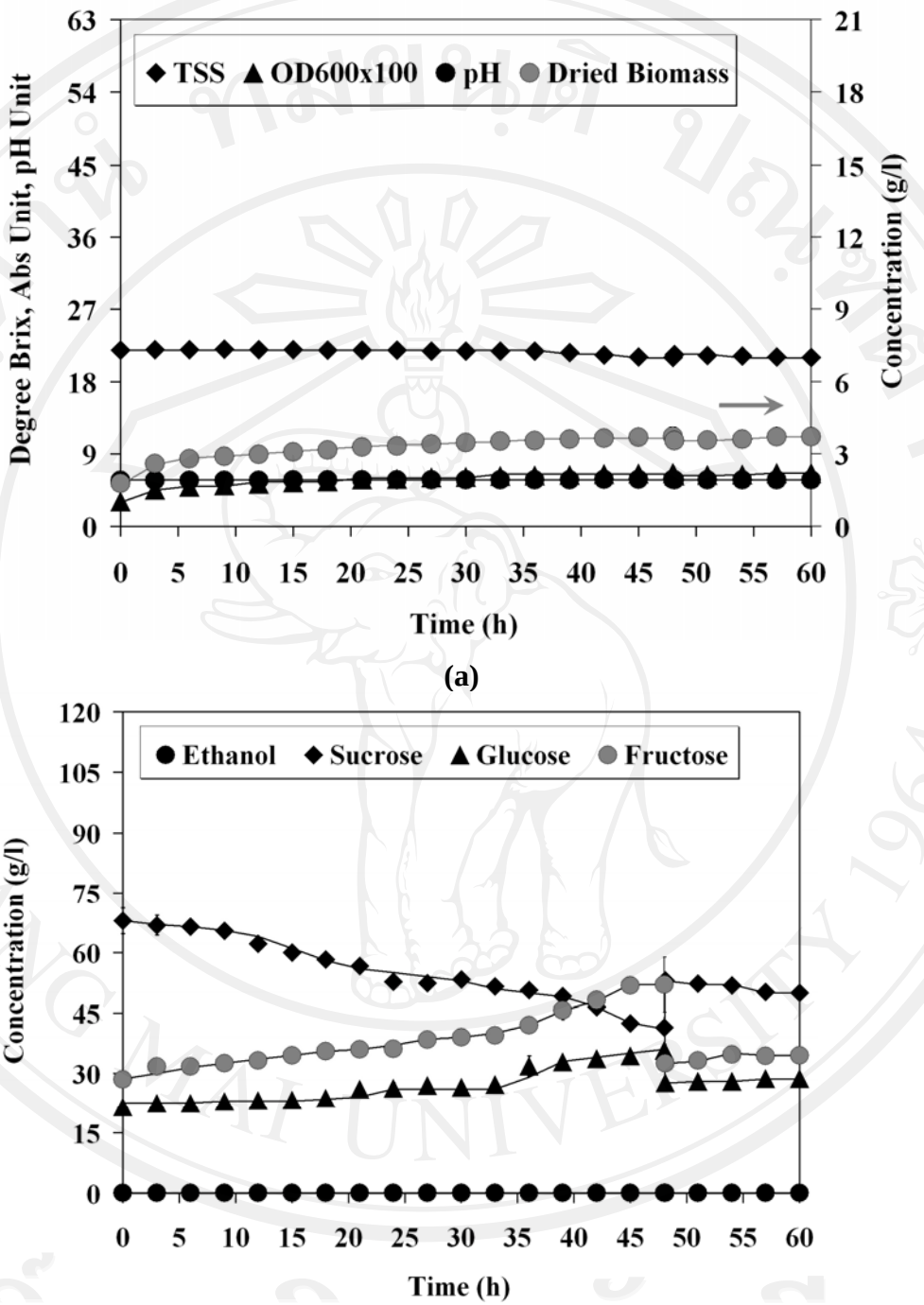
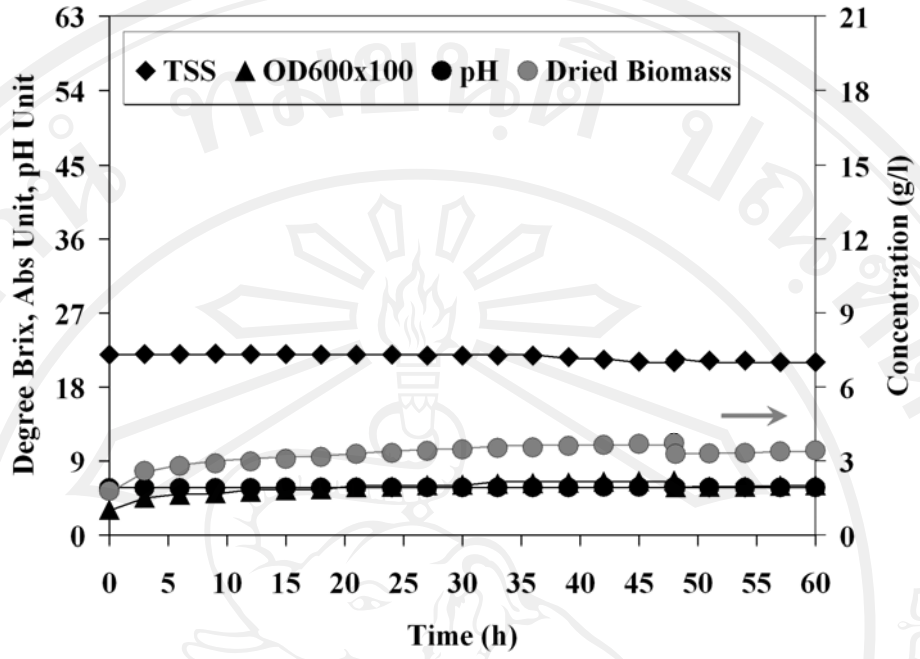
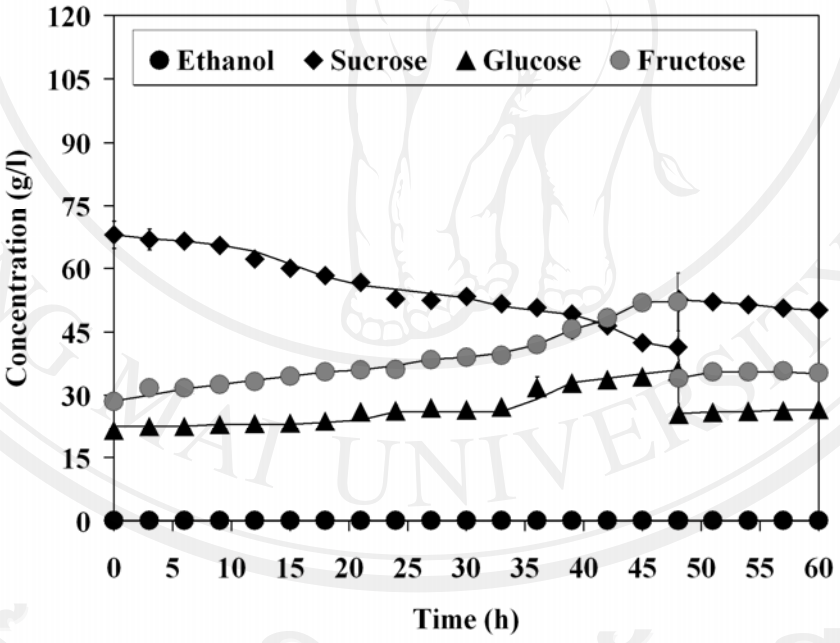


Figure 4.10 Growth kinetics of *C. utilis* UNSW 709400 in fed batch system which utilized DLE media in batch stage for 48 h before feeding of DLE media for the next 24 h during 60 h cultivation period in a static condition at 30°C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g/l); (b) concentration profiles of ethanol, sucrose, glucose and fructose concentrations.

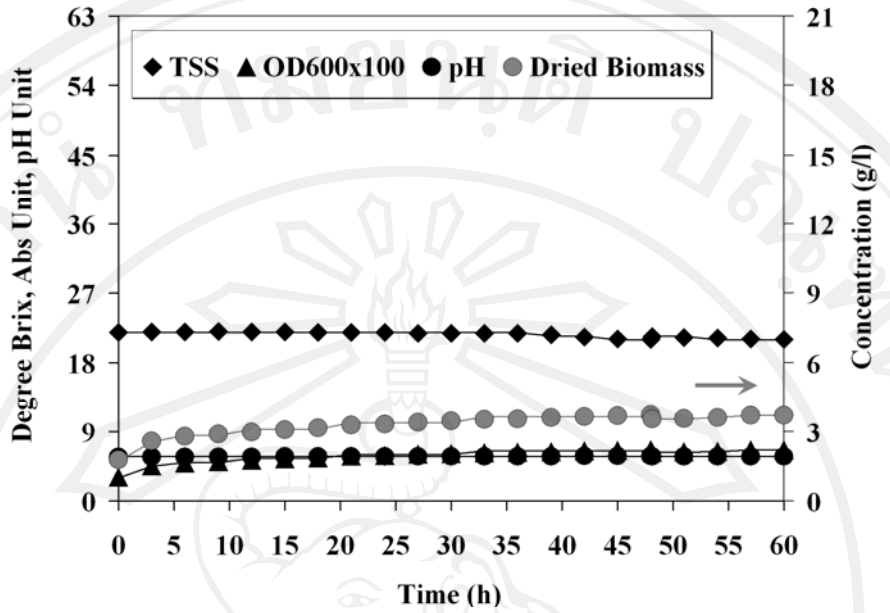


(a)

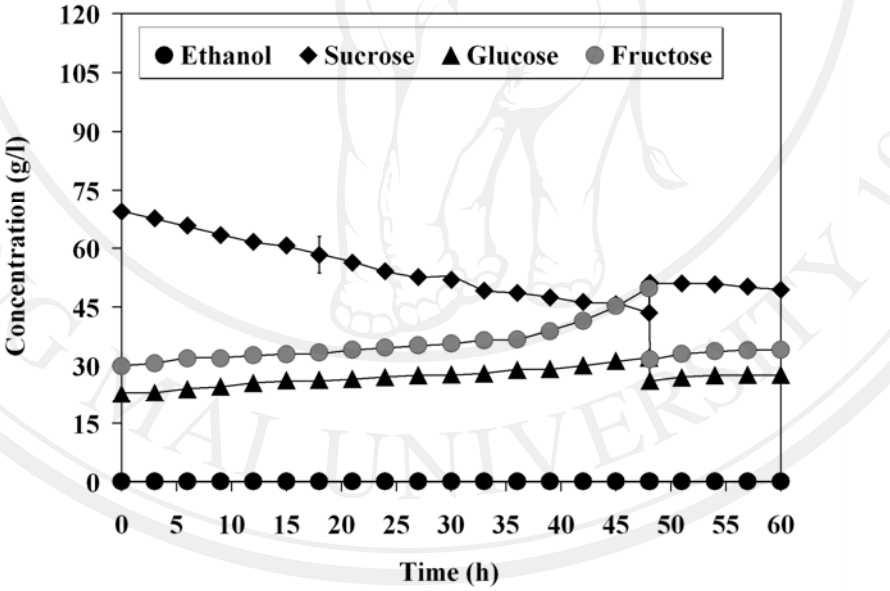


(b)

Figure 4.11 Growth kinetics of *C. utilis* UNSW 709400 in fed batch system which utilized DLE media in batch stage for 48 h before feeding of DDLFH media for the next 24 h during 60 h cultivation period in a static condition at 30°C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g/l); (b) concentration profiles of ethanol, sucrose, glucose and fructose concentrations.



(a)



(b)

Figure 4.12 Growth kinetics of *C. utilis* UNSW 709700 in fed batch system which utilized DLE media in batch stage for 48 h before feeding of DLE media for the next 24 h during 60 h cultivation period in a static condition at 30°C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g/l); (b) concentration profiles of ethanol, sucrose, glucose and fructose concentrations.

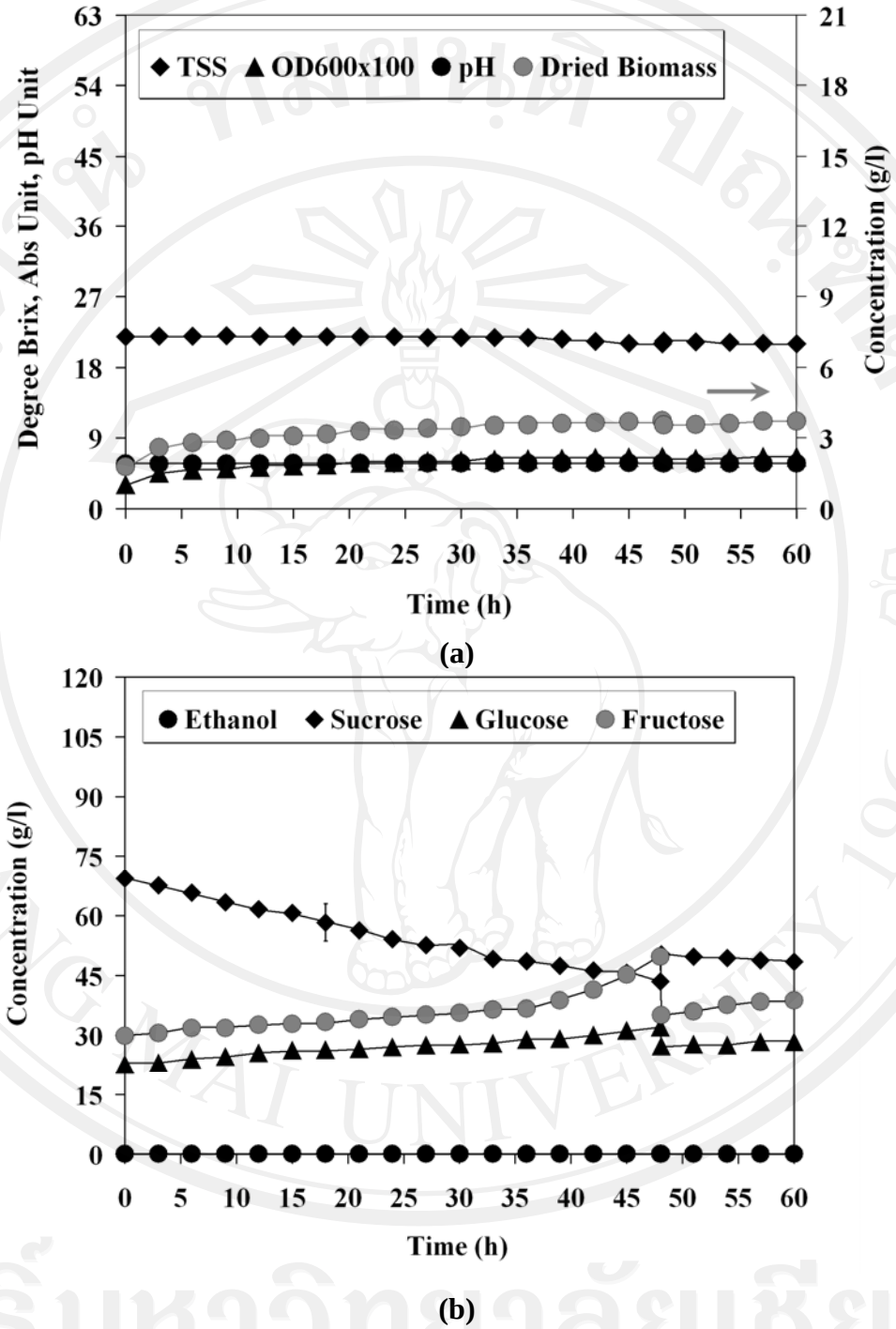


Figure 4.13 Growth kinetics of *C. utilis* UNSW 709700 in fed batch system which utilized DLE media in batch stage for 48 h before feeding of DDLFH media for the next 24 h during 60 h cultivation period in a static condition at 30°C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g/l); (b) concentration profiles of ethanol, sucrose, glucose and fructose concentrations.

Table 4.11 The differences in TSS, OD600 and dried biomass (X) concentration (g/l) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 30°C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
TSS decreasing level	7.80 ± 0.03	I	2.94 ± 0.09	II	2.66 ± 0.10	III
OD600 increasing level	32.10 ± 0.01	I	11.24 ± 0.01	II	4.68 ± 0.01	III
X production level	7.04 ± 0.07	I	2.46 ± 0.07	II	1.02 ± 0.07	III

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.12 The differences in TSS, OD600 and dried biomass (X) concentration (g/l) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709400 at 30°C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
TSS decreasing level	0.90 ± 0.08	I	0.40 ± 0.08	II	0.34 ± 0.09	II
OD600 increasing level	3.52 ± 0.02	I	0.32 ± 0.02	II	0.24 ± 0.01	III
X production level	1.95 ± 0.08	I	0.18 ± 0.07	II	0.13 ± 0.08	II

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.13 The differences in TSS, OD600 and dried biomass (X) concentration (g/l) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709700 at 30°C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
TSS decreasing level	0.16 ± 0.08	I	0.10 ± 0.06	I	0.06 ± 0.07	I
OD600 increasing level	3.53 ± 0.01	I	0.30 ± 0.01	II	0.21 ± 0.02	III
X production level	1.74 ± 0.07	I	0.12 ± 0.07	I, II	0.09 ± 0.07	II

The numbers with the same Roman numeral (I - III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.14 The average pH level, average TSS decreasing rate (^{o}Brix/h), average OD600 increasing rate (ODU/h), average dried biomass (X) concentration increasing rate (g/l/h), average specific growth rate (per h) and average doubling time (h) during 60 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 30^{o}C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
pH level	5.391 ± 0.018	I	5.387 ± 0.009	I	5.422 ± 0.013	I
TSS decreasing rate	0.167 ± 0.030	I	0.253 ± 0.050	I	0.211 ± 0.081	I
OD600 increasing rate	0.635 ± 0.162	I	0.930 ± 0.211	II	0.422 ± 0.089	III
X increasing rate	0.139 ± 0.035	I	0.204 ± 0.046	II	0.092 ± 0.019	III
Specific growth rate	0.040 ± 0.013	I	0.026 ± 0.007	I, II	0.014 ± 0.003	II
Doubling time	17.1 ± 5.7	I	26.6 ± 7.3	I, II	47.9 ± 11.4	II

The numbers with the same Roman numeral (I - III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.15 The average pH level, average TSS decreasing rate (^{o}Brix/h), average OD600 increasing rate (ODU/h), average dried biomass (X) concentration increasing rate (g/l/h), average specific growth rate (per h) and average doubling time (h) during 60 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709400 at 30^{o}C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
pH level	5.805 ± 0.005	I	5.777 ± 0.009	I	5.780 ± 0.008	I
TSS decreasing rate	0.021 ± 0.014	I	0.042 ± 0.042	I	0.043 ± 0.043	II
OD600 increasing rate	0.073 ± 0.034	I	0.025 ± 0.016	I	0.017 ± 0.010	I
X increasing rate	0.040 ± 0.019	I	0.014 ± 0.009	II	0.009 ± 0.005	II
Specific growth rate	0.016 ± 0.008	I	0.008 ± 0.002	I, II	0.003 ± 0.002	II
Doubling time	44.19 ± 23.75	I	184.99 ± 117.40	I	248.96 ± 143.83	I

The numbers with the same Roman numeral (I - II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.16 The average pH level, average TSS decreasing rate (^{o}Brix/h), average OD600 increasing rate (ODU/h), average dried biomass (X) concentration increasing rate (g/l/h), average specific growth rate (per h) and average doubling time (h) during 60 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709700 at 30^{o}C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
pH level	5.793 ± 0.003	I	5.765 ± 0.009	I	5.769 ± 0.008	I
TSS decreasing rate	0.002 ± 0.002	I	0.008 ± 0.008	I	0.000 ± 0.000	I
OD600 increasing rate	0.073 ± 0.021	I	0.025 ± 0.008	I	0.025 ± 0.008	I
X increasing rate	0.035 ± 0.011	I	0.010 ± 0.003	II	0.003 ± 0.001	II
Specific growth rate	0.012 ± 0.004	I	0.011 ± 0.004	I, II	0.003 ± 0.001	II
Doubling time	59.55 ± 18.70	I	254.66 ± 85.08	I	226.19 ± 75.58	I

The numbers with the same Roman numeral (I - II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.17 The maximum TSS decreasing rate (^{o}Brix/h), maximum OD600 increasing rate (ODU/h), maximum dried biomass (X) concentration increasing rate (g/l/h), maximum specific growth rate (per h) and minimum doubling time (h) during 60 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 30^{o}C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
TSS decreasing rate	0.300 ± 0.033	I	0.339 ± 0.006	I	0.339 ± 0.006	I
OD600 increasing rate	1.433 ± 0.251	I	1.193 ± 0.359	I, II	0.511 ± 0.178	II
X increasing rate	0.314 ± 0.055	I	0.260 ± 0.078	I, II	0.111 ± 0.039	II
Specific growth rate	0.102 ± 0.028	I	0.035 ± 0.012	II	0.018 ± 0.007	II
Doubling time	6.83 ± 1.85	I	19.7 ± 6.8	II	38.3 ± 14.2	III

The numbers with the same Roman numeral (I - III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.18 The maximum TSS decreasing rate (^{o}Brix/h), maximum OD600 increasing rate (ODU/h), maximum dried biomass (X) concentration increasing rate (g/l/h), maximum specific growth rate (per h) and minimum doubling time (h) during 60 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709400 at 30^{o}C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
TSS decreasing rate	0.067 ± 0.041	I	0.083 ± 0.083	I	0.086 ± 0.086	I
OD600 increasing rate	0.233 ± 0.067	I	0.050 ± 0.017	II	0.034 ± 0.001	II
X increasing rate	0.129 ± 0.038	I	0.027 ± 0.009	II	0.019 ± 0.000	II
Specific growth rate	0.050 ± 0.020	I	0.007 ± 0.002	II	0.006 ± 0.000	II
Doubling time	13.81 ± 5.64	I	92.50 ± 29.85	II	124.48 ± 3.20	III

The numbers with the same Roman numeral (I - III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.19 The maximum TSS decreasing rate (^{o}Brix/h), maximum OD600 increasing rate (ODU/h), maximum dried biomass (X) concentration increasing rate (g/l/h), maximum specific growth rate (per h) and minimum doubling time (h) during 60 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709700 at 30^{o}C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
TSS decreasing rate	0.007 ± 0.007	I	0.017 ± 0.017	I	0.000 ± 0.000	I
OD600 increasing rate	0.167 ± 0.000	I	0.034 ± 0.001	II	0.034 ± 0.001	II
X increasing rate	0.086 ± 0.004	I	0.014 ± 0.000	II	0.015 ± 0.000	II
Specific growth rate	0.030 ± 0.003	I	0.004 ± 0.000	II	0.004 ± 0.000	II
Doubling time	23.13 ± 2.55	I	187.80 ± 5.34	II	166.66 ± 4.89	III

The numbers with the same Roman numeral (I - III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

4.2.1 Total soluble solid (TSS)

Total soluble solids (TSS) in fruit juices constituted mainly of sugars, while acid, minerals, nitrogenous compound, pectins and minor soluble substances contributes the remaining composition of TSS (Verma and Joshi, 2000; Ladaniya, 2008).

As indicated in Fig. 4.8(a) – 4.9(a), the maximum change in TSS level for *S. cerevisiae* TISTR 5606 prior to feeding was 7.80 ± 0.03°Brix. This was significantly different ($p \leq 0.05$) from the addition of DLE media during 48 – 60 h (DLE(48 - 60)) and DDLFH media during the same period (DDLFH(48 – 60)) as indicated in Table 4.11. TSS change of DLE(48 – 60) was 2.94 ± 0.09°Brix which was also significantly different ($p \leq 0.05$) from 2.66 ± 0.10°Brix for DDLFH (48 – 60), which was similar in trend with *C. utilis* UNSW 709400 and 709700 as showed in Fig. 4.10(a) – 4.13(a) and Table 4.12 – 4.13. The maximum level of TSS change ($p \leq 0.05$) for *C. utilis* UNSW 709400 and 709700 prior to feeding were of 0.90 ± 0.08 and 0.16 ± 0.08°Brix, respectively. TSS change of DLE(48 – 60) were 0.40 ± 0.08 and 0.10 ± 0.06°Brix, respectively which were also significantly different ($p \leq 0.05$) from 0.34 ± 0.09 and 0.06 ± 0.07°Brix, respectively for DDLFH (48 – 60).

The average rate of TSS decreasing for *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 prior to feeding were 0.167 ± 0.030, 0.021 ± 0.014 and 0.002 ± 0.002°Brix/h, respectively. These were not significantly different ($p > 0.05$) from DLE(48 - 60) (0.253 ± 0.050, 0.042 ± 0.042 and 0.008 ± 0.008°Brix/h, respectively) and DDLFH (48 – 60) (0.211 ± 0.081, 0.043 ± 0.043 and 0.000 ± 0.000°Brix/h, respectively) as indicated in Table 4.14 – 4.16

The maximum rate of TSS decreasing for *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 prior to feeding were 0.300 ± 0.033, 0.067 ± 0.041 and 0.007 ± 0.007°Brix/h, respectively. These were not significantly different ($p > 0.05$) from DLE(48 - 60) (0.339 ± 0.006, 0.083 ± 0.083 and 0.017 ± 0.017°Brix/h, respectively) and DDLFH (48 – 60) (0.339 ± 0.006, 0.086 ± 0.086 and 0.000 ± 0.000°Brix/h, respectively) as indicated in Table 4.17 – 4.19.

The depletion of TSS represented the bioconversion extent in soluble solids, mainly sugars, to other metabolite compounds. Furthermore, Li *et al*. (2009) mentioned that dried longan extract did not only contain sucrose but other compounds such as impurities, fiber, organic acids, phenolic compound, amino acids and pectin which could affect the translucency of the extract and contributed to the level of total soluble solids.

4.2.2 OD600 (Optical density at 600 nm)

The method OD600 is used to determinate the absorbance at 600 nm. The optical density of a suspension of cells is directly to cell mass or cell number after construction and calibration of a standard curve (Todar, 2008).

From Fig. 4.8(a) and 4.9(a), the maximum change in OD600 level for *S. cerevisiae* TISTR 5606 of 32.1 ± 0.01 ODU prior to feeding as indicated in Table 4.11 was significantly higher ($p \leq 0.05$) than DLE(48 – 60) of 11.2 ± 0.01 ODU. These were significantly different ($p \leq 0.05$) from DDLFH(48 – 60) with the corresponding values of 4.68 ± 0.01 ODU. The maximum change in OD600 level for *C. utilis* UNSW 709400 of 3.52 ± 0.02 ODU prior to feeding (Fig. 4.10(a) – 4.11(a) and Table 4.12) was significantly higher ($p \leq 0.05$) than DLE(48 – 60) of 0.32 ± 0.02 ODU. This was significantly different ($p \leq 0.05$) from DDLFH(48 – 60) with the corresponding values of 0.24 ± 0.01 ODU. The maximum change in OD600 level for *C. utilis* UNSW 709700 of 3.53 ± 0.01 ODU prior to feeding (Fig. 4.12(a) – 4.13(a) and Table 4.13) was significantly higher ($p \leq 0.05$) than DLE(48 – 60) of 0.30 ± 0.01 ODU. These were significantly higher ($p \leq 0.05$) than DDLFH(48 – 60) of 0.21 ± 0.02 ODU.

The comparison of OD600 increasing rate suggested that the increasing rate in OD600 level for *S. cerevisiae* TISTR 5606 DLE(48 – 60) of 0.930 ± 0.211 ODU/h was significantly higher ($p \leq 0.05$) than prior to feeding and DDLFH(48 – 60) of 0.635 ± 0.162 and 0.422 ± 0.089 ODU/h, respectively. The increasing rate in OD600 level for *C. utilis* UNSW 709400 and 709700 all conditions were not different statistically ($p > 0.05$) with the corresponding range of rates between 0.017 – 0.073 ODU/h (Table 4.14 – 4.16).

In addition, the highest maximum OD600 increasing rate in Table 4.17 – 4.19 belonged to DLE(48 – 60) and DDLFH(48 – 60) of 0.034 – 1.193 ODU/h. These were different statistically ($p \leq 0.05$) from prior to feeding (0.167 – 1.433 ODU/h).

4.2.3 pH level

The presence of weak acid in the cultivation medium could influence the pH value. The subsequent increase in pH level might be caused by the presence of total soluble protein in a higher concentration with additional nitrogen sources. The release of amine group could increase pH level under limited amount of carbon source (Lopez *et al*., 1970; Agustina *et al*., 2009).

The cultivation with microbial strains yielded the increasing pH level for *C. utilis*. The resistance to pH change for *C. utilis* UNSW 709400 throughout the cultivation period was generally observed prior to feeding at 5.81 ± 0.05 (Fig. 4.10(a) – 4.11(a) and Table 4.15), which was not significantly higher ($p > 0.05$) than DLE(48 – 60) and DDLFH(48 – 60) with the corresponding values of 5.78 ± 0.01 and 5.78 ± 0.01, respectively. The pH change for *C. utilis* UNSW 709700 was observed prior to feeding at 5.79 ± 0.003 (Fig. 4.12(a) – 4.13(a) and Table 4.16), which was not significantly higher ($p > 0.05$) than DLE(48 – 60) and DDLFH(48 – 60) with the corresponding values of 5.77 ± 0.009 and 5.77 ± 0.008, respectively. However, *S. cerevisiae* TISTR 5606 also showed decreasing pH level. The pH change for *S. cerevisiae* TISTR 5606 prior to feeding was 5.39 ± 0.02. This was not significantly different ($p > 0.05$) from DLE(48 - 60) (5.39 ± 0.009) and DDLFH(48 – 60) (5.42 ± 0.013) as shown in Fig. 4.8(a) – 4.9(a) and Table 4.14.

The increase in pH level after cultivation of *C. utilis*, might be caused not only by the activity of proteolytic enzymes (protease and peptidases) which were naturally presence to breakdown of amino acid (Barrett, 2001) but also the actively growing of the microbial cells during cultivation and fermentation (Giovanni and Rossi, 2007). In addition, *C. utilis* also did not generate organic acids at a relatively high level during cultivation in static condition (Poodtatep *et al*., 2009).

The decline of pH level was possibly due to the results of sugar utilization. Microbial strains were able to generate the weak acids through their metabolic pathways. Weak acids were naturally presence in the dried longan extract and small quantities of several organic acids could be produced during primary fermentation

(such acetic acid (Schlegel, 1993; Lea and Piggott, 2003; Rodrigues *et al*., 2005), oxalic acid (Cleland and Johnson, 1955) and propionic acid (Barbiratto *et al*., 1997)). The number of hydrogen ions were produced when the weak acids were dissociated (Rao, 1992). The buffering extract contributed to the counteraction of pH changes (Doonan, 1996).

4.2.4 Dried biomass concentration

The microbial growth was influenced by the presence of carbon and nitrogen sources to provide the adequate source of nutrition. The role of carbon source was to support the formation of ATP gained from microbial metabolism which was used as the energy for the cells to drive the energy required processes (Bergman, 1999) while the nitrogen source stimulated the growth of microbial cells, product formation and other metabolites (Torija *et al*., 2003; Albers *et al*., 1996).

As indicated in Fig. 4.8(a) and 4.9(a), the maximum change in dried biomass concentration for *S. cerevisiae* TISTR 5606 prior to feeding was 7.04 ± 0.07 g/l. This was significantly different ($p \leq 0.05$) from DLE(48 - 60) and DDLFH(48 – 60) as indicated in Table 4.11. Dried biomass concentration change of DLE(48 – 60) was 2.46 ± 0.07 g/l which was also significantly different ($p \leq 0.05$) from 1.02 ± 0.07 g/l for DDLFH (48 – 60). The maximum change in dried biomass concentration for *C. utilis* UNSW 709400 of 1.95 ± 0.08 prior to feeding were the significantly different ($p \leq 0.05$) from DLE(48 – 60) and DDLFH(48 – 60) (0.18 ± 0.07 and 0.13 ± 0.08 g/l, respectively) as shown in Fig. 4.10(a) – 4.11(a) and Table 4.12. Dried biomass concentration change for *C. utilis* UNSW 709700 of 1.74 ± 0.07 prior to feeding was not the significantly different ($p \leq 0.05$) from DLE(48 – 60) (0.12 ± 0.07 g/l) which was not significantly different ($p > 0.05$) from 0.09 ± 0.07 g/l for DDLFH(48 – 60) as showed in Fig. 4.12(a) – 4.13(a) and Table 4.13.

The average increasing rate of dried biomass concentration for *S. cerevisiae* TISTR 5606 in Table 4.14 suggested that prior to feeding were 0.139 ± 0.035 g/l/h. This was different statistically ($p \leq 0.05$) from 0.024 ± 0.046 g/l/h for DLE(48 – 60) which was different statistically ($p > 0.05$) from DDLFH(48 – 60) (0.092 ± 0.019 g/l/h). The average increasing rate of dried biomass concentration for *C. utilis* UNSW 709400 prior to feeding were 0.040 ± 0.019 g/l/h. This was different statistically ($p \leq 0.05$) from DLE(48 – 60) (0.014 ± 0.009 g/l/h) which was not significantly different ($p > 0.05$) from DDLFH(48 – 60) (0.009 ± 0.005 g/l/h) as indicated in Table 4.15. The average increasing rate of dried biomass concentration for *C. utilis* UNSW 709700 prior to feeding were 0.035 ± 0.011 g/l/h which were

different statistically ($p \leq 0.05$) from DLE(48 – 60) (0.010 ± 0.003 g/l/h) and DDLFH(48 – 60) (0.003 ± 0.001 g/l/h) as indicated in Table 4.16.

The highest maximum increasing rate of dried biomass concentration for *S. cerevisiae* TISTR 5606 in Table 4.17 was 0.260 ± 0.078 g/l/h for DLE(48 – 60). This was not significantly different ($p > 0.05$) from prior to feeding and DDLFH(48 – 60) (0.314 ± 0.055 and 0.111 ± 0.039 g/l/h, respectively). The highest maximum increasing rate of dried biomass concentration for *C. utilis* UNSW 709400 and 709700 suggested that prior to feeding was 0.129 ± 0.038 and 0.086 ± 0.004 g/l/h, respectively which were different statistically ($p \leq 0.05$) from DLE(48 – 60) (0.027 ± 0.009 and 0.014 ± 0.000 g/l/h, respectively) and DDLFH(48 – 60) (0.019 ± 0.000 and 0.015 ± 0.000 g/l/h, respectively) as indicated in Table 4.18 – 4.19.

These results were similar to Chen *et al*. (2000) who studied the effects of cell fermentation time and biomass drying strategies on the recovery of poly-3-hydroxyalkanoates from *Alcaligenes eutrophus* using a surfactant-chelate aqueous system. Dried biomass concentration would directly proportional to fermentation time when the microbe was subjected to adequate level of carbon and nitrogen sources.

4.2.5 Specific growth rate

Specific growth rate is defined as the increase in cell mass per unit time. This was calculated according to reciprocal hours (h^{-1}).

According to Table 4.14 – 4.16, the strain which was able to generate the highest average and maximum specific growth rate was *S. cerevisiae* TISTR 5606. The highest average specific growth rate for *S. cerevisiae* TISTR 5606 prior to feeding was 0.040 ± 0.013 per h, which was not significantly different ($p > 0.05$) from DLE(48 – 60) (0.026 ± 0.007 per h). This was significantly different ($p \leq 0.05$) from DDLFH(48 – 60) (0.014 ± 0.003 per h).

This was similar trend with the highest average specific growth rate for *C. utilis* UNSW 709400 and 709700 with the corresponding range of rates between 0.003 – 0.016 per h.

The statistical analyses of maximum specific growth rate (μ_{max}) for *S. cerevisiae* TISTR 5606 indicated that the value of prior to feeding was the highest at 0.102 ± 0.028 per h. This was significantly different ($p \leq 0.05$) from DLE(48 – 60) and DDLFH(48 – 60) (0.035 ± 0.012 and 0.018 ± 0.007 per h, respectively) as indicated in Table 4.17 – 4.19. This was similar trends with the highest maximum specific growth rate for *C. utilis* UNSW 709400 and 709700 with the corresponding range of rates between 0.004 – 0.050 per h.

The results from the current study was in agreement with Van Hoek *et al*. (1998) who investigated the effect of specific growth rate on fermentative capacity of baker's yeast and found that *S. cerevisiae* strain had high specific growth rate.

The different specific growth rates between *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 might stem from different characteristics of sugar utilization and product formation during the cultivation condition of each microbial strain. Basically, the more sugars consumed could be translated into higher level of specific growth rate (Albers *et al*., 1996).

4.2.6 Doubling time

Doubling time (t_d) is inversely proportional to the specific growth (Koch, 1979; Perry, 2008).

According to Table 4.14 – 4.16, *S. cerevisiae* TISTR 5606 had a shorter doubling time than *C. utilis* UNSW 709400 and 709700. The shortest period of average doubling time for *S. cerevisiae* TISTR 5606 occurred with the kinetics profile prior to feeding at 17.1 ± 5.7 h which could be compared to the longest period of doubling time for DDLFH(48 – 60) at 47.9 ± 11.4 h which were different statistically ($p \leq 0.05$) from DLE(48 – 60) (26.6 ± 7.31 h) as shown in Table 4.14. These were compared to the average doubling time for *C. utilis* UNSW 709400 and 709700. The results in all conditions were not different statistically ($p > 0.05$) with the corresponding values between 44.2 – 254.7 h as shown in Table 4.15 – 4.16.

In case of minimum doubling time ($t_{d,\ min}$) for *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700, prior to feeding had the lowest value of 6.83 ± 1.85, 13.8 ± 5.6 and 23.1 ± 2.6 h, respectively which were different statistically ($p \leq 0.05$) from DLE(48 – 60) (19.7 – 188 h) and DDLFH(48 – 60) (39.3 – 167 h).

Table 4.20 The differences in sugars (sucrose, glucose and fructose) concentration levels (g/l), ethanol concentration levels (g/l), lag time (sucrose, glucose, fructose and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
Sucrose decreasing level	82.9 ± 2.0	I	25.2 ± 0.1	II	12.6 ± 1.6	III
Glucose decreasing level	33.0 ± 0.8	I	12.6 ± 0.1	II	9.5 ± 1.4	II
Fructose decreasing level	38.0 ± 0.7	I	10.3 ± 0.4	II	10.6 ± 0.9	II
Ethanol production level	61.9 ± 5.5	I	15.3 ± 1.6	II	9.5 ± 2.7	III
Sucrose lag time	45.0 ± 4.5	I	0.00 ± 0.00	II	0.00 ± 0.00	III
Glucose lag time	33.0 ± 3.3	I	0.00 ± 0.00	II	0.00 ± 0.00	III
Fructose lag time	36.0 ± 3.6	I	57.0 ± 5.7	II	0.00 ± 0.00	III
Ethanol lag time	0.00 ± 0.00	I	48.1 ± 4.8	II	48.1 ± 4.8	II
$Y_{P/S}$	0.403 ± 0.036	I	0.318 ± 0.032	II	0.292 ± 0.084	III

The numbers with the same Roman numeral (I - III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.21 The differences in sugars (sucrose, glucose and fructose) concentration levels (g/l), ethanol concentration levels (g/l), lag time (sucrose, glucose, fructose and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709400 at 25.6°C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
Sucrose decreasing level	26.6 ± 3.4	I	3.07 ± 0.32	II	2.50 ± 0.37	II
Glucose decreasing level	14.3 ± 1.6	I	1.02 ± 0.94	II	1.11 ± 0.78	II
Fructose decreasing level	23.6 ± 1.2	I	2.04 ± 1.33	II	1.21 ± 0.96	II
Ethanol production level	0.00 ± 0.00	I	0.00 ± 0.00	I	0.00 ± 0.00	I
Sucrose lag time	24.0 ± 2.4	I	51.0 ± 5.1	II	0.00 ± 0.00	III
Glucose lag time	21.0 ± 2.1	I	51.0 ± 5.1	II	51.0 ± 5.1	II
Fructose lag time	24.0 ± 2.4	I	57.0 ± 5.7	II	54.0 ± 5.4	II
Ethanol lag time	48.0 ± 4.8	I	48.1 ± 4.8	I	48.1 ± 4.8	I
$Y_{P/S}$	0.00 ± 0.00	I	0.00 ± 0.00	I	0.00 ± 0.00	I

The numbers with the same Roman numeral (I - III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.22 The differences in sugars (sucrose, glucose and fructose) concentration levels (g/l), ethanol concentration levels (g/l), lag time (sucrose, glucose, fructose and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709700 at 25.6°C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
Sucrose decreasing level	25.9 ± 1.5	I	1.73 ± 0.24	II	1.93 ± 0.30	II
Glucose decreasing level	9.4 ± 1.3	I	1.44 ± 0.94	II	1.03 ± 0.27	II
Fructose decreasing level	19.9 ± 1.0	I	2.42 ± 1.16	II	3.65 ± 0.90	II
Ethanol production level	0.00 ± 0.00	I	0.00 ± 0.00	I	0.00 ± 0.00	I
Sucrose lag time	42.0 ± 4.2	I	51.0 ± 5.1	II	51.0 ± 5.1	II
Glucose lag time	0.00 ± 0.00	I	51.0 ± 5.1	II	51.0 ± 5.1	II
Fructose lag time	9.00 ± 0.90	I	57.0 ± 5.7	II	57.0 ± 5.7	II
Ethanol lag time	48.0 ± 4.8	I	48.1 ± 4.8	I	48.1 ± 4.8	I
$Y_{P/S}$	0.00 ± 0.00	I	0.00 ± 0.00	I	0.00 ± 0.00	I

The numbers with the same Roman numeral (I - II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.23 The average sugars (sucrose, glucose and fructose) consumption rate (g/l/h), average ethanol production rate (g/l/h), average specific rate of sugars consumption and production (Avg Q_s and Avg Q_p g/g/h) and average specific rate of ethanol production (Avg Q_p, g/g/h) during 60 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
Sucrose consumption rate	1.73 ± 0.43	I	2.12 ± 0.76	I	1.05 ± 0.08	II
Glucose production rate	0.688 ± 0.177	I	1.05 ± 0.15	I	0.797 ± 0.077	I
Fructose production rate	0.792 ± 0.172	I	0.849 ± 0.404	I	0.886 ± 0.169	I
Ethanol production rate	1.29 ± 0.30	I	1.51 ± 0.09	II	0.800 ± 0.162	III
Avg Q_s of sucrose	0.555 ± 0.149	I	0.275 ± 0.108	II	0.164 ± 0.014	II
Avg Q_s of glucose	0.232 ± 0.065	I	0.132 ± 0.021	II	0.124 ± 0.010	II
Avg Q_s of fructose	0.261 ± 0.057	I	0.113 ± 0.057	II	0.140 ± 0.031	II
Avg Q_p of ethanol	0.410 ± 0.107	I	0.187 ± 0.006	II	0.126 ± 0.027	III

The numbers with the same Roman numeral (I - III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.24 The average sugars (sucrose, glucose and fructose) consumption rate (g/l/h), average ethanol production rate (g/l/h), average specific rate of sugars consumption and production (Avg Q_s and Avg Q_p g/g/h) and average specific rate of ethanol production (Avg Q_p, g/g/h) during 60 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709400 at 25.6°C for three conditions.

Investigated parameters	**Conditions**					
	Before 48 h		**DLE (48 – 60 h)**		**DDLFH (48 – 60 h)**	
Sucrose consumption rate	0.552 ± 0.109	I	0.253 ± 0.160	II	0.210 ± 0.041	II
Glucose production rate	0.292 ± 0.109	I	0.085 ± 0.049	II	0.085 ± 0.049	II
Fructose production rate	0.490 ± 0.111	I	0.168 ± 0.180	II	0.088 ± 0.148	II
Ethanol production rate	0.000 ± 0.000	I	0.000 ± 0.000	I	0.000 ± 0.000	I
Avg Q_s of sucrose	0.169 ± 0.032	I	0.070 ± 0.044	II	0.063 ± 0.012	II
Avg Q_p of glucose	0.087 ± 0.033	I	0.024 ± 0.014	II	0.026 ± 0.015	II
Avg Q_p of fructose	0.142 ± 0.036	I	0.047 ± 0.050	II	0.027 ± 0.045	II
Avg Q_p of ethanol	0.000 ± 0.000	I	0.000 ± 0.000	I	0.000 ± 0.000	I

The numbers with the same Roman numeral (I - II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.25 The average sugars (sucrose, glucose and fructose) consumption rate (g/l/h), average ethanol production rate (g/l/h), average specific rate of sugars consumption and production (Avg Q_s and Avg Q_p g/g/h) and average specific rate of ethanol production (Avg Q_p, g/g/h) during 60 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709700 at 25.6°C for three conditions.

Investigated parameters	**Conditions**					
	Before 48 h		**DLE (48 – 60 h)**		**DDLFH (48 – 60 h)**	
Sucrose consumption rate	0.542 ± 0.089	I	0.125 ± 0.080	II	0.170 ± 0.070	II
Glucose production rate	0.188 ± 0.034	I	0.128 ± 0.082	II	0.126 ± 0.080	II
Fructose production rate	0.417 ± 0.154	I	0.213 ± 0.109	II	0.295 ± 0.105	II
Ethanol production rate	0.000 ± 0.000	I	0.000 ± 0.000	I	0.000 ± 0.000	I
Avg Q_s of sucrose	0.173 ± 0.028	I	0.034 ± 0.022	II	0.047 ± 0.020	II
Avg Q_p of glucose	0.057 ± 0.010	I	0.036 ± 0.023	II	0.035 ± 0.022	II
Avg Q_p of fructose	0.122 ± 0.032	I	0.060 ± 0.031	II	0.082 ± 0.029	II
Avg Q_p of ethanol	0.000 ± 0.000	I	0.000 ± 0.000	I	0.000 ± 0.000	I

The numbers with the same Roman numeral (I - II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.26 The maximum sugars (sucrose, glucose and fructose) consumption rate (g/l/h), maximum ethanol production rate (g/l/h), maximum specific rate of sugars consumption and production (Max Q_s and Max Q_p g/g/h) and maximum specific rate of ethanol production (Max Q_p, g/g/h) during 60 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three conditions.

Investigated parameters	Conditions					
	Before 48 h		DLE (48 – 60 h)		DDLFH (48 – 60 h)	
Sucrose consumption rate	4.25 ± 0.59	I	2.54 ± 0.89	II	1.12 ± 0.04	III
Glucose production rate	1.63 ± 0.21	I	1.18 ± 0.10	II	0.841 ± 0.090	III
Fructose production rate	1.67 ± 0.12	I	1.13 ± 0.41	II	0.980 ± 0.215	II
Ethanol production rate	2.96 ± 0.32	I	1.29 ± 0.24	II	0.956 ± 0.062	II
Max Q_s of sucrose	1.41 ± 0.28	I	0.454 ± 0.076	II	0.178 ± 0.003	III
Max Q_s of glucose	0.618 ± 0.074	I	0.163 ± 0.005	II	0.131 ± 0.010	II
Max Q_s of fructose	0.546 ± 0.037	I	0.195 ± 0.069	II	0.154 ± 0.039	II
Max Q_p of ethanol	0.993 ± 0.204	I	0.195 ± 0.003	II	0.152 ± 0.012	II

The numbers with the same Roman numeral (I - III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.27 The maximum sugars (sucrose, glucose and fructose) consumption rate (g/l/h), maximum ethanol production rate (g/l/h), maximum specific rate of sugars consumption and production (Max Q_s and Max Q_p g/g/h) and maximum specific rate of ethanol production (Max Q_p, g/g/h) during 60 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709400 at 25.6°C for three conditions.

Investigated parameters	**Conditions**					
	Before 48 h		**DLE (48 – 60 h)**		**DDLFH (48 – 60 h)**	
Sucrose consumption rate	0.958 ± 0.172	I	0.506 ± 0.161	II	0.169 ± 0.002	III
Glucose production rate	0.792 ± 0.315	I	0.170 ± 0.003	II	0.170 ± 0.003	II
Fructose production rate	0.792 ± 0.105	I	0.224 ± 0.024	II	0.175 ± 0.342	II
Ethanol production rate	0.000 ± 0.000	I	0.000 ± 0.000	I	0.000 ± 0.000	I
Max Q_s of sucrose	0.302 ± 0.053	I	0.140 ± 0.043	II	0.067 ± 0.016	III
Max Q_p of glucose	0.242 ± 0.079	I	0.047 ± 0.002	II	0.051 ± 0.002	II
Max Q_p of fructose	0.276 ± 0.067	I	0.063 ± 0.068	II	0.054 ± 0.103	II
Max Q_p of ethanol	0.000 ± 0.000	I	0.000 ± 0.000	I	0.000 ± 0.000	I

The numbers with the same Roman numeral (I - III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 4.28 The maximum sugars (sucrose, glucose and fructose) consumption rate (g/l/h), maximum ethanol production rate (g/l/h), maximum specific rate of sugars consumption and production (Max Q_s and Max Q_p g/g/h) and maximum specific rate of ethanol production (Max Q_p, g/g/h) during 60 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *C. utilis* UNSW 709700 at 25.6°C for three conditions.

Investigated parameters	**Conditions**					
	Before 48 h		**DLE (48 – 60 h)**		**DDLFH (48 – 60 h)**	
Sucrose consumption rate	0.917 ± 0.144	I	0.250 ± 0.083	II	0.226 ± 0.059	II
Glucose production rate	0.333 ± 0.001	I	0.256 ± 0.089	II	0.253 ± 0.080	II
Fructose production rate	1.13 ± 0.20	I	0.284 ± 0.117	II	0.393 ± 0.054	II
Ethanol production rate	0.000 ± 0.000	I	0.000 ± 0.000	I	0.000 ± 0.000	I
Max Q_s of sucrose	0.297 ± 0.032	I	0.068 ± 0.023	II	0.063 ± 0.017	II
Max Q_p of glucose	0.097 ± 0.014	I	0.072 ± 0.025	II	0.070 ± 0.021	II
Max Q_p of fructose	0.308 ± 0.054	I	0.079 ± 0.033	II	0.110 ± 0.075	II
Max Q_p of ethanol	0.000 ± 0.000	I	0.000 ± 0.000	I	0.000 ± 0.000	I

The numbers with the same Roman numeral (I - II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

4.2.7 Sugars decreasing

The decreasing levels of each sugar for all conditions being investigated are illustrated in Fig. 4.8(b) – 4.13(b) and Table 4.20 – 4.22. The highest sucrose concentration levels for *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 prior to feeding were 82.9 ± 2.0, 26.6 ± 3.4 and 25.9 ± 1.5 g/l, respectively. These concentration levels were significantly different ($p \leq 0.05$) after the addition of DDLFH with corresponding values of 12.6 ± 1.6, 2.50 ± 0.37 and 1.93 ± 0.30 g/l during 48th - 60th h cultivation period, respectively.

The highest average sucrose decreasing rate (Table 4.23 – 4.25) for *S. cerevisiae* TISTR 5606 after the addition of DLE was 2.12 ± 0.76 g/l/h during 48th - 60th h cultivation period. This was significantly different ($p \leq 0.05$) from the condition of DDLFH(48 – 60) of 1.05 ± 0.08 g/l/h. *C. utilis* UNSW 709400 and 709700 cultivated in the condition of DLE(48 – 60) were able to consume the highest average sucrose consumption rates at 0.253 ± 0.160 and 0.125 ± 0.080 g/l/h, respectively which were not significantly different ($p > 0.05$) from the condition of DDLFH(48 – 60) at 0.210 ± 0.041 and 0.170 ± 0.070 g/l/h, respectively.

In the situation of the maximum sucrose decreasing rates (Table 4.26 – 4.28), *S. cerevisiae* TISTR 5606 and *C. utilis* UNSW 709400 cultivated in the condition of DLE(48 – 60) were able to consume the highest maximum sucrose consumption rates at 2.54 ± 0.89 and 0.506 ± 0.161 g/l/h. These were significantly different ($p \leq 0.05$) from the condition of DDLFH(48 – 60) at 1.12 ± 0.04 and 0.169 ± 0.002 g/l/h, respectively. *C. utilis* UNSW 709700 cultivated in the condition of DLE(48 – 60) was able to consume the maximum sucrose consumption rate at 0.250 ± 0.083 g/l/h which was not significantly different ($p > 0.05$) from the condition of DDLFH(48 – 60) at 0.226 ± 0.059 g/l/h.

In term of glucose consumption, *S. cerevisiae* TISTR 5606 was able to uptake this sugar at the highest level of 33.0 ± 0.8 g/l while the increases in glucose concentration were observed for both *C. utilis* UNSW 709400 and 709700 instead.

In term of the average glucose decreasing rate (Table 4.20 – 4.22) for *S. cerevisiae* TISTR 5606, the consumption rate of glucose in the condition of DLE(48 – 60) was at the highest level at 1.05 ± 0.15 g/l/h which was not significantly different ($p > 0.05$) from the condition of DDLFH(48 – 60) at 0.797 ± 0.077 g/l/h. In the situation of the average glucose increasing rates for *C. utilis* UNSW 709400 and 709700, the production rates of glucose in the condition of DLE(48- 60) were at the highest level of 0.085 ± 0.049 and 0.128 ± 0.082 g/l/h, respectively. These were not significantly different ($p > 0.05$) from the conditions of DDLFH (48 - 60) at 0.085 ± 0.049 and 0.126 ± 0.080 g/l/h, respectively.

In term of the maximum glucose decreasing rate for *S. cerevisiae* TISTR 5606, the consumption rate of glucose in the condition of DLE(48 - 60) was at the highest level of 1.18 ± 0.10 g/l/h which was significantly different ($p \leq 0.05$) from the condition of DDLFH(48 - 60) at 0.841 ± 0.090 g/l/h. For the maximum glucose increasing rates of *C. utilis* UNSW 709400 and 709700, the production rates of glucose in the condition of DLE(48 - 60) were at the highest levels of 0.170 ± 0.003 and 0.256 ± 0.089 g/l/h, respectively. These were not significantly different ($p > 0.05$) from the condition of DDLFH(48 - 60) at 0.170 ±0.003 and 0.253 ± 0.080 g/l/h, respectively.

In term of fructose consumption, *S. cerevisiae* TISTR 5606 cultivated in the condition of DLE(48 – 60) was able to consume the maximum level of fructose at 10.6 ± 0.9 g/l which was not significantly different ($p > 0.05$) from DDLFH(48 – 60) of 10.3 ± 0.4 g/l. This was in contrast to *C. utilis* UNSW 709400 and 709700 where the fructose levels had elevated during the time course.

In the situation of the highest average fructose decreasing rate, *S. cerevisiae* TISTR 5606 cultivated in the condition of DDLFH(48 - 60) was able to consume the highest average fructose consumption rate at 0.886 ± 0.169 g/l/h which was not significantly different ($p > 0.05$) from DLE(48 – 60) of 0.849 ± 0.404 g/l/h. The highest average fructose increasing rates of *C. utilis* UNSW 709400 and 709700 for DLE medium were not significantly different ($p > 0.05$) from DDLFH medium with the corresponding values between 0.088 - 0.295 g/l/h.

The maximum fructose decreasing rates of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 for DLE medium were not significantly different ($p > 0.05$) from DDLFH medium with the corresponding values between of 0.175 - 1.13 g/l/h.

The elevation of glucose and fructose concentrations in comparison to the initial level throughout 60 h cultivation profiles of *C. utilis* strains as evident from Fig. 4.10(b) – 4.13(b) might be explained by the invertase activity that converted sucrose to glucose and fructose (Takeshige *et al*., 1995).

Prior to feeding, *S. cerevisiae* TISTR 5606 revealed the least lag time of 45.0 ± 4.5 h for sucrose consumption. This was significantly different ($p \leq 0.05$) from DLE(48 – 60) and DDLFH(48 – 60) in which the lag time period was absent for this sugar. *C. utilis* UNSW 709400 in the condition of DLE(48 - 60) revealed the least lag time of 51.0 ± 5.1 h for sucrose consumption. This was significantly different ($p \leq 0.05$) from the condition of DDLFH(48 – 60) in which the lag time period was absent for this sugar. *C. utilis* UNSW 709700 in the conditions of DLE(48 – 60) and DDLFH(48 – 60) had the similar lag time period of 51.0 ± 5.1 h for sucrose consumption.

Prior to feeding, *S. cerevisiae* TISTR 5606 revealed the least lag time of 33.0 ± 3.3 h for glucose consumption. This was significantly different ($p \leq 0.05$) from the conditions of DLE(48 – 60) and DDLFH(48 – 60) in which the lag time period was absent for this sugar. *C. utilis* UNSW 709400 and 709700 in the conditions of DLE(48 – 60) and DDLFH(48 – 60) had the similar lag time period of 51.0 ± 5.1 h for glucose production.

S. cerevisiae TISTR 5606 cultivated in the condition of DLE(48 - 60) revealed the least lag time of 57.0 ± 5.7 h for fructose consumption. This was significantly different ($p \leq 0.05$) from the condition of DDLFH(48 – 60) in which the lag time period was absent for this sugar. On the other hand, the lag time periods of *C. utilis* UNSW 709400 and 709700 cultivated in DLE medium were not significantly different ($p > 0.05$) from those of DDLFH medium.

In term of the highest average specific rates of sugars (sucrose, glucose and fructosee) consumption for *S. cerevisiae* TISTR 5606, the consumption of sugars in the condition of DLE(48 - 60) were at the highest levels of 0.275 ± 0.108, 0.132 ±0.021 and 0.113 ± 0.057 g/l/h, respectively. These were not significantly different ($p > 0.05$) from the condition of DDLFH(48 – 60) at 0.164 ± 0.014, 0.124 ± 0.010 and 0.140 ± 0.031 g/l/h, respectively. The similar trend was observed for the highest average sugars consumption/production rates for *C. utilis* UNSW 709400 and 709700.

In the situation of the maximum specific rates of sucrose consumption, *S. cerevisiae* TISTR 5606 and *C. utilis* UNSW 709400 cultivated in the condition of DLE(48 – 60) was able to consume the highest maximum specific rates of sucrose consumption at 0.454 ± 0.076 and 0.140 ± 0.043 g/l/h, respectively. These were significantly different ($p \leq 0.05$) from the condition of DDLFH(48 – 60) at 0.178 ± 0.003 and 0.067 ± 0.016 g/l/h, respectively. *C. utilis* UNSW 709700 cultivated in the condition of DLE(48 – 60) was able to consume the highest maximum specific rates of sucrose consumption at 0.068 ± 0.023 g/l/h which was not significantly different ($p > 0.05$) from the condition of DDLFH at 0.063 ± 0.017 g/l/h.

In case of the maximum specific rates of sugars (glucose and fructose) consumption for *S. cerevisiae* TISTR 5606, the consumption of these sugars in the condition of DLE(48 - 60) were at the highest levels of 0.163 ± 0.005 and 0.195 ± 0.069 g/l/h, respectively. These were not significantly different ($p > 0.05$) from the condition of DDLFH(48 – 60) at 0.131 ± 0.010 and 0.154 ± 0.039 g/l/h, respectively. The similar trend was observed for the highest average sugars consumption/production rates for *C. utilis* UNSW 709400 and 709700.

4.2.8 Ethanol increasing

Fig. 4.8(b) – 4.13(b) portray the increasing trends of ethanol production. At the beginning of feeding step on the 48th h, the rapid decrease in ethanol concentration was generally observed due to the dilution of the cultivation culture by the feeding medium. This was followed by the increasing trend until the 60th h.

The analyses of ethanol production levels are illustrated in Table 4.20 – 4.22. The highest ethanol concentration levels for *S. cerevisiae* TISTR 5606 prior to feeding was 61.9 ± 5.5 g/l. This concentration level was significantly different ($p \leq 0.05$) from the condition of DLE(48 – 60) and DDLFH(48 – 60) at 15.3 ± 1.6 and 9.53 ± 2.66 g/l, respectively. However, the ethanol production level after feeding with DLE media was significantly different ($p \leq 0.05$) from DDLFH media. In the situation of the highest ethanol concentration levels for *C. utilis* UNSW 709400 and 709700, the cultivation condition in this study stimulated cells production in the absence of ethanol production. The absence of ethanol production for *C. utilis* UNSW 709400 and 709700 were evident which might be the result of the strains' characteristics. It was well recognized that the effects were strongly dependent on the yeast strain. Bruinenberg *et al*. (1983) and Verduyn *et al*. (1984) stated that *C. utilis* was not able to produce ethanol from glucose under the aerobic condition, however, it could slowly generate ethanol in the anaerobic condition. Furthermore, these results were similar to Tangsuntornkhan *et al*. (2010) who investigated the growth kinetics of six *C. utilis* strains in 150 ml DLE. The average ethanol production time for six *C. utilis* strains was 90.2 ± 30.2th h. Thus, *C. utilis* UNSW 709400 and 709700 in this study were not able to produce ethanol during 1st – 60th cultivation period.

The lag time periods of ethanol production for all microbial strains after feeding with DLE medium were not significantly different ($p > 0.05$) from DDLFH medium.

The highest average ethanol production rate for *S. cerevisiae* TISTR 5606 (Table 4.23) cultivated in the condition of DLE(48 – 60) was 1.51 ± 0.09 g/l/h. This

was significantly different ($p \leq 0.05$) from the condition of DDLFH(48 – 60) at 0.800 ± 0.162 g/l/h. The comparison of maximum ethanol production rates are given in Table 4.26. The production rate of ethanol in the condition of DLE(48 – 60) was at the highest level of 1.29 ± 0.24 g/l/h which was significantly different ($p \leq 0.05$) from the condition of DDLFH(48 –60) at 0.956 ± 0.062 g/l/h.

The average specific rate of ethanol production for *S. cerevisiae* TISTR 5606 (Table 4.23) in the condition of DLE(48 – 60) was at the highest level of 0.187 ± 0.006 g/l/h. This was significantly different ($p \leq 0.05$) from the condition of DDLFH(48 – 60) at 0.126 ± 0.027 g/l/h. In term of maximum specific rate of ethanol production (Table 4.26), *S. cerevisiae* TISTR 5606 cultivated in the condition of DLE(48 – 60) was able to produce the highest maximum ethanol production rate at 0.195 ± 0.003 g/l/h. This was not significantly different ($p > 0.05$) from the condition of DDLFH(48 – 60) at 0.152 ± 0.012 g/l/h.

The highest ethanol yield for *S. cerevisiae* TISTR 5606 prior to feeding were 0.40 ± 0.04 g ethanol/ g sugars consumed. This was significantly different ($p \leq 0.05$) from the condition of DLE(48 – 60) and DDLFH(48 – 60) of 0.32 ± 0.03 and 0.29 ± 0.08 g ethanol/ g sugars consumed, respectively (Table 4.20).

However, the ethanol yield after feeding with DLE medium was significantly different ($p \leq 0.05$) from DDLFH medium. Although these medium (DLE and DDLFH) had the similar initial sugar, but DDLFH medium feeding had slower increasing trend DLE medium was observed. Such phenomenon might illustrate the toxicity of DDLFH medium which slowed down the ethanol production rate. The production of DDLFH with a heat treatment step might generate various toxic compounds, namely, furans, furfural and hydroxymethylfurfural (HMF) which inhibited microbial growth (Pienkos *et al*., 2009).

The difference in ethanol yield levels between the conditions of DLE(48 – 60) and DDLFH(48 – 60) might be the results of relation between $Y_{X/S}$ to glucose consumption and $Y_{P/S}$ to sucrose consumption. According to Table 4.20, *S. cerevisiae* TISTR 5606 in the condition of DLE(48 – 60) possessed higher level of

$Y_{X/S}$ and Qs of glucose as well as $Y_{P/S}$ and Q_S of sucrose than in the condition of DDLFH(48 – 60). Wang *et al*. (2004) mentioned that glucose had beneficial effect on biomass production while sucrose was more beneficial in the ethanol production.

4.3 Biotransformation of PAC

The experiment of two phases emulsion system for PAC biotransformation was conducted by adopting whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 cultivated in three conditions, namely, the batch cultivation with dried longan extract at 48th h of fermentation period, fed batch cultivation with DLE and DDLFH at 60th h of fermentation period (from section 4.2). The whole cells concentration were adjusted to dried biomass equivalent level of 12.24 g/l. In order to investigate the extent of PAC and by-product produced, substrate consumption, pH level increasing and volume ratio are shown in Fig. 4.14 – 4.25 and Table 4.29 – 4.41.

4.3.1 pH level

Since PAC biotransformation was a reaction with proton consumption, pH level will gradually increase throughout the biotransformation time course (Rosche *et al*., 2002; Rosche *et al*., 2004; Leksawasdi *et al*., 2005).

The pH level after 72 h of biotransformation with whole cells PDC from *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 are shown in Fig. 4.14 and Table 4.29.

The attained pH level of PAC biotransformation using the whole cells from *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 resulted in the higher level of pH (starting from pH = 6.0). The highest level of pH (6.60 ± 0.01) was obtained from the whole cells of *S. cerevisiae* TISTR 5606 in the condition of batch cultivation with DLE at 48th h fermentation period. This was significantly different ($p \leq 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE and DDLFH at 60th h fermentation period of 6.47 ± 0.01 and 6.46 ± 0.01, respectively. The highest level of pH for *S. cerevisiae* TISTR 5606 in this study was significantly lower ($p \leq 0.05$) than Achawasamit (2010) who employed the whole cells from the similar microbial strain and biotransformation system. The whole cells from *S. cerevisiae* TISTR 5606 in DLE and FLE generated the highest pH level of 6.75 ± 0.07 and 6.74 ± 0.01, respectively.

The whole cells from *C. utilis* UNSW 709400 revealed the highest pH level of 6.25 ± 0.01 in the condition of batch cultivation with DLE at 48th h fermentation period which was not significantly different ($p > 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE and DDLFH at 60th h fermentation period of 6.24 ± 0.02 and 6.23 ± 0.01, respectively.

In term of the pH levels of the whole cells from *C. utilis* UNSW 709700, the condition of batch cultivation with DLE at 48thh fermentation period and fed batch cultivation with DLE at 60th h fermentation period reached at the highest levels of 6.39 ± 0.01 and 6.37 ± 0.01, respectively. These were significantly different ($p \leq 0.05$) from the whole cells harvested from condition of fed batch cultivation with DDLFH at 60th h fermentation period of 6.24 ± 0.02.

The highest level of pH for *C. utilis* UNSW 709400 and 709700 in this study was significantly lower ($p \leq 0.05$) than Achawasamit (2010) who used whole cells from *C. utilis* TISTR 5020 in FLE with the highest pH level of 6.72 ± 0.01. In addition, Leksawasdi (2004) who employed the whole cells from similar microbial strain and similar biotransformation condition, stated that partially purified PDC extracted from *C. utilis* revealed the pH level of 8.5. The higher pH level was caused by the proton consumption during the PAC biotransformation (Rosche *et al*., 2002; Rosche *et al*., 2004; and Leksawasdi *et al*., 2005).

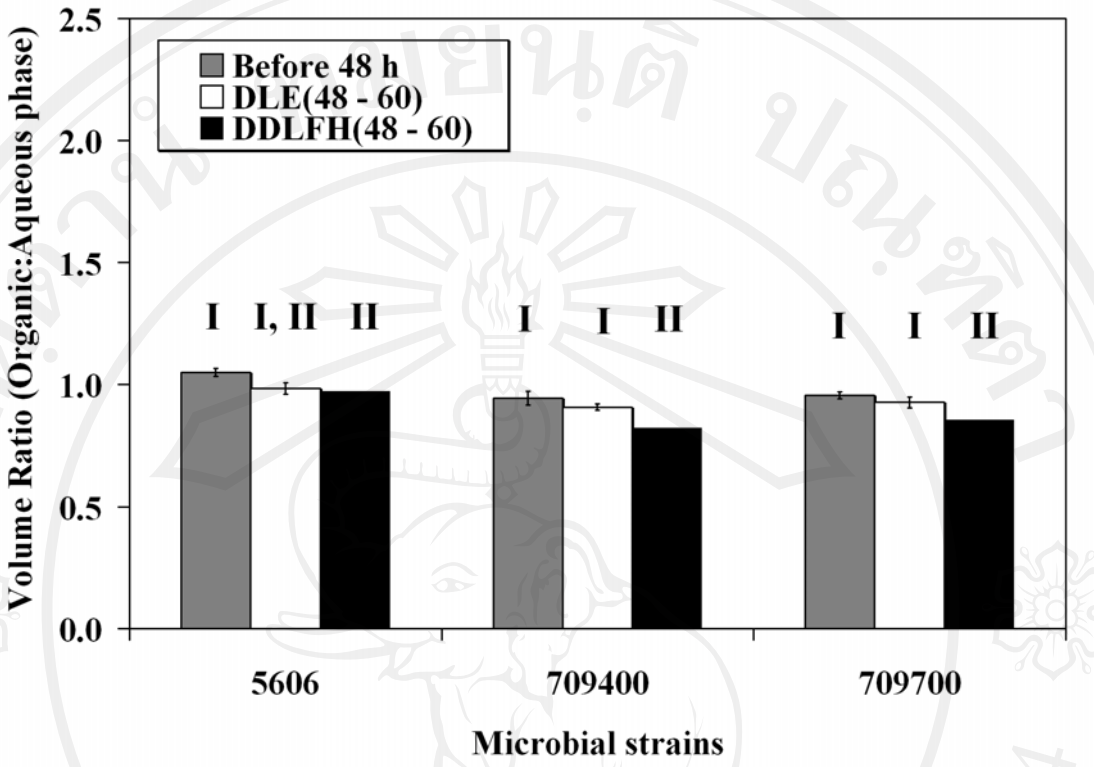


Figure 4.14 The pH level in both phases after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.29 The statistical comparison of pH level for two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	pH level (no unit) in both phases								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	6.60 ± 0.01	A	I	6.47 ± 0.01	A	II	6.46 ± 0.01	A	II
C. utilis UNSW 709400	6.25 ± 0.01	B	I	6.24 ± 0.02	B	I	6.23 ± 0.01	B	I
C. utilis UNSW 709700	6.39 ± 0.01	C	I	6.37 ± 0.01	C	I	6.24 ± 0.02	B	II

The number with the same Roman numeral (I – II) and alphabet (A – B) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

4.3.2 Volume Ratio

The initial volume ratio of organic to aqueous was 1:1 with the total volume of 10 ml. The subsequent whole cells PDC addition and biotransformation for 72 h resulted in the altered volume ratio of various biphasic systems (Agustina *et al*., 2009).

The determined volume ratio of the system which employed whole cells PDC from *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 are shown in Fig. 4.15 and Table 4.30.

After 72 h biotransformation, the highest level of volume ratio in the system which utilized the whole cells PDC from *S. cerevisiae* TISTR 5606 in the condition of batch cultivation with DLE at 48^{th} h fermentation period corresponded to the highest volume ratio of 1.05 ± 0.02 which was not significantly different ($p > 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE at 60^{th} h fermentation period of 0.984 ± 0.035. However, this volume ratio was significantly different ($p \leq 0.05$) from the whole cells harvested from condition of fed batch cultivation with DDLFH at 60^{th} h fermentation period of 0.973 ± 0.024.

The whole cells from *C. utilis* UNSW 709400 and 709700 in the condition of batch cultivation with DLE at 48^{th} h fermentation period revealed the highest pH levels of 0.944 ± 0.029 and 0.956 ± 0.014, respectively which were not significantly different ($p > 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE at 60^{th} h fermentation period of 0.908 ± 0.025 and 0.927 ± 0.011, respectively. These volume ratios were significantly different ($p \leq 0.05$) from the whole cells harvested from condition of fed batch cultivation with DDLFH at 60^{th} h fermentation period of 0.822 ± 0.012 and 0.855 ± 0.022, respectively.

The changing in volume ratio might be the direct effect of adding whole cells PDC (Agustina *et al*., 2009).

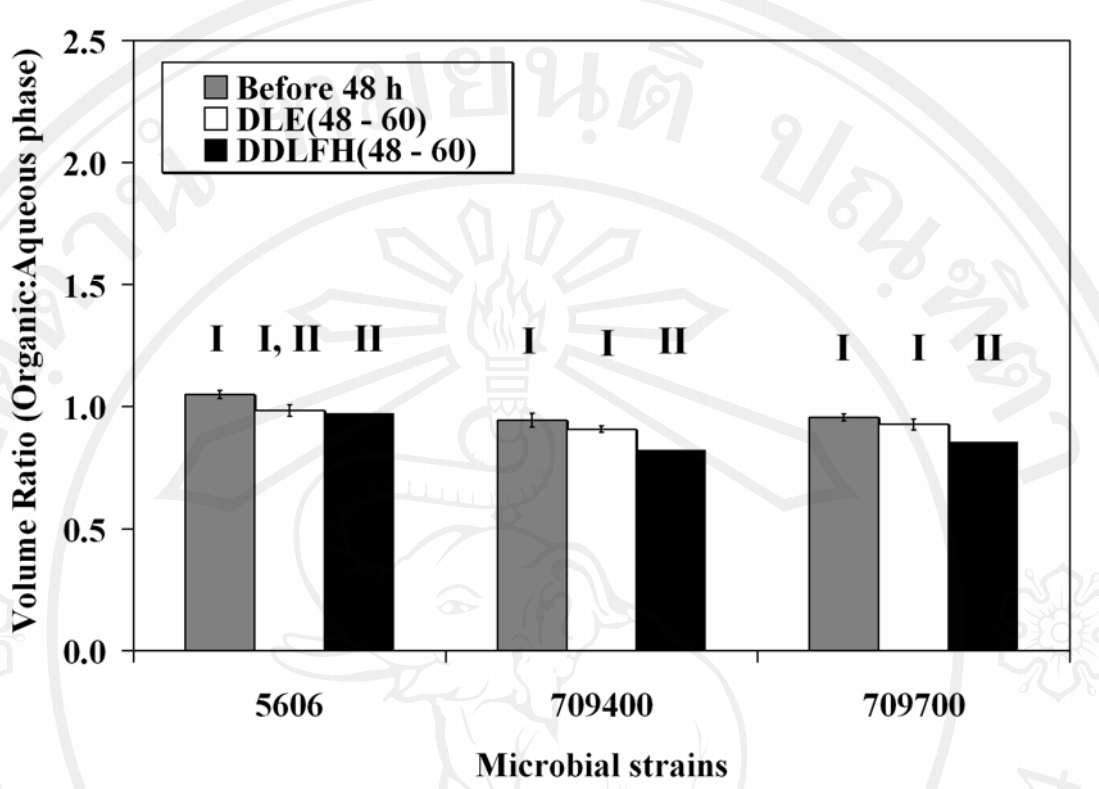


Figure 4.15 The volume ratio in both phases after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.30 The statistical comparison of volume ratio for two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	volume ratio in both phases for								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	1.05 ± 0.02	A	I	0.984 ± 0.035	A	I, II	0.973 ± 0.024	A	II
C. utilis UNSW 709400	0.944 ± 0.029	B	I	0.908 ± 0.025	A	I	0.822 ± 0.012	B	II
C. utilis UNSW 709700	0.956 ± 0.014	B	I	0.927 ± 0.011	A	I	0.855 ± 0.022	B	II

The number with the same Roman numeral (I – II) and alphabet (A – B) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

4.3.3 PAC

Biotransformation of PAC was performed with 1.75 M benzaldehyde and 300 mM pyruvate within 72 h with adopting whole cells from 3 microbial strains cultivated in various conditions for two phase emulsion system.

As indicated in Fig. 4.16 and Table 4.31, the highest average PAC concentration level of whole cells from *S. cerevisiae* TISTR 5606 in the condition of batch cultivation with DLE at 48th h fermentation period was 15.6 ± 0.5 mM. This concentration level was significantly different ($p \leq 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE and DDLFH at 60th h fermentation period (13.5 ± 0.7 and 11.7 ± 0.4 mM, respectively).

The whole cells from *C. utilis* UNSW 709400 in the condition of batch cultivation with DLE at 48th h fermentation period and the condition of fed batch cultivation with DLE at 60th h fermentation period were able to produce PAC at the highest levels of 7.90 ± 0.76 and 7.59 ± 1.00 mM, respectively. These were significantly different ($p \leq 0.05$) from the whole cells harvested from the condition of fed batch cultivation with DDLFH at 60th h fermentation period of 2.25 ± 0.06 mM.

The similar trend was observed for the highest average PAC concentration level using whole cells from *C. utilis* UNSW 709700 with the corresponding values between 3.43 – 10.1 mM.

These were in contrast to Tangsuntornkhan *et al*. (2010) who employed the same biotransformation condition and reported the production of PAC using whole cells of *C. utilis* TISTR 5198 at 6.12 g/l dried biomass equivalent harvested at 192nd h in DLE and DDLFH media. The PAC level of 1.76 ± 0.06 mM was observed for the whole cells collected from DDLFH medium which was significantly higher than that from DLE medium (0.19 ± 0.01 mM).

The whole cells of *S. cerevisiae* TISTR 5606 was able to produce PAC level at 15.6 ± 0.5, 13.5 ± 0.7 and 11.7 ± 0.4 mM for the conditions of batch cultivation with DLE at 48th h fermentation period, fed batch cultivation with DLE and DDLFH at 60th h fermentation period, respectively. These were significantly different ($p \leq 0.05$) from whole cells of *C. utilis* UNSW 709400 and 709700 with the PAC production range of 2.25 – 10.1 mM.

The overall PAC production with whole cells from *S. cerevisiae* TISTR 5606 was significantly ($p \leq 0.05$) higher than Chaweekunlayakun *et al*. (2010) who employed the whole cells from the similar microbial strain in two – phase separated biotransformation system but did not result in any PAC production. The absence of PAC production was evident which might be the result of separated organic/aqueous phase which minimized the exposure of whole cells to benzaldehyde substrate.

The study performed by Achawasamit (2010), who also employed the whole cells from the similar microbial strain in two – phase separated biotransformation system, indicated that whole cells from *S. cerevisiae* TISTR 5606 in DLE and Fresh Longan Extract (FLE) could produce PAC concentration levels at 31.5 ± 0.7 and 28.2 ± 1.2 mM, respectively. These were significantly ($p \leq 0.05$) higher than PAC obtained from the whole cells from *C. utilis* TISTR 5020 in FLE (25.8 ± 1.0 mM).

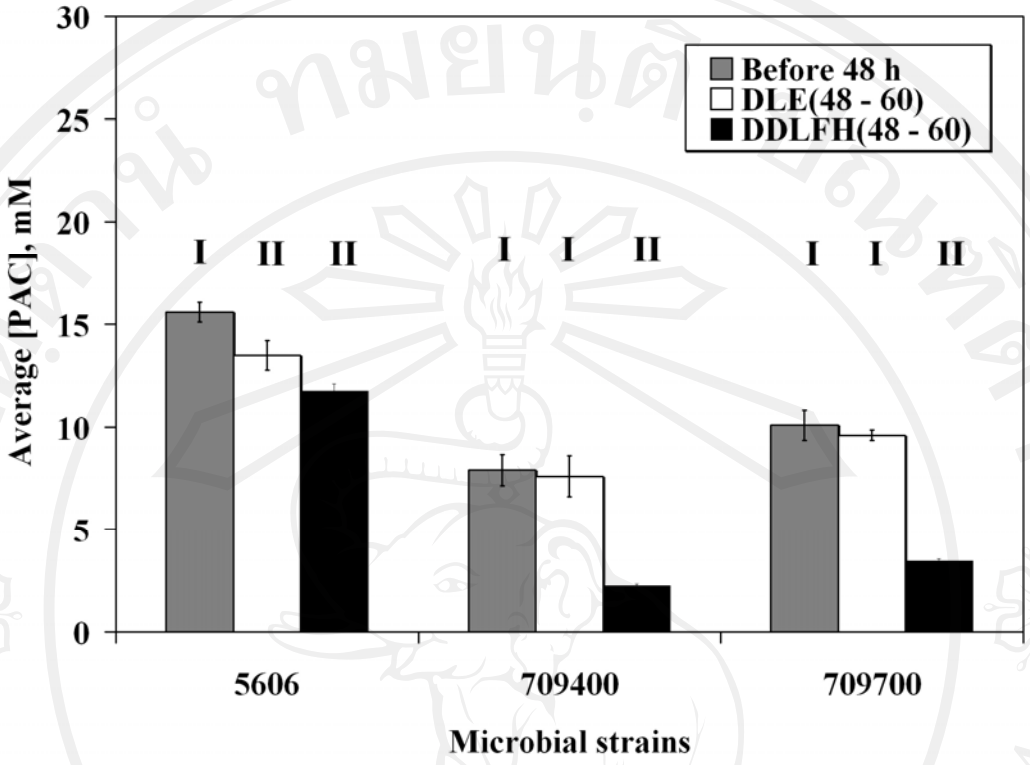


Figure 4.16 The overall PAC concentration (mM) in both phases after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.31 The statistical comparison of overall PAC concentration for two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	[PAC] (mM) in both phases								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	15.6 ± 0.5	A	I	13.5 ± 0.7	A	II	11.7 ± 0.4	A	II
C. utilis UNSW 709400	7.90 ± 0.76	B	I	7.59 ± 1.00	B	I	2.25 ± 0.06	B	II
C. utilis UNSW 709700	10.1 ± 0.7	B	I	7.60 ± 0.26	B	I	3.43 ± 0.12	C	II

The number with the same Roman numeral (I – II) and alphabet (A – C) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

4.3.4 Acetaldehyde

During PAC biotransformation, acetaldehyde was one of the by - product. The formation of acetaldehyde was inevitable yet could be minimized. After pyruvate was decarboxylated using TPP and Mg^{2+} as cofactors, active acetaldehyde was produced. Active acetaldehyde performed two reactions: (1) react as nucleophile with added benzaldehyde to form PAC and/or (2) take up a proton and be released as acetaldehyde (Rosche *et al*., 2001; Rosche *et al*., 2002; Leksawasdi, 2004).

The formation of acetaldehyde in both phases (aqueous and organic) in the biotransformation of PAC by using whole cells PDC from *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 after 72 h is given in Fig. 4.17 and Table 4.32.

The highest level of acetaldehyde for whole cells of *S. cerevisiae* TISTR 5606 in the condition of batch cultivation with DLE at 48th h fermentation period was 5.50 ± 0.28 mM. This was significantly different ($p \leq 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE and DDLFH at 60th h fermentation period at 4.62 ± 0.25 and 4.51 ± 0.16 mM, respectively.

The whole cells from *C. utilis* UNSW 709400 in the condition of batch cultivation with DLE at 48th h fermentation period and the condition of fed batch cultivation with DLE at 60th h fermentation period were able to produce acetaldehyde at the highest levels of 4.54 ± 0.25 and 4.35 ± 0.39 mM, respectively. These were significantly different ($p \leq 0.05$) from the whole cells harvested from the condition of fed batch cultivation with DDLFH at 60th h fermentation period of 3.98 ± 0.20 mM.

The whole cells from *C. utilis* UNSW 709700 in the condition of batch cultivation with DLE at 48th h fermentation period and the condition of fed batch cultivation with DLE at 60th h fermentation period were able to produce acetaldehyde at the highest levels of 5.06 ± 0.16 and 4.90 ± 0.23 mM, respectively. These were significantly different ($p \leq 0.05$) from the whole cells harvested from the condition of fed batch cultivation with DDLFH at 60th h fermentation period of 4.50 ± 0.19 mM.

Acetaldehyde was released when the active acetaldehyde produced from non-oxidative decarboxylation by PDC consumed the available proton. The direct correlation between high level of PAC and acetaldehyde produced might be used as supportive evidence of such process (Agustina *et al*., 2009).

The highest acetaldehyde level of 5.50 ± 0.28 mM (Table 4.32) was related to the highest level of PAC produced (14.5 ± 0.4 mM, Table 4.31) during PAC biotransformation.

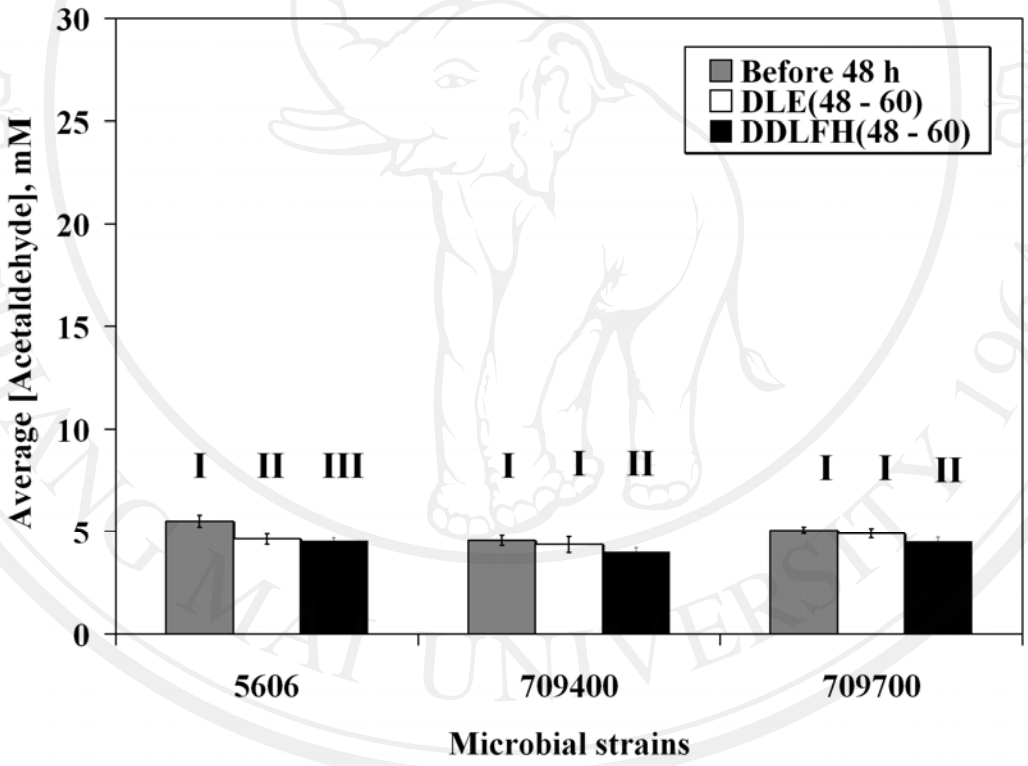


Figure 4.17 The overall acetaldehyde concentration (mM) in both phases after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.32 The statistical comparison of overall acetaldehyde concentration for two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	[acetaldehyde] (mM) in both phases								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	5.50 ± 0.28	A	I	4.62 ± 0.25	A	I	4.51 ± 0.16	A	III
C. utilis UNSW 709400	4.54 ± 0.25	B	I	4.35 ± 0.39	A	I	3.98 ± 0.20	B	II
C. utilis UNSW 709700	5.06 ± 0.16	A, B	I	4.90 ± 0.23	A	I	4.50 ± 0.19	A, B	II

The number with the same Roman numeral (I – II) and alphabet (A – B) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

4.3.5 Acetoin

Acetoin is another by-product of PAC biotransformation when the acetaldehyde produced is reacted with active acetaldehyde (Rosche *et al*., 2002). During this study, acetoin was not detected.

4.3.6 Benzoic acid

Benzoic acid is the oxidized by-product of benzaldehyde (Rogers and Shin, 1997) which was relatively constant during the biotransformation process and being maintained at the level lesser than 6.85 ± 0.07 mM in the organic phase and 29.1 ± 0.2 mM in the aqueous phase.

4.3.7 Benzyl alcohol

Benzyl alcohol is the by-product of benzaldehyde reduction by ADH which is naturally presence in the cells (Rogers and Shin, 1997; Rosche *et al*., 2001; Satianegara *et al*., 2006). Benzyl alcohol was not detected in this study.

4.3.8 Benzaldehyde

Benzaldehyde was the substrate for PAC biotransformation. Whole cells PDC added performed carboligation between benzaldehyde and decarboxylated pyruvate to produce PAC. Gunawan (2007) stated that both PAC and benzaldehyde strongly partitioned into the organic phase and thereby the enzyme in the aqueous phase was protected from high interfacial benzaldehyde concentrations. The result was due to mass transfer limitation of benzaldehyde from organic phase to the aqueous phase which was dependent on the partition efficient, the degree of turbulence and the ratio of interfacial area to aqueous phase volume (Leksawasdi, 2004).

The utilization of whole cells from same microbial strain in the condition of fed batch cultivation with DLE and DDLFH at 60^{th} h fermentation period was assumed to consume substrates (benzaldehyde and pyruvate) and produce PAC at the highest level. The condition of fed batch was a production technique after batch cultivation to increase the productivity of desired product or biomass (Longobardi, 1994). The result of increase productivity was stemmed from the decreasing of (1) substrate inhibition, (2) the utilization of sugars for ethanol production which resulted in the increased cells concentration, and (3) contamination from other microbes (Chotipattana and Tanthawichet, 1992).

Although DLE and DDLFH medium had the similar initial sugars concentration level, DDLFH medium contained toxic compounds which were generated from a heat treatment step, namely, furans, furfural, and hydroxyl-methylfurfural (HMF). These compounds could significantly inhibited microbial growth (Pienkos *et al*., 2009). The whole cells cultivated in DDLFH medium thus consumed substrates (benzaldehyde and pyruvate) and produced PAC at the lower levels than whole cells cultivated in DLE medium.

The average benzaldehyde concentrations after 72 h biotransformation period with whole cells PDC from *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 are shown in Fig. 4.18 – 4.21 and Table 4.33 – 4.37.

As indicated in Table 4.33, the benzaldehyde concentration in aqueous phase prior to addition of whole cells was 20.1 ± 0.3 mM. The benzaldehyde concentration in organic phase prior to addition of whole cells was 1,708 ± 33 mM. The overall benzaldehyde concentration in two phase prior to addition of whole cells was 864 ± 16 mM. The lowest level of remnant benzaldehyde concentration in aqueous phase (8.57 ± 0.38 mM) was remained when the whole cells of *S. cerevisiae* TISTR 5606 in the condition of batch cultivation with DLE at 48th h fermentation period was used (Fig. 4.18 and Table 4.34). This result was not significantly different ($p > 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE and DDLFH at 60th h fermentation period (12.4 ± 2.4 and 14.1 ± 4.4 mM, respectively). The similar trend was observed for the lowest level of remnant benzaldehyde concentration in aqueous phase when the whole cells of *C. utilis* UNSW 709400 and 709700 were used with the corresponding values between 13.9 – 18.0 mM.

Table 4.33 The banzaldehyde concentration in two phase PAC biotransformation system prior to addition of whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Phase	**[benzaldehyde] (mM)**
Aqueous phase	20.1 ± 0.3
Organic phase	1,708 ± 33
Overall	864 ± 16

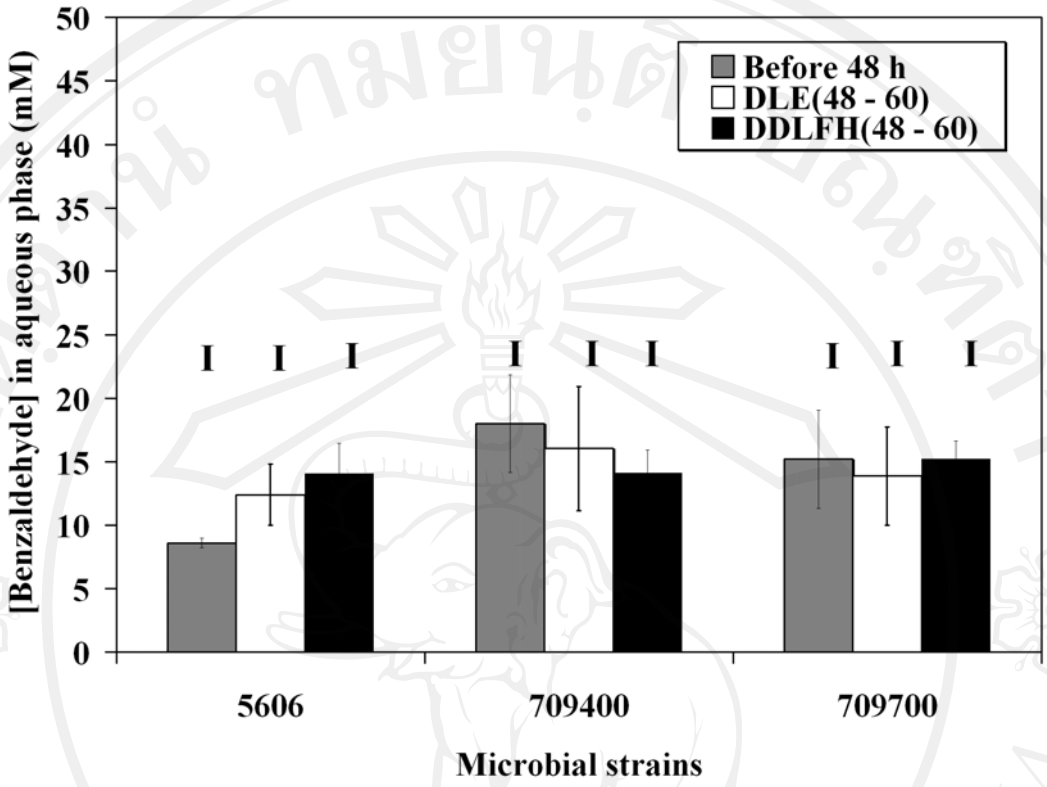


Figure 4.18 The remnant benzaldehyde concentration (mM) in aqueous phase after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.34 The statistical comparison of remnant benzaldehyde concentration in aqueous phase for two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	[benzaldehyde] (mM) in aqueous phase								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	8.57 ± 0.38	A	I	12.4 ± 2.4	A	I	14.1 ± 4.4	A	I
C. utilis UNSW 709400	18.0 ± 3.8	B	I	16.1 ± 4.9	A	I	14.1 ± 6.8	A	I
C. utilis UNSW 709700	15.2 ± 3.8	B	I	13.9 ± 3.9	A	I	15.2 ± 1.4	A	I

The number with the same Roman numeral (I) and alphabet (A – B) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

The lowest level of remnant benzaldehyde concentration in organic phase (805 ± 22 mM, Fig. 4.19 and Table 4.35) was achieved when the whole cells of *S. cerevisiae* TISTR 5606 in the condition of batch cultivation with DLE at 48th h fermentation period was used. This result was not significantly different ($p > 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE and DDLFH at 60th h fermentation period (813 ± 60 and 857 ± 8 mM, respectively). The similar trend was observed for the lowest level of remnant benzaldehyde concentration in organic phase when the whole cells of *C. utilis* UNSW 709400 and 709700 were used with the corresponding values between 863 – 962 mM.

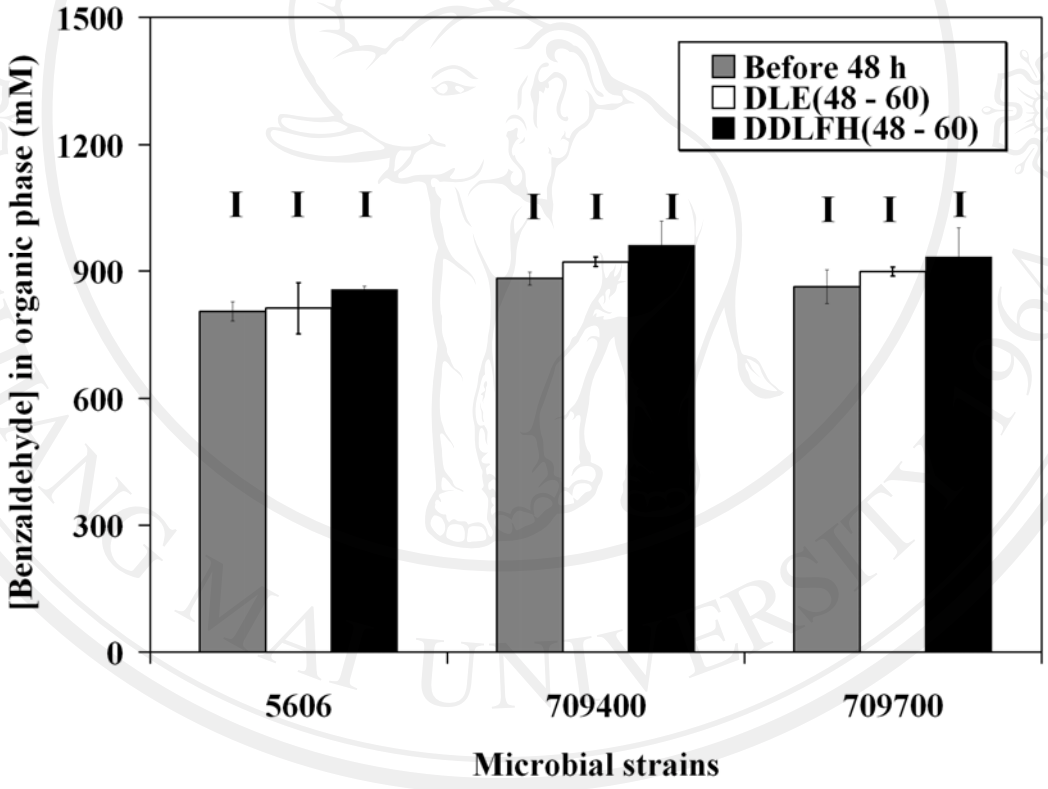


Figure 4.19 The remnant benzaldehyde concentration (mM) in organic phase after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.35 The statistical comparison of remnant benzaldehyde concentration in organic phase for two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	[benzaldehyde] (mM) in organic phase								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	805 ± 22	A	I	813 ± 60	A	I	857 ± 8	A	I
C. utilis UNSW 709400	883 ± 15	B	I	922 ± 11	A	I	962 ± 58	A	I
C. utilis UNSW 709700	863 ± 40	A, B	I	899 ± 11	A	I	934 ± 69	A	I

The number with the same Roman numeral (I) and alphabet (A – B) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

The highest level of overall consumed/unaccounted benzaldehyde concentration in both phases (468 ± 14 mM, Fig. 4.20 and Table 4.36) was remained when the whole cells of *S. cerevisiae* TISTR 5606 in the condition of batch cultivation with DLE at 48th h fermentation period was used. This was not significantly different ($p > 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE at 60th h fermentation period of 448 ± 15 mM. However, the highest level of overall consumed/unaccounted benzaldehyde concentration in both phases was significantly different ($p \leq 0.05$) to the whole cells harvested from condition of fed batch cultivation with DDLFH at 60th h fermentation period of 423 ± 12 mM.

The similar trend was observed for the whole cells of *C. utilis* UNSW 709400 and 709700 with the corresponding values between 340 – 415 mM.

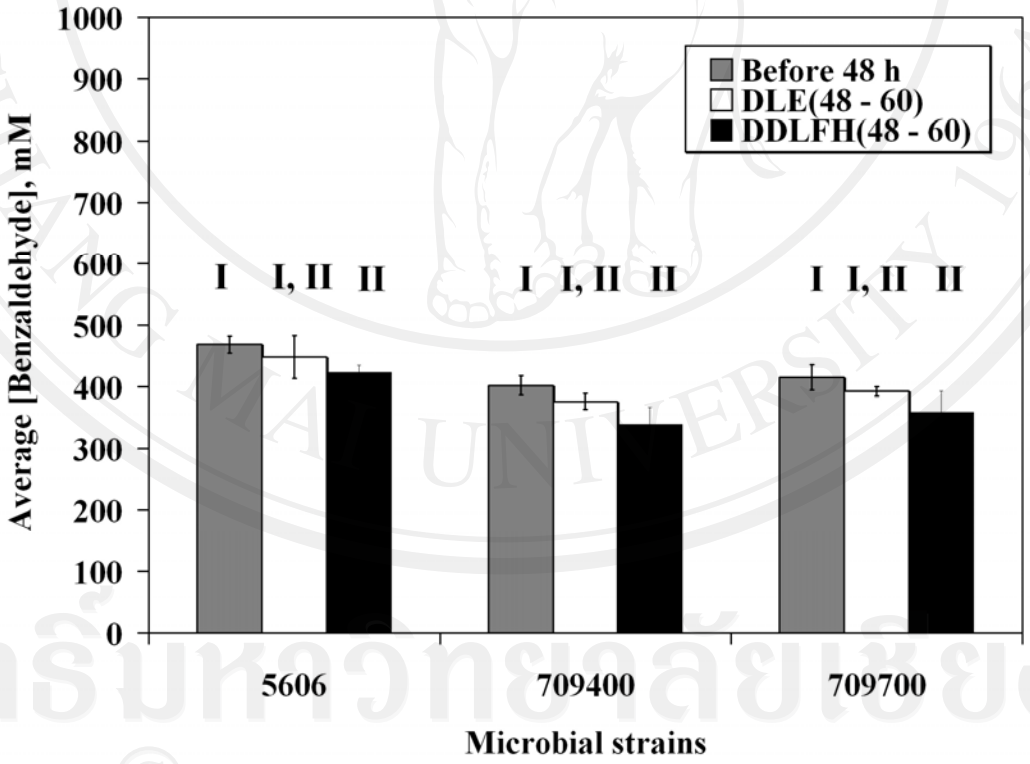


Figure 4.20 The consumed/unaccounted benzaldehyde concentration (mM) in both phases after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.36 The statistical comparison of average consumed/unaccounted for benzaldehyde concentration in both phases for two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	[benzaldehyde] (mM) in both phases								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	468 ± 14	A	I	448 ± 15	A	I, II	423 ± 12	A	II
C. utilis UNSW 709400	402 ± 16	B	I	376 ± 13	B	I, II	340 ± 17	B	II
C. utilis UNSW 709700	415 ± 21	B	I	392 ± 8	B	I, II	359 ± 14	B	II

The number with the same Roman numeral (I – II) and alphabet (A – B) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

The benzaldehyde balances obtained from PAC biotransformation (Fig. 4.21 and Table 4.37) were not significantly different ($p > 0.05$) with the corresponding values between 48.3 – 51.4% (PAC 0.66 – 3.33% were produced in absence of benzoic acid).

The highest of benzaldehyde balance from this study (52.0 ± 1.7%, Table 4.41) was lower than the benzaldehyde balance determined by Gunawan (2007) who employed the whole cells from the similar microbial strain, cultivated in the medium containing yeast extract in the similar biotransformation condition with 20 mM 3-[N-morpholino] propanesulfonic acid (MOPS) and 2.5 M dipropylene glycol (DPG) at 20°C with pH control at the level of 7.0. This was resulted in 70.0% benzaldehyde balance (mole basis) where 62.0% of benzaldehyde was used to produce PAC, 8.0% was remained, and 30.0% was lost/not detected (standard errors were not reported). These were compared to the current study where 1.0 ± 0.2% (mole basis) of benzaldehyde was used to produce PAC, 49.4 ± 0.5% benzaldehyde was remained and 49.6 ± 0.4% was lost/not detected.

This might be due to the utilization of MOPS and DPG. The addition MOPS and DPG as stabilizing agents was studied whether these compounds contributed to the efficiency of PAC production (Leksawasdi, 2004 and Gunawan, 2007). Leksawasdi (2004) and Rosche *et al*., (2002) stated that MOPS and DPG were found to have a beneficial effect on enhancing PDC stability. Furthermore, Leksawasdi *et al*. (2005) reported that the implementation of stabilizing agents in the two-phase aqueous/octanol-benzaldehyde system was associated with higher aqueous phase benzaldehyde levels in comparison to the condition with lower stabilizing agent concentrations. It was noted that prolonged cells contact with benzaldehyde has negative impacts on cells permeability, viability and growth (Long and Ward 1989).

It was possible that the undetected benzaldehyde had joined with the whole cells used in the biotransformation system and formed the suspension between the interfacial layer of aqueous/organic phase. Since the current system generated a relatively smaller amount of PAC, the amount of benzaldehyde remained in such layer was thus still in the relatively larger amount. It was thus recommended in the future study to rectify the benzaldehyde loss problem to improve the benzaldehyde balance.

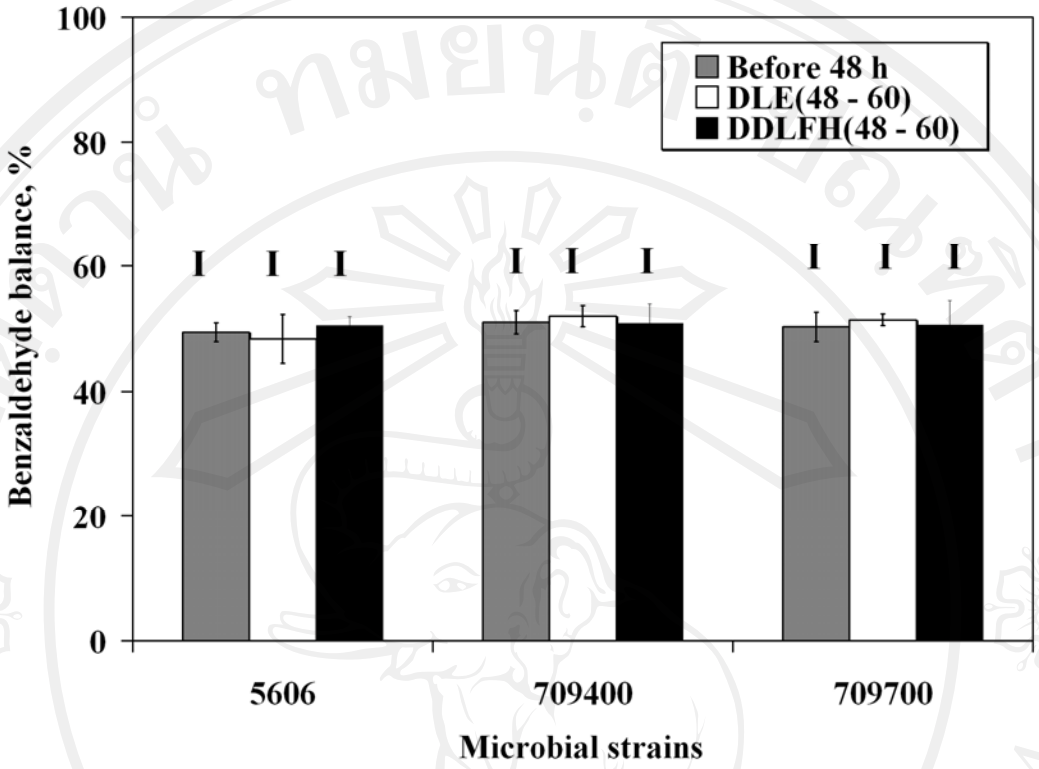


Figure 4.21 The average benzaldehyde balance (%) in both phases after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.37 The statistical comparison of benzaldehyde balance (%) in two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	benzaldehyde balance (%) in both phases								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	49.4 ± 1.5	A	I	48.3 ± 3.9	A	I	50.5 ± 1.4	A	I
C. utilis UNSW 709400	51.0 ± 1.9	A	I	52.0 ± 1.7	A	I	50.8 ± 3.1	A	I
C. utilis UNSW 709700	50.2 ± 2.4	A	I	51.4 ± 0.9	A	I	50.5 ± 3.9	A	I

The number with the same Roman numeral (I) and alphabet (A) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

4.3.9 Pyruvate

During PAC biotransformation, pyruvate was the substrate which was decarboxylated into active acetaldehyde and ligated with benzaldehyde to PAC. In this study, pyruvate added was in the concentration of 300 mM to a lower concentration level due to the formation of PAC and acetaldehyde.

The average pyruvate concentrations after 72 h biotransformation period with whole cells PDC from *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 are shown in Fig. 4.22 – 4.25 and Table 4.38 – 4.41.

The lowest level of remnant pyruvate concentration in aqueous phase (187 ± 3 mM, Fig. 4.22 and Table 4.38) was achieved when the whole cells of *S. cerevisiae* TISTR 5606 in the condition of batch cultivation with DLE at 48^{th} h fermentation period was used. This result was not significantly different ($p > 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE at 60^{th} h fermentation period of 191 ± 2 mM. This lowest level was significantly different ($p \leq 0.05$) from the whole cells harvested from condition of fed batch cultivation with DDLFH at 60^{th} h fermentation period of 196 ± 2 mM.

The lowest level of remnant pyruvate concentration in aqueous phase (202 ± 3 mM) was resulted when the whole cells of *C. utilis* UNSW 709400 was used. This result was not significantly different ($p > 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE and DDLFH at 60^{th} h fermentation period of 207 ± 4 and 210 ± 4 mM, respectively. The similar trend was observed for the level of remnant pyruvate concentration in aqueous phase when the whole cells of *C. utilis* UNSW 709700 was used with the corresponding values between 199 – 209 mM.

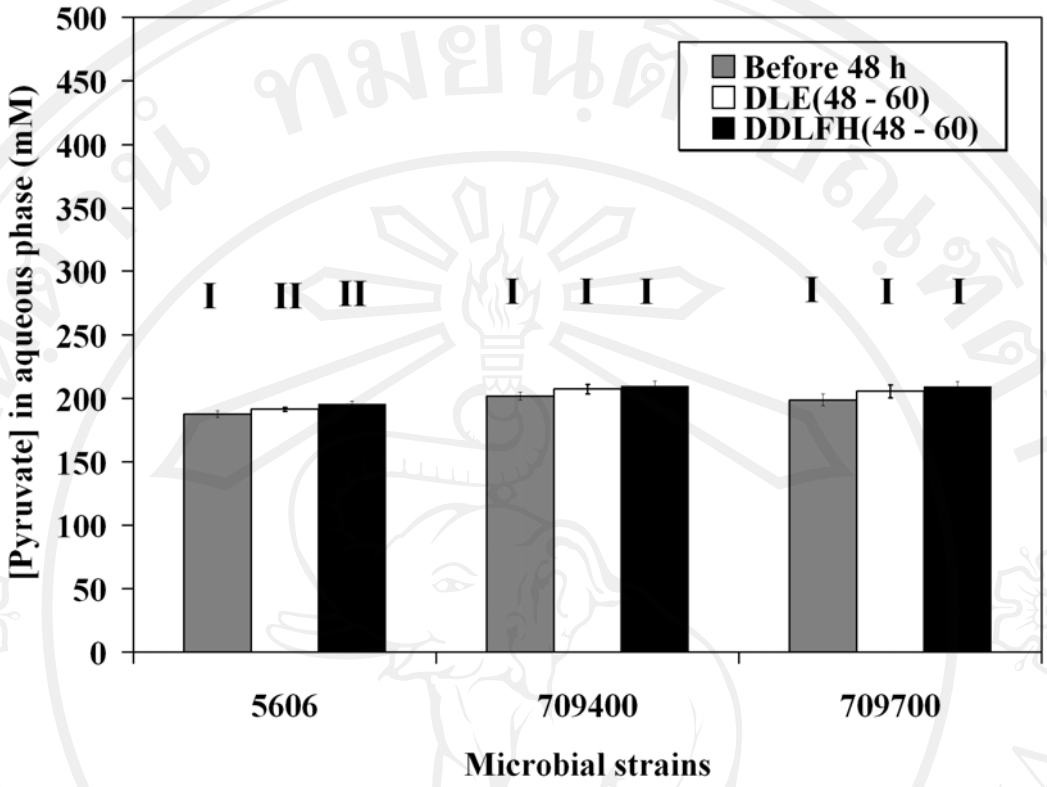


Figure 4.22 The remnant pyruvate concentration (mM) in aqueous phases after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.38 The statistical comparison of remnant pyruvate concentration in aqueous phase for two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	**[pyruvate] (mM) in both phases**								
	Before 48 h			**DLE (48 – 60 h)**			**DDLFH (48 – 60 h)**		
S. cerevisiae TISTR 5606	187 ± 3	A	I	191 ± 2	A	I, II	196 ± 2	A	II
C. utilis UNSW 709400	202 ± 3	B	I	207 ± 4	B	I, II	210 ± 4	B	II
C. utilis UNSW 709700	199 ± 5	B	I	206 ± 5	B	I, II	209 ± 4	B	II

The number with the same Roman numeral (I – II) and alphabet (A – B) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

As indicated in Fig. 4.23 and Table 4.39, the whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 revealed the level of remnant pyruvate concentration in organic phase of 0.02 – 0.08 mM.

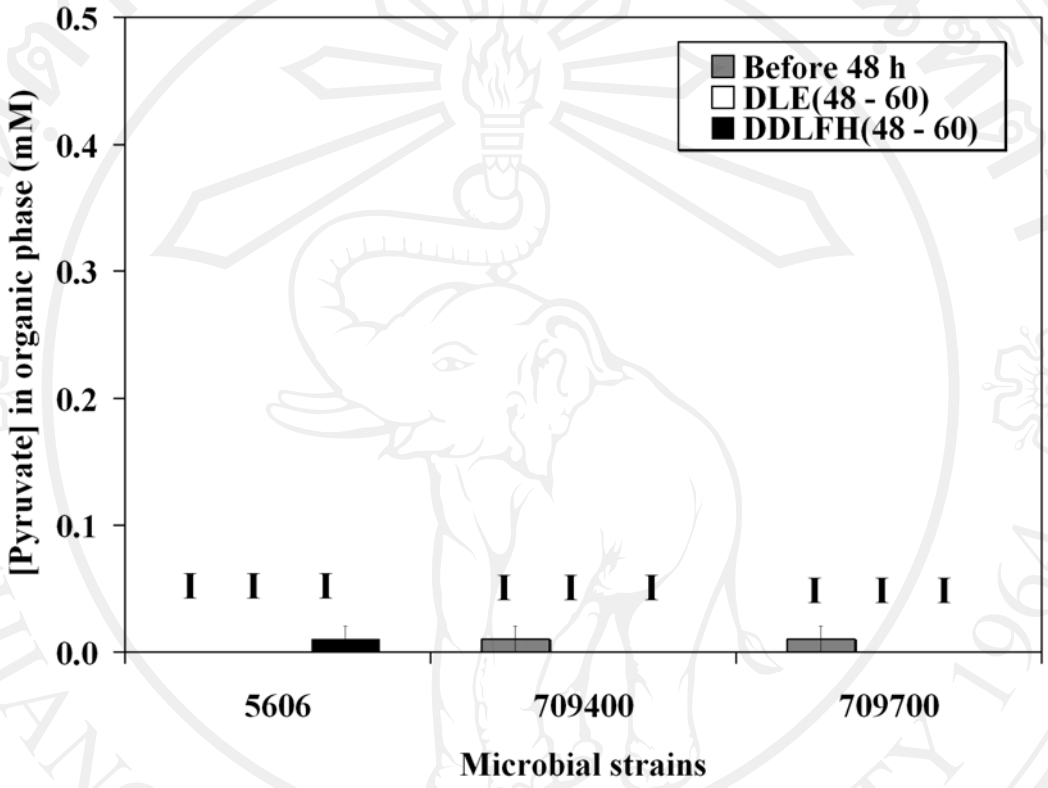


Figure 4.23 The remnant pyruvate concentration (mM) in organic phases after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.39 The statistical comparison of remnant pyruvate concentration in organic phase for two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	[pyruvate] (mM) in organic phase								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	0.02 ± 0.02	A	I	0.03 ± 0.03	A	I	0.03 ± 0.03	A	I
C. utilis UNSW 709400	0.05 ± 0.05	A	I	0.07 ± 0.05	A	I	0.08 ± 0.05	A	I
C. utilis UNSW 709700	0.02 ± 0.02	A	I	0.04 ± 0.02	A	I	0.05 ± 0.04	A	I

The number with the same Roman numeral (I) and alphabet (A) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

The highest level of overall consumed/unaccounted pyruvate concentration in both phases (54.9 ± 1.4 mM, Fig. 4.24 and Table 4.40) was achieved when the whole cells of *S. cerevisiae* TISTR 5606 in the condition of batch cultivation with DLE at 48th h fermentation period was used. This result was not significantly different ($p > 0.05$) from the whole cells harvested from condition of fed batch cultivation with DLE and DDLFH at 60th h fermentation period of 54.8 ± 1.3 and 52.9 ± 1.2 mM, respectively. The similar trend was observed for whole cells of *C. utilis* UNSW 709400 and 709700 with the corresponding values of overall consumed/unaccounted pyruvate concentration in the range of 52.1 – 55.3 mM.

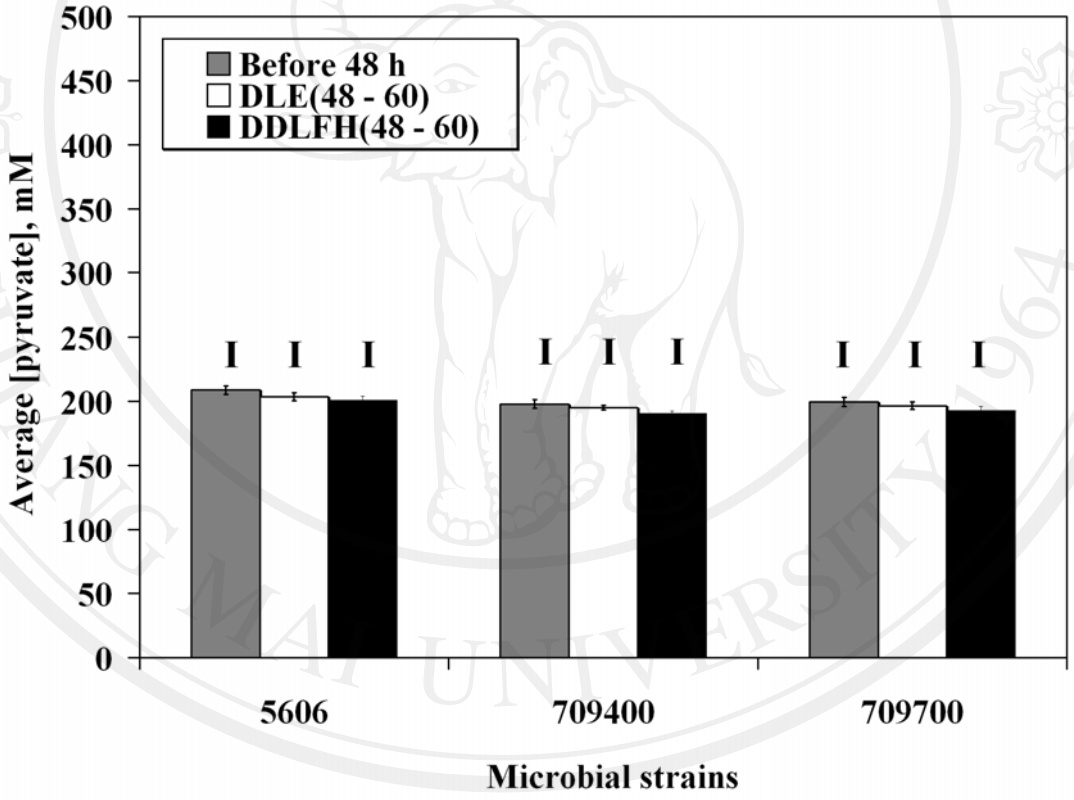


Figure 4.24 The overall consumed/unaccounted pyruvate concentration (mM) in both phases after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.40 The statistical comparison of consumed/unaccounted pyruvate concentration in both phase for two phases PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	[pyruvate] (mM) in both phases								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	209 ± 3	A	I	204 ± 4	A	I	201 ± 3	A	I
C. utilis UNSW 709400	198 ± 3	A	I	195 ± 3	A	I	191 ± 2	B	I
C. utilis UNSW 709700	199 ± 4	A	I	196 ± 2	A	I	193 ± 3	A, B	I

The number with the same Roman numeral (I) and alphabet (A – B) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.

The pyruvate balances (Fig. 4.25 and Table 4.41) obtained from PAC biotransformation using three microbial strains in the all conditions as the sources of PDC for PAC biotransformation were not significantly different ($p > 0.05$) with the corresponding values between 75.0 – 78.7% (6.05 ± 0.97% PAC and 3.11 ± 0.10% acetaldehyde were produced in absence of acetoin).

The highest of pyruvate balance for *S. cerevisiae* TISTR 5606 resulted from this study (77.0 ± 1.1%, Table 4.41) was not significantly different ($p > 0.05$) from the pyruvate balance determined by Agustina (2009) who employed the whole cells from the similar microbial strain in two – phase separated biotransformation system. In that study, whole cells of *S. cerevisiae* TISTR 5606 resulted in 77.9 ± 3.2% pyruvate balance where 17.5% of PAC and 6.80% acetaldehyde were produced (standard errors were not reported) from the consumed pyruvate in absence of acetoin.

The highest pyruvate balances for *C. utilis* UNSW 709400 and 709700 resulted from this study (78.7 ± 0.8 and 77.9 ± 1.3%, respectively) were lower than the pyruvate balance determined by Rosche *et al*. (2005) who employed the resting cells from *C. utilis* UNSW 709400 in the similar biotransformation condition (1.44 M benzaldehyde and 1.43 M pyruvate).

In that study, resting cells from *C. utilis* UNSW 709400 resulted in 89.0% pyruvate balance where 72.0% of pyruvate was used to produce PAC, 2.05% acetaldehyde, 5.78% acetoin, 9.17% of pyruvate was remained, and 11.0% was lost/not detected (standard errors were not reported). These were compared to the current study where 4.54 ± 0.88% of pyruvate was used to produce PAC, 3.06 ± 0.11% acetaldehyde, 69.8 ± 0.9% pyruvate was remained, and 22.6 ± 0.3% was lost/not detected in absence of acetoin. In addition, Rosche *et al*. (2005) also stated that the loss of pyruvate might be due to evaporative loss of by – product acetaldehyde during biotransformation.

However, the highest of pyruvate balance for *C. utilis* UNSW 709400 and 709700 in this study was higher than the pyruvate balance determined by Agustina (2009) who employed the whole cells from the similar microbial strain in two – phase separated biotransformation system. In that study, whole cells from *C. utilis* TISTR 5198 resulted in 77.9 ± 3.2% pyruvate balance where 55.9% of pyruvate was used to produce PAC, 12.0% acetaldehyde, as well as 10.0% was remained, and 22.1% was lost/not detected (standard errors were not reported) in absence of acetoin.

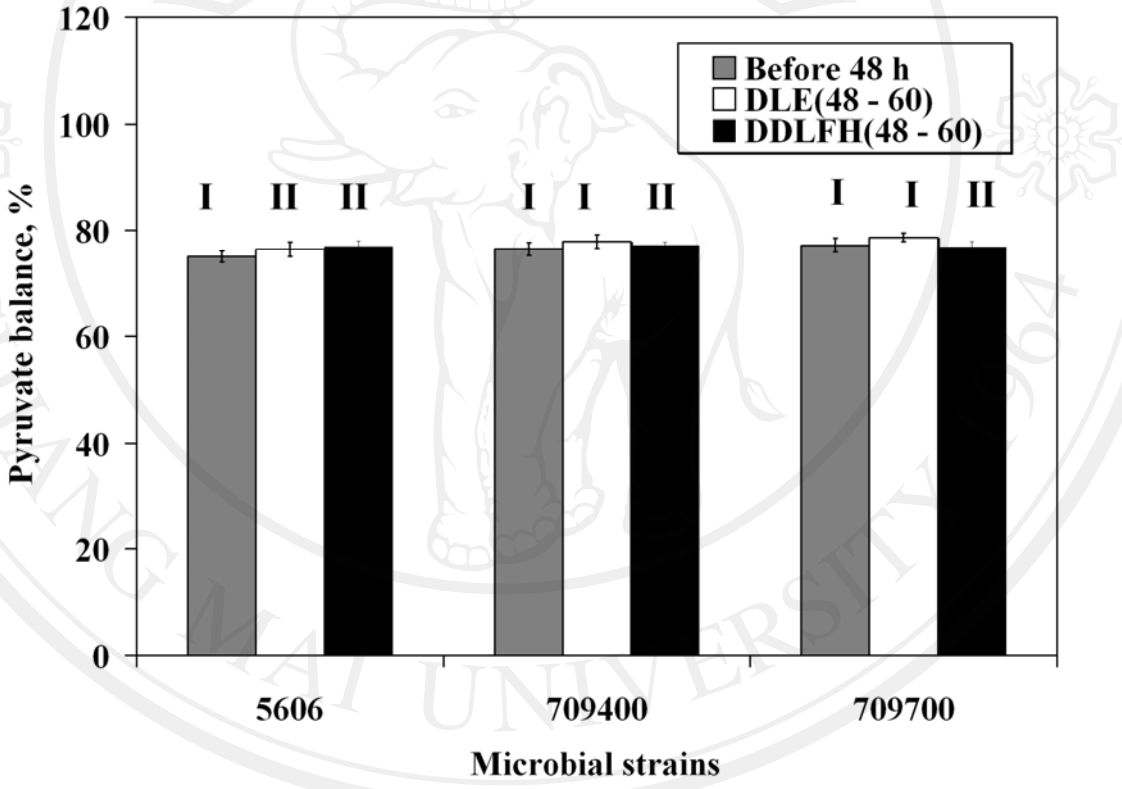


Figure 4.25 The average pyruvate balance (%) in both phases after 72 h biotransformation with adopting whole cells from 3 microbial strains cultivated in various conditions.

Table 4.41 The statistical comparison of pyruvate balance (%) in two phase PAC biotransformation system using whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700.

Investigated parameters	pyruvate balance (%) in both phases								
	Before 48 h			DLE (48 – 60 h)			DDLFH (48 – 60 h)		
S. cerevisiae TISTR 5606	75.0 ± 1.1	A	I	76.4 ± 1.4	A	I	77.0 ± 1.1	A	I
C. utilis UNSW 709400	76.5 ± 1.2	A	I	77.9 ± 1.3	A	I	77.1 ± 0.7	A	I
C. utilis UNSW 709700	77.2 ± 1.3	A	I	78.7 ± 0.8	A	I	76.8 ± 1.1	A	I

The number with the same Roman numeral (I) and alphabet (A) indicated no significant difference ($p > 0.05$) for comparison between different columns of the same row and different rows of the same column, respectively.