CHAPTER 4

RESULTS

4.1 Component of sugar depleted dried longan (SDDL)

Initial sugar type and sugar content in SDDL was measured by HPLC and DNS method. DNS analysis revealed that dried longan contained 2.72 ± 0.55 g/l or 9.08 ± 1.82 mg/g dried substrate of reducing sugar. The results showed that three types of sugar (sucrose, glucose and fructose) were found in SDDL (Table 4.1). The maximum content was glucose (1.70 ± 1.31 mg/g) followed by fructose (0.95 ± 0.18 mg/g) and sucrose (0.36 ± 0.01 mg/g). From these results, SDDL had low sugar which was not available to be used in ethanol fermentation by yeast.

Table 4.1: Amount of sugar in sugar depleted dried longan

Sugar	**Concentration (g/l)**	**Concentration (mg/g dried substrate)**
Sucrose	0.11 ± 0.01	0.36 ± 0.01
Glucose	0.51 ± 0.39	1.70 ± 1.31
Fructose	0.28 ± 0.05	0.95 ± 0.18

Moreover, some of the main composition of SDDL were analyzed i.e. cellulose and hemicelluloses by modified method from T 203 om-88, lignin by modified method from T 222 om-88, pectin by modified method from Kertez (1951) and starch (Chow and Landhausser, 2004). Results were shown in Table 4.2., indicated that SDDL contained hemicellulose as the major content about 31.71 ± 3.25 % (w/w) followed by lignin and starch content at 24.24 ± 0.64 % (w/w) and 21.59 ± 0.79 % (w/w), respectively.

Table 4.2: Chemical composition of sugar depleted dried longan (% dry weight basis)

Component	% Dry weight basis
Cellulose	15.01 ± 0.65
Hemicellulose	31.71 ± 3.25
Lignin	24.24 ± 0.64
Pectin	8.38 ± 0.24
Starch	21.59 ± 0.79

4.2 Enzyme activities from fungal fermentation

Five fungal strains (*Aspergillus niger* TISTR 3063 and 3089, *A. foetidus* TISTR 3461 and *Trichoderma reesei* TISTR 3080 and 3081) were used to treat SDDL for 10 days in order to produce reducing sugar. Enzyme activities (cellulase, xylanase, mannanase, pectinase and amylase) were observed. The results showed that all fungal strains could grow and produce enzymes on SDDL (Figure 4.1, 4.2, 4.3, 4.4 and 4.5 and Table F.1, F.2, F.3, F.4 and F.5). The strains capable of producing the highest cellulase, xylanase, mannanase, pectinase and amylase activity are shown in Table 4.3.

Results showed that, *A. niger TISTR* 3063 showed maximum cellulase activity, it was about 1.93 ± 0.56 U/gds at 8 day-cultivation. *A. niger TISTR* 3089 showed maximum xylanase activity (5.98 ± 0.11 U/gds) and mananase activity (3.80 ± 0.72 U/gds) after culturing for 2 and 8 days, respectively. *A. foetidus* TISTR 3461 showed maximum pectinase activity (14.59 ± 0.82 U/gds) at 9 day-cultivation and amylase activity (21.49 ± 1.41 U/gds) at 2 day-cultivation but cellulase activity was not found.

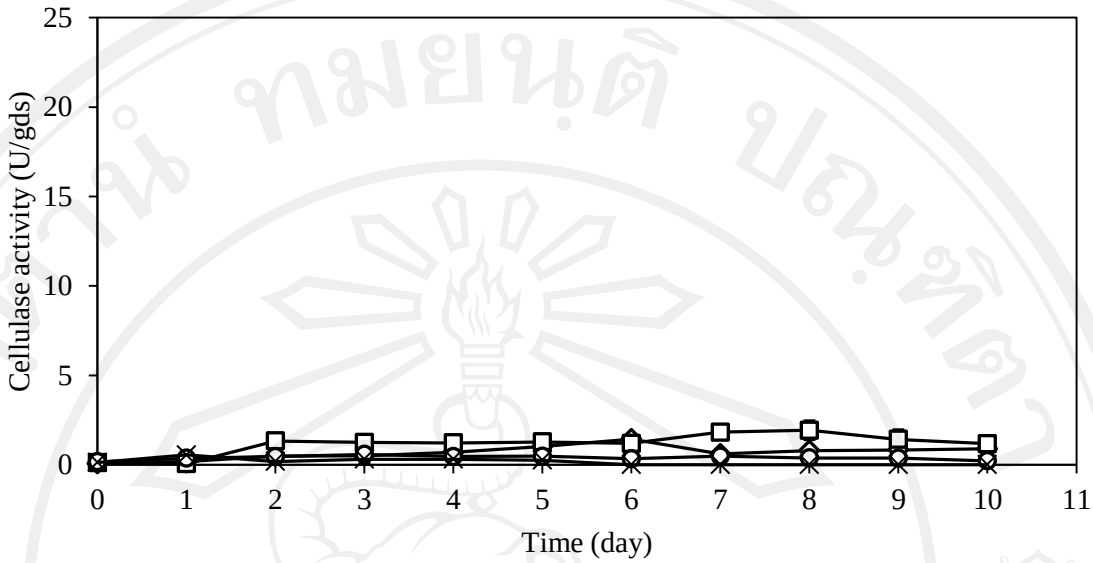

Figure 4.1: Cellulase activity during 10 days cultivation: (□) *A. niger* TISTR 3063; (◇) *A. niger* TISTR 3089; (△) *A. foetidus* TISTR 3461; (×) *T. reesei* TISTR 3080; (○) *T. reesei* TISTR 3081

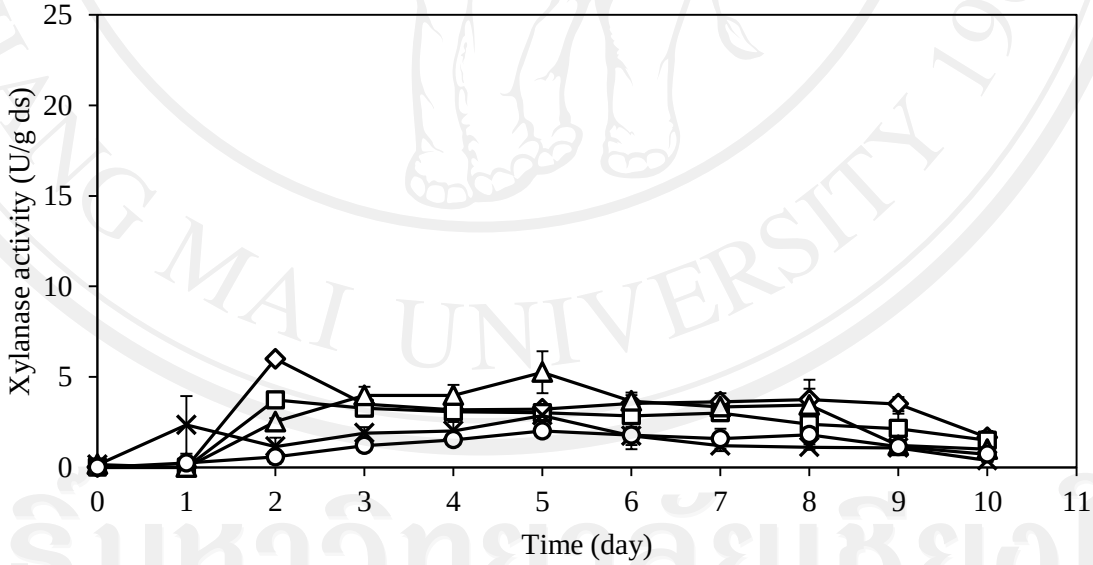

Figure 4.2: Xylanase activity during 10 days cultivation: (□) *A. niger* TISTR 3063; (◇) *A. niger* TISTR 3089; (△) *A. foetidus* TISTR 3461; (×) *T. reesei* TISTR 3080; (○) *T. reesei* TISTR 3081.

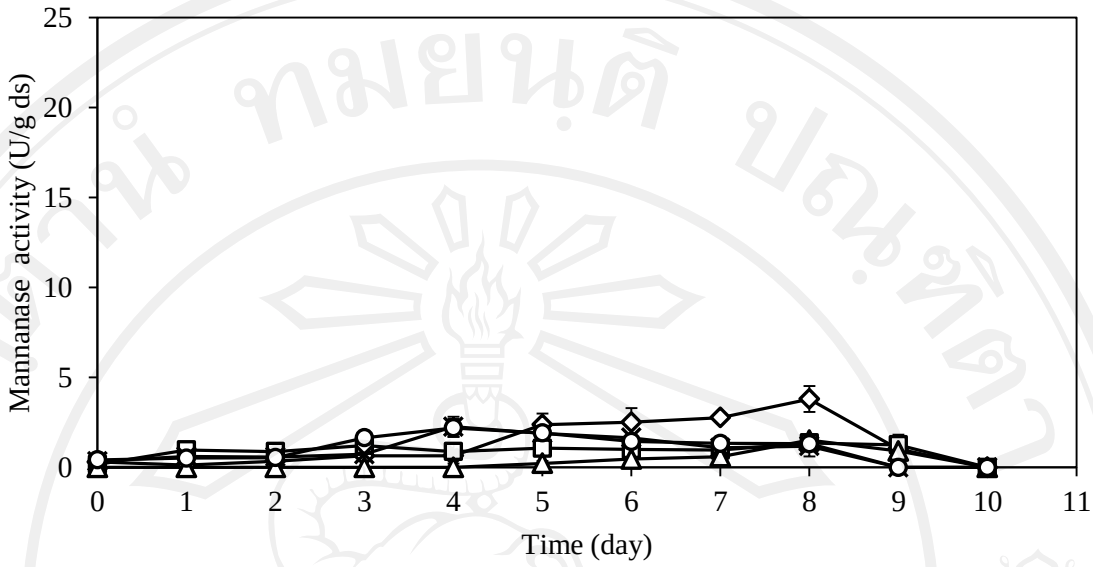

Figure 4.3: Mannanase activity during 10 days cultivation: (□) *A. niger* TISTR 3063; (◇) *A. niger* TISTR 3089; (△) *A. foetidus* TISTR 3461; (×) *T. reesei* TISTR 3080; (○) *T. reesei* TISTR 3081.

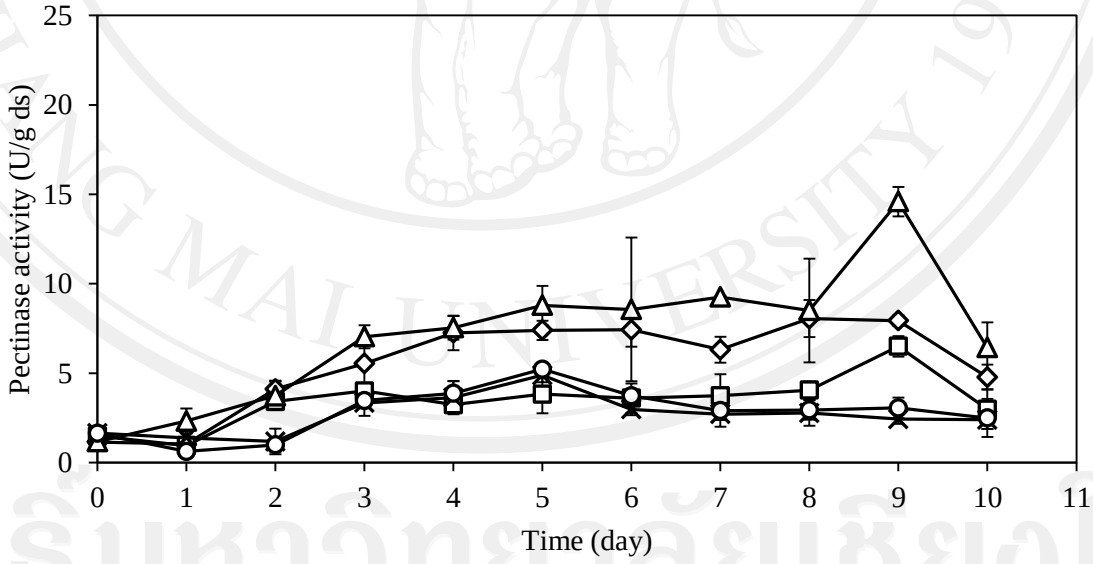

Figure 4.4: Pectinase activity during 10 days cultivation: (□) *A. niger* TISTR 3063; (◇) *A. niger* TISTR 3089; (△) *A. foetidus* TISTR 3461; (×) *T. reesei* TISTR 3080; (○) *T. reesei* TISTR 3081.

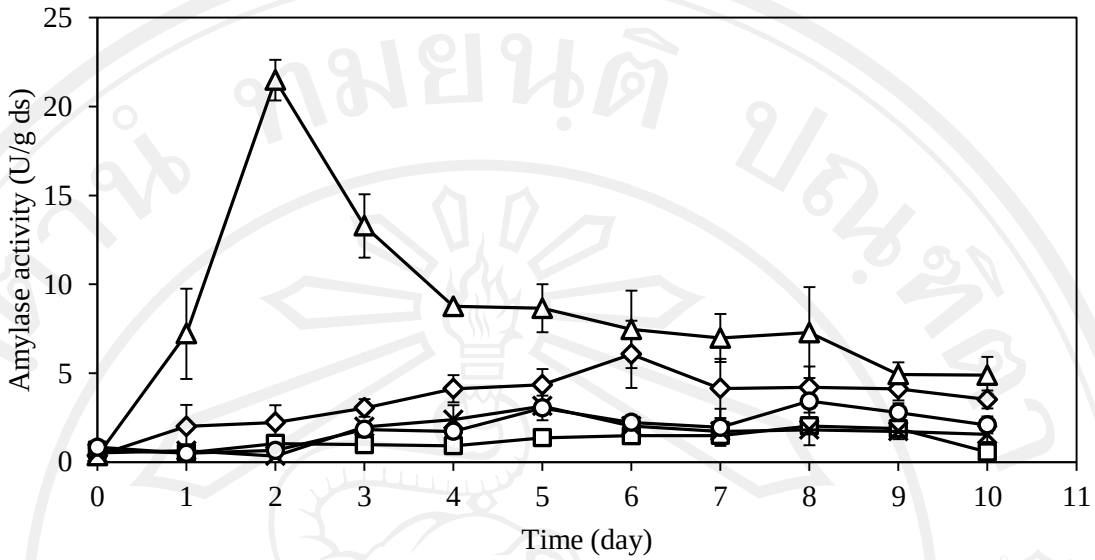

Figure 4.5: Amylase activity during 10 days cultivation: (□) *A. niger* TISTR 3063; (◇) *A. niger* TISTR 3089; (△) *A. foetidus* TISTR 3461; (×) *T. reesei* TISTR 3080; (○) *T. reesei* TISTR 3081.

Table 4.3: Maximum cellulase, xylanase, mannanase, pectinase and amylase activities of 5 strains of fungi.

Enzyme	**Fungi**	**Activity (U/gds)**	**Cultivation time (day)**
Cellulase	*A. niger* TISTR 3063	1.93 ± 0.56	8
Xylanase	*A. niger* TISTR 3089	5.98 ± 0.11	2
Mannanase	*A. niger* TISTR 3089	3.80 ± 0.72	8
Pectinase	*A. foetidus* TISTR 3461	14.59 ± 0.82	9
Amylase	*A. foetidus* TISTR 3461	21.49 ± 1.41	2

4.3 Screening of 5 strains of fungi for maximum reducing sugar production

During 10-days cultivation, all fungal cultures were extracted with 0.05 M acetate buffer pH 5.0 for enzyme and reducing sugar analysis. Reducing sugar in the extract was analyzed by DNS method. The results showed that all fungal strains could produce reducing sugar from ground SDDL (Figure 4.6 and Table D.1). The maximum reducing sugar contents obtained from fungal fermentation are shown in Table 4.4.

From Figure 4.6, the results showed that the reducing sugar contents of *A. foetidus* TISTR 3461 extract increased rapidly than the other strains. The highest reducing sugar content was obtained when using *A. foetidus* TISTR 3461 to treat SDDL at day 7 (30.84 ± 1.02 g/l or 102.79 ± 3.39 mg/g dried substrate). The second in rank was *A. niger* TISTR 3089 which produced reducing sugar at 25.10 ± 0.55 g/l or 83.67 ± 1.84 mg/g dried substrate.

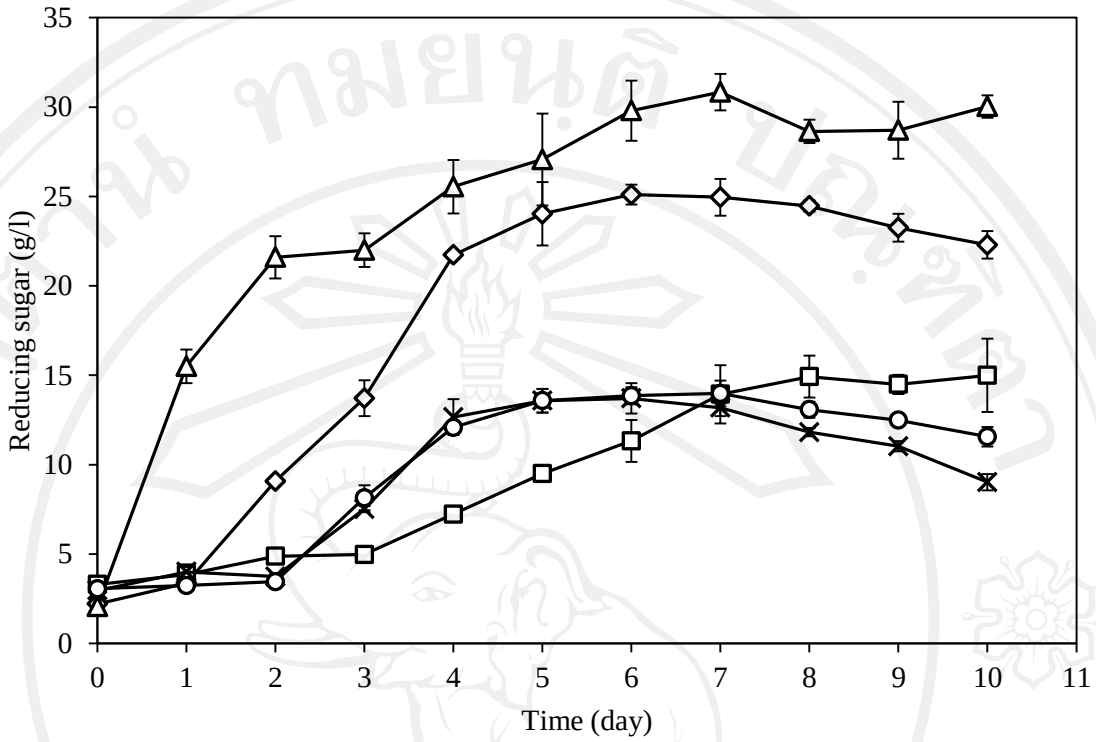

Figure 4.6: Reducing sugar during 10 days cultivation: (□) *A. niger* TISTR 3063; (◇) *A. niger* TISTR 3089; (△) *A. foetidus* TISTR 3461; (×) *T. reesei* TISTR 3080; (○) *T. reesei* TISTR 3081.

Table 4.4: Maximum reducing sugar obtained from fungal fermentation.

Fungal Strains	Time (day)	Reducing sugar (g/l)	Reducing sugar/substrate (mg/g dried substrate)
A. niger TISTR 3063	10	15.00 ± 2.05[a]	49.99 ± 6.83[a]
A. niger TISTR 3089	6	25.10 ± 0.55	83.67 ± 1.84
A. foetidus TISTR 3461	7	30.84 ± 1.02	102.79 ± 3.39
T. reesei TISTR 3080	6	13.71 ± 0.85 [a]	45.69 ± 2.82[a]
T. reesei TISTR 3081	7	13.99 ± 0.72 [a]	46.62 ± 2.42 [a]

The numbers with the same alphabet (a), for comparison between each row of the same column, indicated no significant difference ($p \leq 0.05$).

4.4 Ethanol production from fungal treatment extract

From the result of reducing sugar production, *A. foetidus* TISTR 3461 and *A. niger* TISTR 3089 were selected to produce reducing sugar by SDDL. After fermentation, the reducing sugar was extracted with RO water, concentrated to around 100 g/l and used in ethanol fermentation. YMB with 100 g/l glucose, 100 g/l glucose solution and SDDL without fungal treatment extract with 100 g/l glucose were used as control for ethanol production. YMB with 100 g/l glucose was used for maximum ethanol production possibility because it contained beneficial supplements such as yeast extract, malt extract and peptone. Glucose solution at the concentration of 100 g/l glucose was used to investigate ethanol production by yeast in glucose solution without nitrogen source. SDDL without fungal treatment extract with 100 g/l glucose was used to study components in SDDL which may improve or inhibit ethanol production. All substrates were adjusted pH to 5.0 and sterilized by autoclaving. The final extracts were inoculated by *Saccharomyces cerevisiae* TISTR 5606 and incubated at 30ºC in static condition for 120 h. Samples were taken every 12 h for analysis. Yeast dried cell weight, fermentable reducing sugar content (obtained at the end of ethanol fermentation), glucose concentration and ethanol concentration during cultivation were analyzed and are shown in Figure 4.7, 4.8, 4.9 and 4.10 and Table I.1, I.2, I.3 and I.4, respectively. DCW, glucose consumption, and ethanol prodcution are shown in Table 4.5, 4.6 and 4.7 and the ethanol production yield ($Y_{P/S}$) is shown in Table 4.8.

From Figure 4.7, the dried cell weight of yeast using *A. foetidus* TISTR 3461 extract and *A. niger* TISTR 3089 extract as culture media increased rapidly during 0 to 12 h with slightly increase after that. The specific growth rate using the both fungal extracts (0.078 ± 0.005 h^{-1} for *A. foetidus* TISTR 3461 extract and 0.070 ± 0.021 h^{-1} for *A. niger* TISTR 3089 extract) were not different ($p \leq 0.05$) but both of them were lower than using YMB as media (Table 4.5). The maximum dry cell weight was obtained from using YMB as substrate. In case of fungal extract, there was no significant difference ($p \leq 0.05$) between maximum dry cell weight when using *A. niger* TISTR 3089 extract (1.36 ± 0.04 g/l) and *A. foetidus* TISTR 3461 extract (1.33 ± 0.13 g/l).

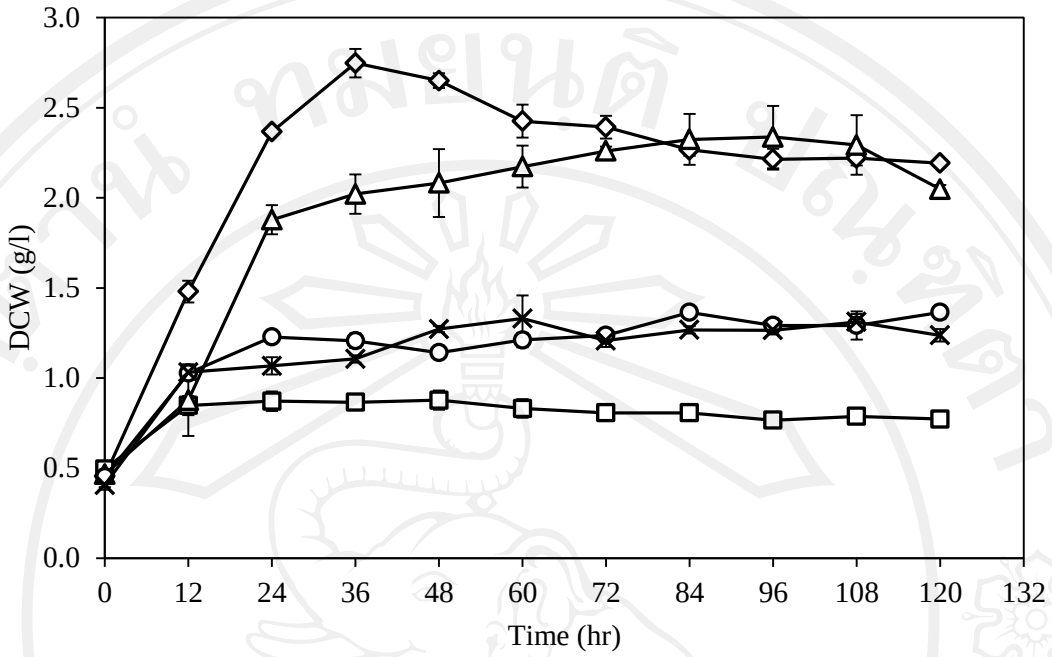

Figure 4.7: Dried cell weight (DCW) of *S. cerevisiae* TISTR 5606 in YMB (◇), glucose solution (□), SDDL without fungal treatment extract with glucose (△), *A. foetidus* TISTR 3461 extract (×) and *A. niger* TISTR 3089 extract (○).

Table 4.5: Comparison of the specific growth rate (μ) and the maximum DCW of *S. cerevisiae* TISTR 5606 in YMB, glucose solution, SDDL (no treatment) extract, *A. foetidus* TISTR 3461 extract and *A. niger* TISTR 3089 extract.

Substrate	**μ**		**Maximum DCW**	
	Time (h)	**(h^{-1})**	**Time (h)**	**(g/l)**
YMB	12	0.098 ± 0.004	36	2.75 ± 0.08
Glucose solution	12	0.045 ± 0.002^{a}	48	0.88 ± 0.05
SDDL extract with glucose	24	0.058 ± 0.002^{ab}	96	2.34 ± 0.17
A. foetidus TISTR 3461 extract	12	0.078 ± 0.005^{c}	60	1.33 ± 0.22^{a}
A. niger TISTR 3089 extract	12	0.070 ± 0.021^{bc}	120	1.36 ± 0.05^{a}

The numbers with the same alphabet (a,b,c), for comparison between each row of the same column, indicated no significant difference ($p \leq 0.05$).

From Figure 4.8 and 4.9, the amount of fermentable reducing sugar detected by DNS method was similar to glucose concentration detected by HPLC. Therefore, the fermentable sugar from fungal extract was glucose. *S. cerevisiae* TISTR 5606 utilized glucose in fungal extract faster than glucose solution but slower than in YMB and in SDDL without fungal treatment extract. The maximum rate of glucose consumption in fungal extract was also higher ($p \leq 0.05$) than glucose solution but lower ($p \leq 0.05$) than using YMB and SDDL without fungal treatment extract (Table 4.6). However, the maximum specific rate of glucose consumption when using both fungal extracts as substrate were not different ($p \leq 0.05$) with using YMB as substrate. Their maximum rate of sugar consumption and maximum specific rate of sugar consumption from using *A. foetidus* TISTR 3461 extract and *A. niger* TISTR 3089 as substrate were not different ($p \leq 0.05$) at 1.64 ± 0.12 g/l/h and 1.46 ± 0.10 g/l/h, and 1.59 ± 0.12 g/l/h and 1.43 ± 0.14 g/l/g, respectively. Glucose concentration from the both fungal extracts was decreased to near-zero at 72-h cultivation.

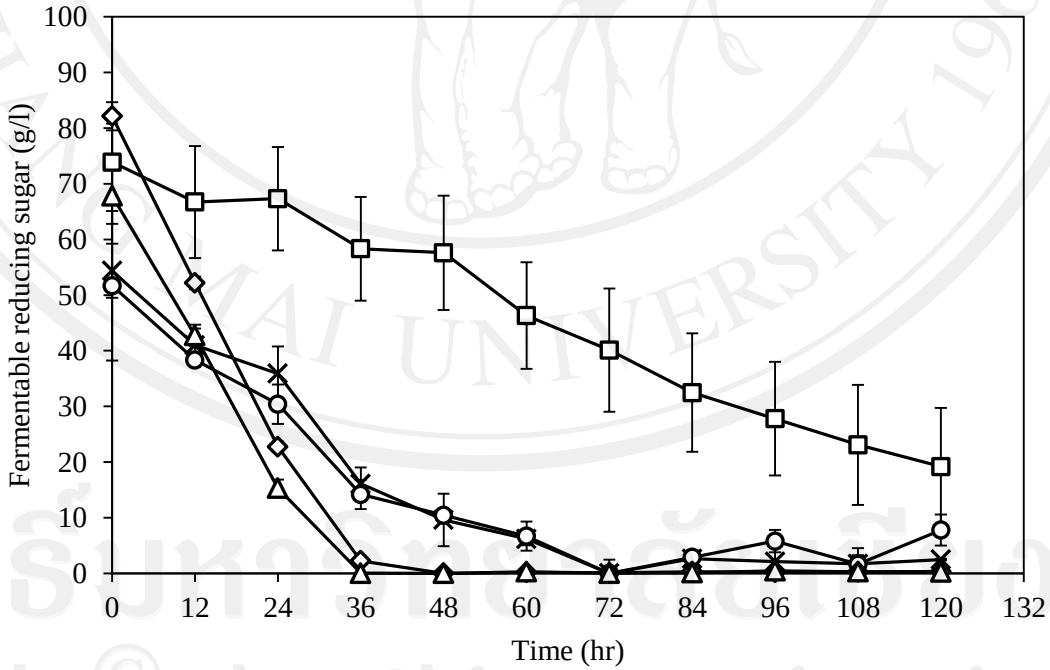

Figure 4.8: Fermentable reducing sugar of *S. cerevisiae* TISTR 5606 in YMB (◇), glucose solution (□), SDDL without fungal treatment extract with glucose (△), *A. foetidus* TISTR 3461 extract (×) and *A. niger* TISTR 3089 extract (○).

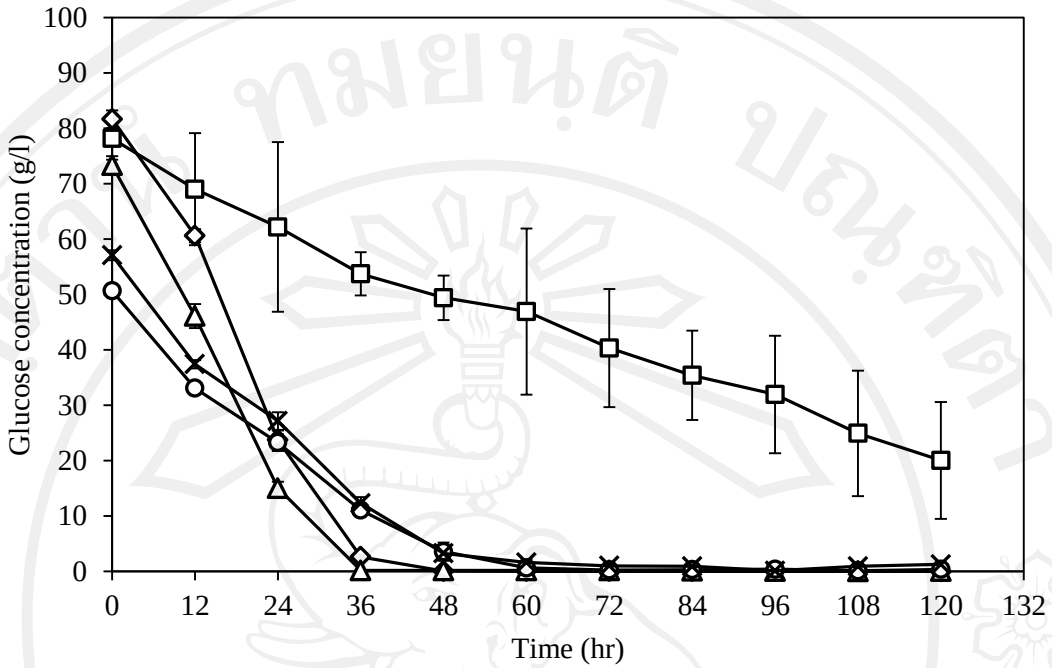

Figure 4.9: Glucose consumption of *S. cerevisiae* TISTR 5606 in YMB (◇), glucose solution (□), SDDL without fungal treatment extract with glucose (△), *A. foetidus* TISTR 3461 extract (×) and *A. niger* TISTR 3089 extract (○).

Table 4.6: Comparison of the maximum rate of glucose consumption (r_{smax}) and the maximum specific rate of glucose consumption (q_{smax}) of *S. cerevisiae* TISTR 5606 in YMB, glucose solution, SDDL (no treatment) extract, *A. foetidus* TISTR 3461 extract and *A. niger* TISTR 3089 extract.

Substrate	**r_{smax}**		**q_{smax}**	
	Time (h)	**(g/l/h)**	**Time (h)**	**(g/g/h)**
YMB	24	2.41 ± 0.07^{a}	12	1.18 ± 0.07^{ab}
Glucose solution	12	0.76 ± 0.39^{c}	12	0.90 ± 0.46^{a}
SDDL extract with glucose	24	2.43 ± 0.16^{a}	12	2.59 ± 0.47^{c}
A. foetidus TISTR 3461 extract	12	1.64 ± 0.12^{b}	12	1.59 ± 0.12^{b}
A. niger TISTR 3089 extract	12	1.46 ± 0.10^{b}	12	1.43 ± 0.14^{ab}

The numbers with the same alphabet (a,b,c), for comparison between each row of the same column, indicated no significant difference ($p \leq 0.05$).

From Figure 4.10, the results show that *S. cerevisiae* TISTR 5606 cultured on *A. foetidus* TISTR 3461 extract and *A. niger* TISTR 3089 extract produced ethanol at slower rate than cultured on YMB and extract of SDDL without fungal treatment with glucose. The slowest ethanol production rate was from glucose solution (0.24 ± 0.02 g/l/h). There were not different in maximum ethanol production rate and maximum specific rate of ethanol production between using *A. foetidus* TISTR 3461 extract and *A. niger* TISTR 3089 extract as substrate (Table 4.7). Moreover, the maximum specific rates of ethanol production from both fungal extracts were similar to YMB but lower than SDDL extract with glucose. For maximum ethanol production, *S. cerevisiae* TISTR 5606 cultured in *A. foetidus* TISTR 3461 extract produced higher ethanol concentration than cultured in *A. niger* TISTR 3089 extract.

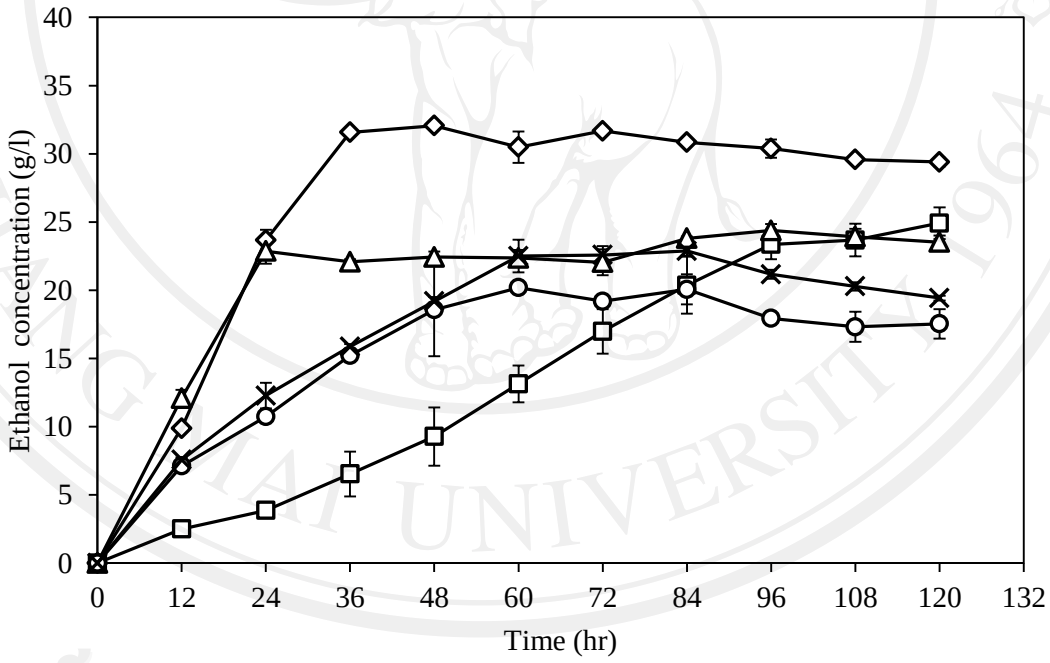

Figure 4.10: Ethanol production of *S. cerevisiae* TISTR 5606 in YMB (◇), glucose solution (□), SDDL without fungal treatment extract with glucose (△), *A. foetidus* TISTR 3461 extract (×) and *A. niger* TISTR 3089 extract (○).

Table 4.7: Comparison of the maximum rate of ethanol production (r_{pmax}), maximum specific rate of ethanol production (q_{pmax}) and the maximum ethanol production of *S. cerevisiae* TISTR 5606 in YMB, glucose solution, SDDL (no treatment) extract, *A. foetidus* TISTR 3461 extract and *A. niger* TISTR 3089 extract.

Substrate	**r_{pmax}**		**q_{pmax}**		**Maximum ethanol concentration**	
	Time (h)	**(g/l/h)**	**Time (h)**	**(g/l/h)**	**Time (h)**	**(g/l)**
YMB	24	0.99 ± 0.03^{a}	12	0.56 ± 0.03^{a}	48	32.06 ± 0.22
Glucose solution	72	0.24 ± 0.02^{c}	96	0.32 ± 0.003	120	24.92 ± 1.16^{a}
SDDL extract with glucose	12	1.01 ± 0.05^{a}	12	1.15 ± 0.06	96	24.38 ± 0.49^{a}
A. foetidus TISTR 3461 extract	12	0.63 ± 0.02^{b}	12	0.61 ± 0.01^{a}	84	22.88 ± 0.34
A. niger TISTR 3089 extract	12	0.59 ± 0.02^{b}	12	0.58 ± 0.04^{a}	60	20.19 ± 0.43

The numbers with the same alphabet (a,b,c), for comparison between each row of the same column, indicated no significant difference ($p \leq 0.05$).

From Table 4.8, the results showed that there was no significant difference ($p \leq 0.05$) between $Y_{p/s}$ and $Y_{p/s}$ at maximum ethanol production of using *A. foetidus* TISTR 3461 extract (0.35 ± 0.007g/g and 0.41 ± 0.003 g/g at 60 h) and *A. niger* TISTR 3089 extract (0.35 ± 0.02 g/g and 0.40 ± 0.005 g/g at 84 h) as substrate. Moreover, the ethanol production yield using *A. foetidus* TISTR 3461 extract and *A. niger* TISTR 3089 extract as substrate were not significant difference ($p \leq 0.05$) with using YMB and SDDL extract with glucose. Similarly the ethanol production yield at maximum ethanol production was not significant difference ($p \leq 0.05$) with using YMB and glucose solution.

Table 4.8: The ethanol production yield ($Y_{P/S}$) of *S. cerevisiae* TISTR 5606 in YMB, glucose solution, SDDL (not hydrolyzed by fungi) extract, *A. foetidus* TISTR 3461 extract and *A. niger* TISTR 3089 extract.

Substrate	**$Y_{P/S}$ (g/g)**	**$Y_{P/S}$ at maximum production time**	
		Time (h)	**(g/g)**
YMB	0.36 ± 0.01^{b}	48	0.39 ± 0.003^{a}
Glucose solution	0.43 ± 0.02^{c}	120	0.43 ± 0.02^{b}
SDDL extract with glucose	0.32 ± 0.01^{a}	96	0.33 ± 0.005^{c}
A. foetidus TISTR 3461 extract	0.35 ± 0.007^{ab}	84	0.41 ± 0.003^{ab}
A. niger TISTR 3089 extract	0.35 ± 0.02^{ab}	60	0.40 ± 0.005^{ab}

The numbers with the same alphabet (a,b,c), for comparison between each row of the same column, indicated no significant difference ($p \leq 0.05$).

4.5 Comparison of sterilized and non-sterilized extract on ethanol production

Sterilization of extract requires equipment and energy, omitting the process will lower the cost of production (Qin *et al*., 2009). Moreover, using non-sterilized substrate may reduce the problem from the Maillard reaction, which leads to the production of unfavorable furfural in sterilization process (Banerjee *et al*., 1981). Therefore, both fungal extracts were compared between sterilized extract with autoclaving and non-sterilization. After cultivation with *S. cerevisiae* TISTR 5606, glucose and ethanol concentrations throughout 120 h of cultivation time were analyzed.

The results obtained from using *A. foetidus* TISTR 3461 extract showed that the ethanol and glucose concentrations under the sterile treatment were similar to non-sterile treatment (Figure 4.11 and Table I.5, I.6). The maximum ethanol production from the non-sterile extract was 23.51 ± 0.76 g/l at 84 h of cultivation, which was not significantly different from sterile extract (22.88 ± 0.34 g/l at 60 h of cultivation) (Table 4.9). Under the sterile treatment, the maximum glucose consumption

(56.99 ± 0.99 at 96 h) was not different ($p \leq 0.05$) from the maximum glucose consumption of the non-sterile treatment (55.82 ± 1.98 g/l at 96 h of cultivation).

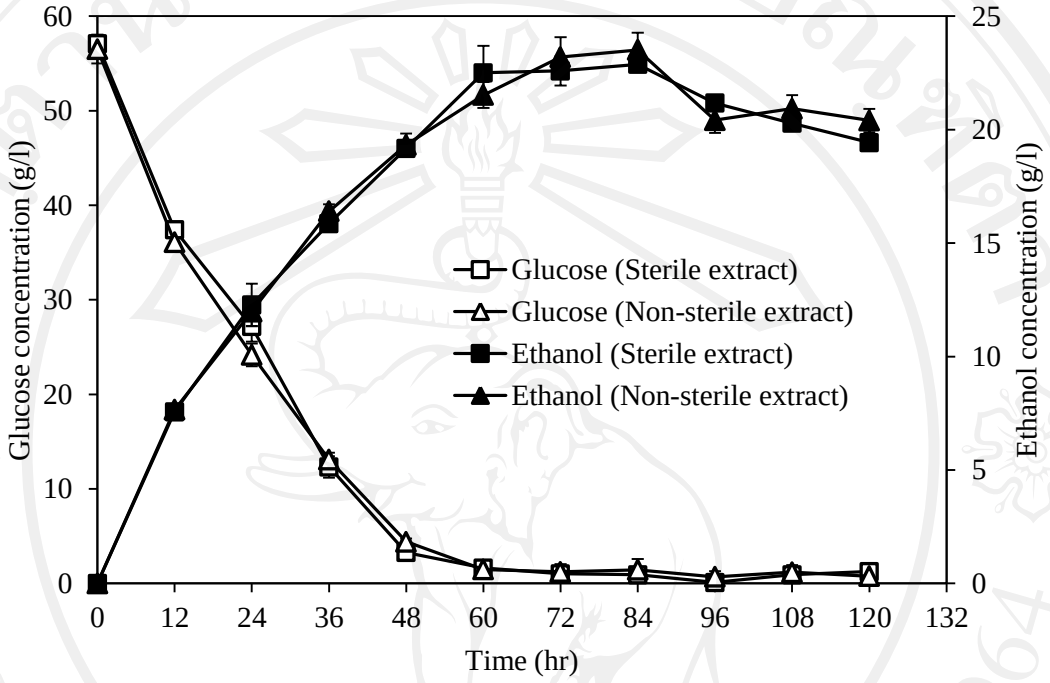

Figure 4.11: Glucose consumption and ethanol production of *S. cerivisiae* TISTR 5606 in the sterile and non-sterile extracts from *A. foetidus* TISTR 3461 cultured in SDDL.

Table 4.9: Comparison of the maximum ethanol production and glucose consumption of *S. cerevisiae* TISTR 5606 in the sterile and non-sterile extracts from *A. foetidus* TISTR 3461 cultured in SDDL.

Substrates	**Ethanol concentration**		**Glucose consumption**	
	Time (h)	**(g/l)**	**Time (h)**	**(g/l)**
Sterilize extract	60	22.88 ± 0.34^{a}	96	56.99 ± 0.99^{a}
Non-sterilize extract	84	23.51 ± 0.76^{a}	96	55.82 ± 1.98^{a}

The numbers with the same alphabet (a), for comparison between each row of the same column, indicated no significant difference ($p \leq 0.05$).

Table 4.10 indicated that the ethanol yield observed at the maximum ethanol production obtained at 84 h of fermentation were 0.43 ± 0.01 g/g when using the non-sterile extract, which were significantly higher ($p \leq 0.05$) than those observed in the sterile extract (0.41 ± 0.003 g/g). However, at the end of fermentation, there was no significant difference between the ethanol yield of sterile (0.35 ± 0.007 g/g) and non-sterile extracts (0.37 ± 0.008 g/g).

Table 4.10: The ethanol production yield ($Y_{P/S}$) of *S. cerevisiae* TISTR 5606 in the sterile and non-sterile extracts from *A. foetidus* TISTR 3461 cultured in SDDL.

Substrates	**$Y_{P/S}$**		**$Y_{P/S}$ at maximum production time**	
	Time (h)	**(g/g)**	**Time (h)**	**(g/g)**
Sterile extract	120	0.35 ± 0.007[a]	84	0.41 ± 0.003
Non-sterile extract	120	0.37 ± 0.008[a]	84	0.43 ± 0.01

The numbers with the same alphabet (a) are indicated no significant difference ($p \leq 0.05$).

Figure 4.12 showed that the profile of ethanol concentration in the sterile extract and non-sterile extract of *A. niger* TISTR 3089 was similar (Table I.5 and I.6). Maximum ethanol production and glucose consumption from the sterile extract were 20.19 ± 0.43 g/l and 50.56 ± 1.07 g/l, respectively and from the non-sterile extract were 18.86 ± 0.33 g/l and 51.51 ± 2.02 g/l, respectively (Table 4.11). However, the maximum ethanol production and the maximum glucose consumption in the sterile extract were not different ($p \leq 0.05$) from the non-sterile extract.

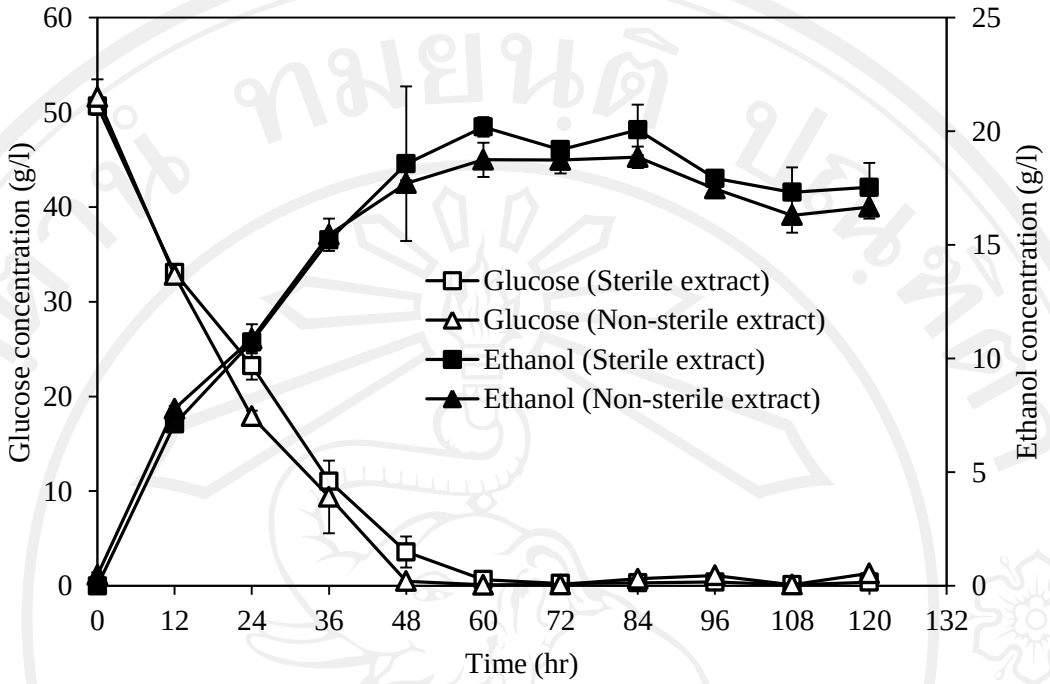

Figure 4.12: Glucose consumption and ethanol production of *S. cerevisiae* TISTR 5606 in the sterile and non-sterile extracts from *A. niger* TISTR 3089 cultured in SDDL.

Table 4.11: Comparison of the maximum ethanol production and glucose consumption of *S. cerevisiae* TISTR 5606 in the sterile and non-sterile extracts from *A. niger* TISTR 3089 cultured in SDDL.

Substrates	**Ethanol concentration**		**Glucose consumption**	
	Time (h)	**(g/l)**	**Time (h)**	**(g/l)**
Sterile extract	60	20.19 ± 0.43^{a}	108	50.56 ± 1.07^{a}
Non-sterile extract	84	18.86 ± 0.33^{a}	60	51.51 ± 2.02^{a}

The numbers with the same alphabet (a) are indicated no significant difference ($p \leq 0.05$).

Table 4.12 showed that no significant difference ($p \leq 0.05$) of ethanol yield between the sterile and non- sterile extract; they were 0.35 ± 0.02 g/g and 0.32 ± 0.004 g/g, respectively. Moreover, the maximum ethanol yield in the sterile extract was 0.40 ± 0.005 g/g which higher ($p \leq 0.05$) than the non-sterile extract (0.36 ± 0.005 g/g).

Table 4.12: The ethanol production yield ($Y_{P/S}$) of *S. cerevisiae* TISTR 5606 in the sterile and non-sterile extracts from *A. niger* TISTR 3089 cultured in SDDL.

Substrates	**$Y_{P/S}$**		**$Y_{P/S}$ at maximum production time**	
	Time (h)	**(g/g)**	**Time (h)**	**(g/g)**
Sterile extract	120	0.35 ± 0.02[a]	60	0.40 ± 0.005
Non-sterile extract	120	0.32 ± 0.004[a]	84	0.36 ± 0.005

The numbers with the same alphabet (a) are indicated no significant difference ($p \leq 0.05$).

4.6 Comparison of pH adjustment of the extract on ethanol production

Both fungal extracts were used to study the effect of pH adjustment on ethanol production by *S. cerevisiae* TISTR 5606 because the pH value of the both extracts were lower than the proper pH (pH 4 - 6) used to produce ethanol from *S. cerevisiae*. There were many research teams which used medium with pH 5 to produce ethanol by *S. cerevisiae* (Marium *et al*., 2009; Thuesombat *et al*., 2007; Zakpaa *et al*., 2009). Therefore, this study compared the ethanol production between pH-adjusted to 5 and no pH adjustment.

Glucose consumption and ethanol production of yeast during fermentation in the pH-adjusted extract and without pH adjusted extract from *A. foetidus* TISTR 3461 cultured in SDDL are shown in Figure 4.13 and Table I.7 and I.8. The maximum ethanol concentration, glucose consumption and pH value at the initial fermentation time are compared in Table 4.13. The ethanol production yield ($Y_{P/S}$) is shown in Table 4.14.

It was found that *S. cerevisiae* TISTR 5606 could grow and produce ethanol in both extracts (with and without pH adjustment) (Figure 4.13). The similar results were obtained i.e. complete utilization of glucose was observe at 72 h of fermentation time, while the ethanol concentration produced in the first 48 h and was relatively constant until the end of fermentation. It seemed that pH adjustment of the extract did not have significant effect on glucose utilization and ethanol production since the maximum glucose consumption in both groups were not significantly different ($p \leq 0.05$) (Table 4.11).

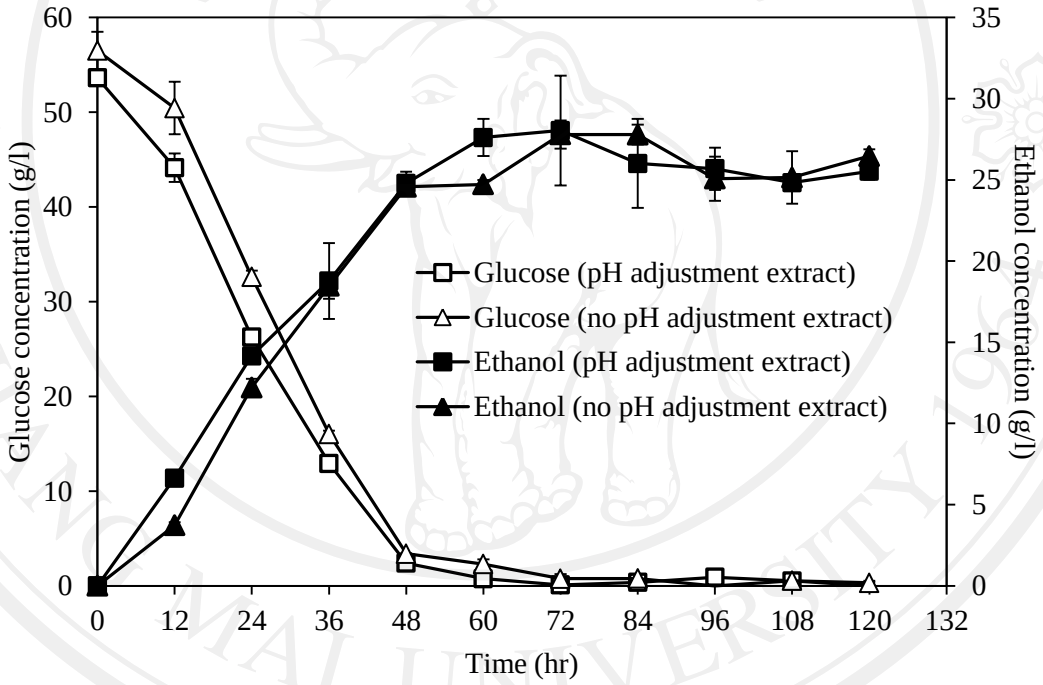

Figure 4.13: Glucose consumption and ethanol production of *S. cerivisiae* TISTR 5606 in the extract with and without pH adjustment from *A. foetidus* TISTR 3461 cultured in SDDL.

Table 4.13: Comparison of the maximum ethanol production and glucose consumption of *S. cerevisiae* TISTR 5606 in the extracts with and without pH-adjustment from *A. foetidus* TISTR 3461 cultured in SDDL.

Conditions	Initial pH	Ethanol concentration		Glucose consumption	
		Time (h)	(g/l)	Time (h)	(g/l)
pH adjustment	5.06 ± 0.03	72	28.04 ± 3.38[a]	120	53.63 ± 0.69[a]
No pH adjustment	3.92 ± 0.03	72	27.80 ± 0.86[a]	96	56.50 ± 2.3[a]

The numbers with the same alphabet (a), for comparison between each row of the same column, indicated no significant difference ($p \leq 0.05$)

Table 4.14 indicated that the ethanol yields of *S. cerevisiae* TISTR 5606 in the extract with and without pH adjustment (0.48 ± 0.01 g/g and 0.47 ± 0.01) were not different ($p \leq 0.05$) at the end of fermentation. Also, at 72 h of fermentation (maximum ethanol concentration), there was no significant difference ($p \leq 0.05$) between pH adjusted and no pH adjustment extract with the value of 0.52 ± 0.07 g/g and 0.50 ± 0.03 g/g, respectively, suggesting that prior pH adjustment is not required for ethanol fermentation when the extract from *A. foetidus* TSTR 3461 is used.

Table 4.14: The ethanol production yield ($Y_{P/S}$) of *S. cerevisiae* TISTR 5606 in the pH-adjusted and no pH adjustment extract from *A. foetidus* TISTR 3461 cultured in SDDL.

Substrates	$Y_{P/S}$		$Y_{P/S}$ at maximum production time	
	Time (h)	(g/g)	Time (h)	(g/g)
pH adjusted extract	120	0.48 ± 0.01[a]	72	0.52 ± 0.07[a]
No pH adjustment extract	120	0.47 ± 0.01[a]	72	0.50 ± 0.03[a]

The numbers with the same alphabet (a), for comparison between each row of the same column, indicated no significant difference ($p \leq 0.05$).

As shown in Figure 4.14, it was found that *S. cerevisiae* TISTR 5606 can produce ethanol only in the pH-adjusted extract from *A. niger* TISTR 3089 (Table I.7 and I8). Complete utilization of glucose was observed at 48 h of cultivation time and the ethanol concentration produced in the first 48 h and was relatively constant until the end of the fermentation. Ethanol and glucose concentration were stable after 48 h. The sugar consumption and ethanol production of *S. cerevisiae* TISTR 5606 in the extract with and without pH adjustment were compared (Table 4.15). The maximum glucose consumption and ethanol production in the pH-adjusted extract were 66.53 ± 2.26 g/l at 72 h and 27.92 ± 0.76 g/l at 60 h, respectively. In case of no pH adjustment extract, there was no ethanol produced by *S. cerevisiae* TISTR 5606 (data not shown).

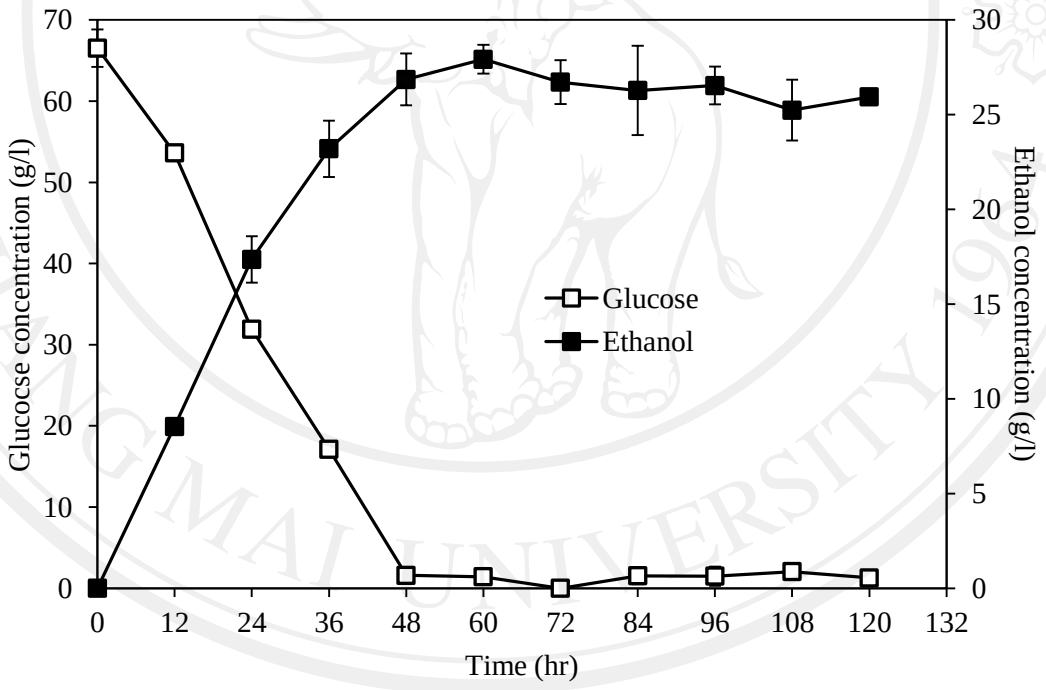

Figure 4.14: Glucose consumption and ethanol production of *S. cerevisiae* TISTR 5606 in the pH-adjusted extract from *A. niger* TISTR 3089 cultured in SDDL.

Table 4.15 Comparison of the maximum ethanol production and glucose consumption of *S. cerevisiae* TISTR 5606 in the extracts with and without pH-adjustment from *A. niger* TISTR 3089 cultured in SDDL.

Conditions	**Initial pH**	**Ethanol concentration**		**Glucose consumption**	
		Time (h)	**(g/l)**	**Time (h)**	**(g/l)**
pH adjustment	5.09 ± 0.01	60	27.92 ± 0.76	72	66.53 ± 2.26
No pH adjustment	2.54 ± 0.04	N/A	N/A	N/A	N/A

From Table 4.16, The ethanol yield of *S. cerevisiae* TISTR 5606 in the pH-adjusted was 0.40 ± 0.02 and 0.43 ± 0.02 g/g of at the maximum yield was obtained at 60 h of fermentation time.

Table 4.16 The ethanol production yield ($Y_{P/S}$) of *S. cerevisiae* TISTR 5606 in the pH-adjusted extract from *A. niger* TISTR 3089 cultured in SDDL.

Substrate	**$Y_{P/S}$**		**$Y_{P/S}$ at maximum production time**	
	Time (h)	**(g/g)**	**Time (h)**	**(g/g)**
pH-adjusted extract	120	0.40 ± 0.02	60	0.43 ± 0.02