APPENDICES

APPENDIX A

Preparation of dried longan extract

Materials

- Dried longan aged 3 years old
- Distilled water
- Boiling pot
- Natural gas tank

Procedure

- Extract of dried longan aged 3 years old was prepared at 30% (w/v).
- Twenty liters of distilled water was heated until boiled.
- Six kilograms of three years old dried longan, and the boling was continued for another 30 min.
- Separation of the extract and insoluble solid as conducted by sieve clothes and strainers.
- The extract was kept in 1 l aliquot at -20°C.

APPENDIX B

Preparation of digested dried longan flesh hydrolysate

Materials

- 3 years old dried longan flesh with low sugar level
- Tray dryer
- Hammer mill

Procedure

- Insoluble solids, which were separated from dried longan extract medium preparation (Appendix A), was dried by tray dryer (Armfield, Model No. UOP8, Ringwood, United Kingdom) at 65°C for 48 h.

65°C for 48 h

- Dried insoluble solid was crushed with hammer and milled with hammer mill (Armfield, Model No. FT2 - P, United Kingdom).

- The digested dried longan flesh hydrolysate was kept at -20°C.

APPENDIX C

Calculation of economical specific sugars production

C.1 Commercial enzyme addition strategy

C.1.1 Cost for preparation of dried longan extract

Table C.1 Cost for preparation of dried longan extract

Item	Cost (Baht)
1. Gas cooker	8,700.00
2. Boiling pot	967.00
3. Ladle	85.00
4. Sieve clothes	20.00
TOTAL	**9.772.00**

Reference: Chaweekunlayakun, 2010

C.1.2 Cost for application of portable pressure sterilizer

Table C.2 Cost for application of portable pressure sterilizer

Item	Cost (Baht)
1. Gas cooker	8,700.00
2. Portable pressure sterilizer	14,000.00
3. Natural gas	0.59
TOTAL	**22,700.59**

Reference: Chaweekunlayakun, 2010

C.1.3 The utilization of natural gas

Table C.3 The utilization of natural gas

Natural gas mass (g)	Cost (Baht)
15,000.00	310.00
1.00	0.02
The experiment was performed at 121°C, 30 min for 8 samples, with natural gas 227.00 g ;	
227.00	4.69
for 1 sample, with natural gas 227.00/8 = 28.38 g ;	
28.38	0.59

Reference: Chaweekunlayakun, 2010

C.1.4 Cost of enzymes

Table C.4 Cost of enzyme

Type of enzyme	Cost (Baht/g)
1. Ronozyme A (DSM Nutritional Product, 2007)	0.60
2. Ronozyme VP (DSM Nutritional Product, 2007)	0.70
3. Ronozyme WX (Roche Vitamins Ltd., 1999)	0.61

C.1.5 Cost of enzyme for each case

Table C.5 Cost of enzyme for each case

Enzyme addition strategy	Cost (Baht/g) for the application enzyme at	
	0.5 g	**1.0 g**
1. no enzyme	0.00	0.00
2. Only A	0.30	0.60
3. Only VP	0.35	0.70
4. Only WX	0.31	0.61
5. A & VP	0.33	0.65
6. A & WX	0.30	0.60
7. VP & WX	0.33	0.65
8. All types of enzyme	0.32	0.64

Therefore,

cost for commercial enzyme addition strategy for non - claving

= cost for preparation of dried longan extract + cost of enzyme in each case

cost for commercial enzyme addition strategy for claving

= cost for preparation of dried longan extract + cost for the application of portable pressure sterilizer + cost of enzyme in each case

C.2 Effects of digestion mixture ratio

C.2.1 Cost of dried longan

Table C.6 Cost of dried longan

Dried longan mass (g)	Cost (Baht)
1,000.00	42.82
10.00	0.43
20.00	0.86
30.00	1.28
50.00	2.14

Reference: Chaweekunlayakun, 2010

C.2.2 Cost of chemicals

Table C.7 Cost of chemicals

Chemical	Cost
Acetic acid (Labscan, 2007)	226 Baht/l
Sodium hydroxide (Labscan, 2007)	320 Baht/kg

APPENDIX D

Investigation of a correlation curve between dried biomass concentration and optical density at 600 nm (OD600) for three microbial strains

Wet biomass of three microbial strains from the cells cultivation without nitrogen source addition to dried longan extract was obtained. The biomass concentrations of *S. cerevisiae* TISTR 5606 was adjusted to 8 levels with 5 replicates using the final volume of 3, 4, 5, 6, 7, 8, 10, and 12 ml, respectively that resulted in the equivalent concentrations of 3.33, 2.50, 2.00, 1.67, 1.42, 1.25, 1.00, and 0.83 times of the original dried biomass concentration, respectively. While the biomass concentrations of *C. utilis* UNSW 709400 and 709700 were adjusted to 4 levels with 5 replicates using the final volume of 1.5, 2.5, 4, and 6 ml, respectively that resulted in the equivalent concentrations of 6.66, 4.00, 2.50, and 1.67 times of the original dried biomass concentration, respectively.

Samples from microbial cultivation were analyzed for the following; (1) optical density at 600 nm (OD600) and (2) dried biomass concentration. The computation of standard errors was based on 5 replicates and a correlation curve which correlated dried biomass concentration and optical density at 600 nm (OD600) was then constructed.

Fig. D.1 – D.3 illustrates correlation curves between dried biomass concentration and optical density at 600 nm (OD600) for *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400, and 709700 without nitrogen source.

Initially, wet biomass of *S. cerevisiae* TISTR 5606 from the cells cultivation without nitrogen source addition to dried longan extract was obtained prior to biomass concentration adjustment to 8 levels with 5 replicates as following; 19.93 ± 0.86, 13.93 ± 0.38, 10.78 ± 0.40, 8.73 ± 0.24, 7.59 ± 0.15, 6.92 ± 0.11, 5.54 ± 0.05, 4.56 ± 0.02 g/l. The value of R^2 from correlation curve was 0.9958 (Fig. D.1).

Initially, wet biomass of *C. utilis* UNSW 709400 from the cells cultivation without nitrogen source addition to dried longan extract was obtained prior to biomass concentration adjustment to 4 levels with 5 replicates as following; 24.03 ± 0.54, 14.83 ± 0.20, 9.74 ± 0.61, 5.76 ± 0.15 g/l. The value of R^2 from correlation curve was 0.9967 (Fig. D.2).

Initially, wet biomass of *C. utilis* UNSW 709700 from the cells cultivation without nitrogen source addition to dried longan extract was obtained prior to biomass concentration adjustment to 4 levels with 5 replicates as following; 25.80 ± 1.49, 13.92 ± 0.54, 8.34 ± 0.18, 6.08 ± 0.09 g/l. The value of R^2 from correlation curve was 0.9944 (Fig. D.3).

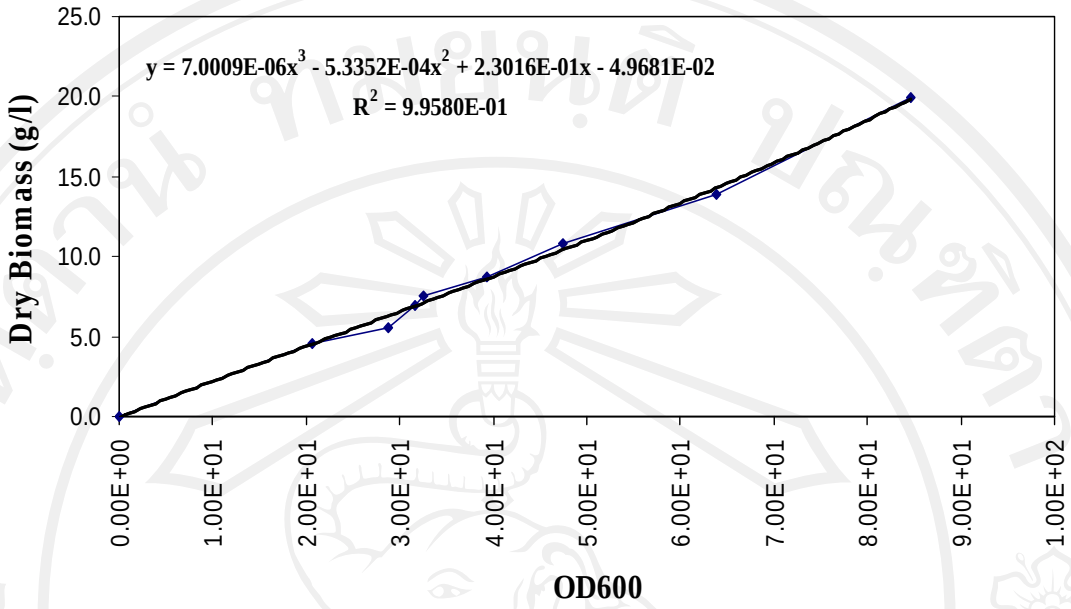

Figure D.1 Correlation curve between dried biomass concentration and optical density at 600 nm for *S. cerevisiae* TISTR 5606

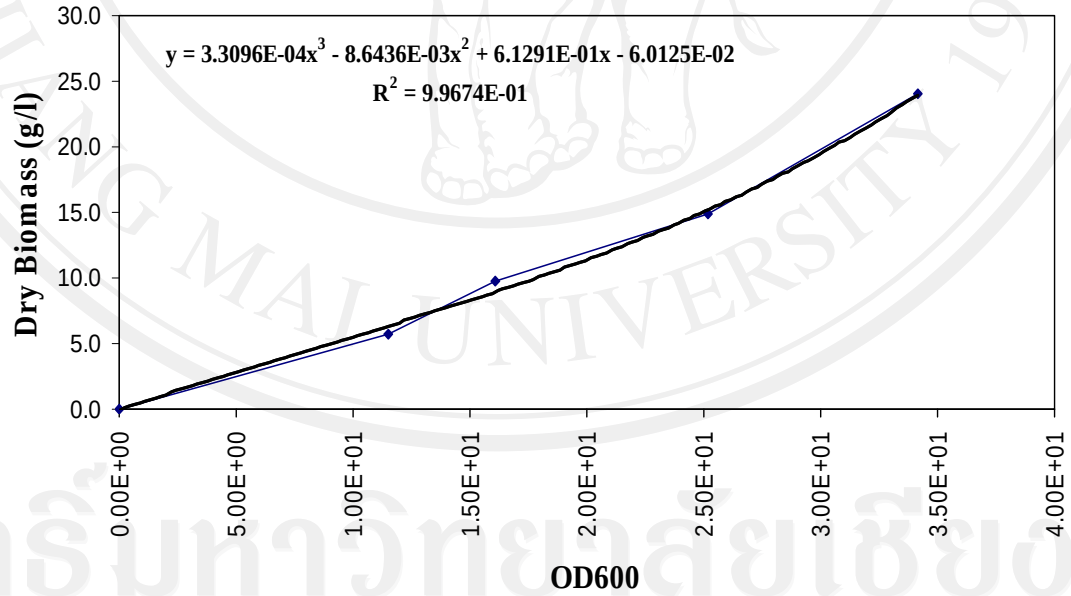

Figure D.2 Correlation curve between dried biomass concentration and optical density at 600 nm for *C. utilis* UNSW 709400

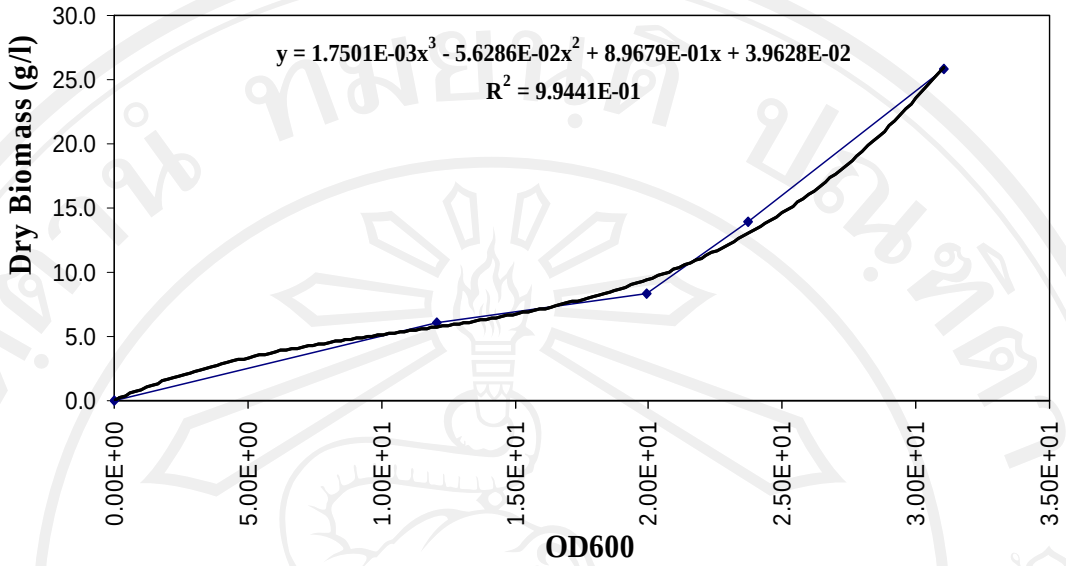

Figure D.3 Correlation curve between dried biomass concentration and optical density at 600 nm for *C. utilis* UNSW 709700

APPENDIX E

Calculation of kinetics parameters

The average specific growth rate

The instantaneous average specific growth rate was determined using:

$$\text{Avg. specific growth rate} = \frac{1}{X_{average}} \frac{dX}{dt}$$

where $X_{average}$ is the average dry biomass weight

$\frac{dX}{dt}$ is the rate of dry biomass production

Calculation sample for using *S.cerevisiae* 5606 after the addition of DLE media during 48 – 60 h to determine the average specific growth rate;

Time (h)	Dried biomass concentration (g/l)
48	6.67
51	7.65
54	8.20
57	8.64
60	9.08

Avg. specific growth rate =

$$\frac{\left(\frac{1}{X_{average}}\frac{dX}{dt}\text{during } 48\text{ - }51\text{ h}\right)+\left(\frac{1}{X_{average}}\frac{dX}{dt}\text{during } 51\text{ - }54\text{ h}\right)}{4}$$

$$+\frac{\left(\frac{1}{X_{average}}\frac{dX}{dt}\text{during } 54\text{ - }57\text{ h}\right)+\left(\frac{1}{X_{average}}\frac{dX}{dt}\text{during } 57\text{ - }60\text{ h}\right)}{4}$$

$$=\frac{\left(\frac{1}{7.16}\times\frac{0.98}{3}\right)+\left(\frac{1}{7.93}\times\frac{0.55}{3}\right)+\left(\frac{1}{8.42}\times\frac{0.44}{3}\right)+\left(\frac{1}{8.86}\times\frac{0.44}{3}\right)}{4}$$

$$= 0.026 \text{ per h.}$$

The maximum specific growth rate

The maximum value for the specific growth rate was calculated over the exponential growth period.

$$\text{Max. specific growth rate} = \frac{1}{X_{average}} \frac{dX_{max}}{dt}$$

Calculation sample for using *S.cerevisiae* 5606 after the addition of DLE media during 48 – 60 h to determine the maximum specific growth rate;

$$\text{Max. specific growth rate} = \frac{1}{X_{average}} \frac{dX_{max}}{dt}$$

$$= \frac{\left(\frac{1}{7.16} \times \frac{0.98}{3}\right) + \left(\frac{1}{7.93} \times \frac{0.55}{3}\right)}{2}$$

$$= 0.035 \text{ per h.}$$

The average specific rate of sucrose consumption

The instantaneous average specific rate of sucrose consumption (Avg. q_s of sucrose) was determined using:

$$\text{Avg. } q_s \text{ of sucrose} = \frac{1}{X_{average}} \frac{dSu}{dt}$$

where $X_{average}$ is the average dry biomass weight

$\frac{dSu}{dt}$ is the rate of sucrose consumption

Calculation sample for using *S.cerevisiae* 5606 after the addition of DLE media during 48 – 60 h to determine the average specific rate of sucrose consumption;

Time (h)	Sucrose concentration (g/l)
48	37.0
51	26.0
54	17.0
57	14.5
60	12.0

$$\text{Avg. } q_s \text{ of sucrose} = \frac{1}{X_{average}} \frac{dSu}{dt}$$

$$= \frac{\left(\frac{1}{7.16} \times \frac{11.0}{3}\right) + \left(\frac{1}{7.93} \times \frac{9.00}{3}\right) + \left(\frac{1}{8.42} \times \frac{2.50}{3}\right) + \left(\frac{1}{8.86} \times \frac{2.50}{3}\right)}{4}$$

$$= 0.275 \text{ g/l/h}$$

The maximum specific rate of sucrose consumption

The maximum value for the maximum specific rate of sucrose consumption (Max. q_s of sucrose) was calculated over the exponential growth period.

$$\text{Max. } q_s \text{ of sucrose} = \frac{1}{X_{average}} \frac{dSu_{max}}{dt}$$

where $X_{average}$ is the average dry biomass weight

$\frac{dSu_{max}}{dt}$ is the maximum rate of sucrose consumption

Calculation sample for using *S.cerevisiae* 5606 after the addition of DLE media during 48 – 60 h to determine the maximum specific rate of sucrose consumption;

$$\text{Max. } q_s \text{ of sucrose} = \frac{1}{X_{average}} \frac{dSu_{max}}{dt}$$

$$= \frac{\left(\frac{1}{7.16} \times \frac{11.0}{3}\right) + \left(\frac{1}{7.93} \times \frac{9.00}{3}\right)}{2}$$

$$= 0.454 \text{ g/l/h}$$

The average specific rate of glucose consumption

The instantaneous average specific rate of glucose consumption (Avg. q_s of glucose) was determined using:

$$\text{Avg. } q_s \text{ of glucose} = \frac{1}{X_{average}} \frac{dGlu}{dt}$$

where $X_{average}$ is the average dry biomass weight

$\frac{dGlu}{dt}$ is the rate of glucose consumption

Calculation sample for using *S.cerevisiae* 5606 after the addition of DLE media during 48 – 60 h to determine the average specific rate of glucose consumption;

Time (h)	Glucose concentration (g/l)
48	18.0
51	14.5
54	11.5
57	7.50
60	5.50

$$\text{Avg. } q_s \text{ of glucose} = \frac{1}{X_{average}} \frac{dGlu}{dt}$$

$$= \frac{\left(\frac{1}{7.16} \times \frac{3.50}{3}\right) + \left(\frac{1}{7.93} \times \frac{3.00}{3}\right) + \left(\frac{1}{8.42} \times \frac{4.00}{3}\right) + \left(\frac{1}{8.86} \times \frac{2.00}{3}\right)}{4}$$

$$= 0.132 \text{ g/l/h}$$

The maximum specific rate of glucose consumption

The maximum value for the maximum specific rate of glucose consumption (Max. q_s of glucose) was calculated over the exponential growth period.

$$\text{Max. } q_s \text{ of glucose} = \frac{1}{X_{average}} \frac{dGlu_{max}}{dt}$$

where $X_{average}$ is the average dry biomass weight

$\frac{dGlu_{max}}{dt}$ is the maximum rate of glucose consumption

Calculation sample for using *S.cerevisiae* 5606 after the addition of DLE media during 48 – 60 h to determine the maximum specific rate of glucose consumption;

$$\text{Max. } q_s \text{ of glucose} = \frac{1}{X_{average}} \frac{dGlu_{max}}{dt}$$

$$= \frac{\left(\frac{1}{7.16} \times \frac{3.50}{3}\right) + \left(\frac{1}{8.42} \times \frac{4.00}{3}\right)}{2}$$

$$= 0.163 \text{ g/l/h}$$

The average specific rate of fructose consumption

The instantaneous average specific rate of fructose consumption (Avg. q_s of fructose) was determined using:

$$\text{Avg. } q_s \text{ of fructose} = \frac{1}{X_{average}} \frac{dFruc}{dt}$$

where $X_{average}$ is the average dry biomass weight

$\frac{dFruc}{dt}$ is the rate of fructose consumption

Calculation sample for using *S.cerevisiae* 5606 after the addition of DLE media during 48 – 60 h to determine the average specific rate of fructose consumption;

Time (h)	Fructose concentration (g/l)
48	19.5
51	14.0
54	11.0
57	9.50
60	9.50

$$\text{Avg. } q_s \text{ of fructose} = \frac{1}{X_{average}} \frac{dFruc}{dt}$$

$$= \frac{\left(\frac{1}{7.16} \times \frac{5.50}{3}\right) + \left(\frac{1}{7.93} \times \frac{3.00}{3}\right) + \left(\frac{1}{8.42} \times \frac{1.50}{3}\right) + \left(\frac{1}{8.86} \times \frac{0.00}{3}\right)}{4}$$

$$= 0.113 \text{ g/l/h}$$

The maximum specific rate of fructose consumption

The maximum value for the maximum specific rate of fructose consumption (Max. q_s of fructose) was calculated over the exponential growth period.

$$\text{Max. } q_s \text{ of fructose} = \frac{1}{X_{average}} \frac{dFruc_{\max}}{dt}$$

where $X_{average}$ is the average dry biomass weight

$\frac{dFruc_{\max}}{dt}$ is the maximum rate of fructose consumption

Calculation sample for using *S.cerevisiae* 5606 after the addition of DLE media during 48 – 60 h to determine the maximum specific rate of fructose consumption;

$$\text{Max. } q_s \text{ of fructose} = \frac{1}{X_{average}} \frac{dFruc_{\max}}{dt}$$

$$= \frac{\left(\frac{1}{7.16} \times \frac{5.50}{3}\right) + \left(\frac{1}{7.93} \times \frac{3.00}{3}\right)}{2}$$

$$= 0.195 \text{ g/l/h}$$

The average specific rate of ethanol production

The instantaneous average specific rate of ethanol production (Avg. q_p of ethanol) was determined using:

$$\text{Avg. } q_p \text{ of ethanol} = \frac{1}{X_{average}} \frac{dEth}{dt}$$

where $X_{average}$ is the average dry biomass weight

$\frac{dEth}{dt}$ is the rate of ethanol production

Calculation sample for using *S.cerevisiae* 5606 after the addition of DLE media during 48 – 60 h to determine the average specific rate of ethanol production;

Time (h)	Ethanol concentration (g/l)
48	52.0
51	56.0
54	60.0
57	65.0
60	70.0

$$\text{Avg. } q_p \text{ of ethanol} = \frac{1}{X_{average}} \frac{dEth}{dt}$$

$$= \frac{\left(\frac{1}{7.16} \times \frac{4.00}{3}\right) + \left(\frac{1}{7.93} \times \frac{4.00}{3}\right) + \left(\frac{1}{8.42} \times \frac{5.00}{3}\right) + \left(\frac{1}{8.86} \times \frac{5.00}{3}\right)}{4}$$

$$= 0.187 \text{ g/l/h}$$

The maximum specific rate of ethanol production

The maximum value for the maximum specific rate of ethanol production (Max. q_p of ethanol) was calculated over the exponential growth period.

$$\text{Max. } q_p \text{ of ethanol} = \frac{1}{X_{average}} \frac{dEth_{max}}{dt}$$

where $X_{average}$ is the average dry biomass weight

$\frac{dEth_{max}}{dt}$ is the maximum rate of ethanol production

Calculation sample for using *S.cerevisiae* 5606 after the addition of DLE media during 48 – 60 h to determine the maximum specific rate of ethanol production;

$$\text{Max. } q_p \text{ of ethanol} = \frac{1}{X_{average}} \frac{dEth_{max}}{dt}$$

$$= \frac{\left(\frac{1}{7.16} \times \frac{4.00}{3}\right) + \left(\frac{1}{8.42} \times \frac{5.00}{3}\right)}{2}$$

$$= 0.195 \text{ g/l/h}$$

APPENDIX F

Effect of preseed and seed cultivation conditions on yeast cells viability

Materials and methods

1. Microorganisms

Saccharomyses cerevisiae TISTR 5606 was obtained from Thailand Institute of Scientific and Technology Research (TISTR). *Candida utilis* UNSW 709400 & 709700 were obtained from The University of New South Wales (UNSW). All of the microbial strains were prepared in the form of working culture stock kept in 16% (v/v) glycerol solution. Each stock was kept in the 1.0 ml aliquot of 1.5 ml eppendorf tube and stored at -20°C before used.

2. Cultivation medium preparation

Dried longan extract (DLE) medium which was also used as a preseed and seed inoculum contained (per litre): 300 g dried longan flesh aged 3 yrs old (Sanpathong District, Chiang Mai, Thailand). The extraction was done in boiling water with mass to volume extraction ratio of 30.0 g dried longan per 100 ml distilled water for 30 min (Agustina *et al*. 2009) prior to removal of insoluble solids by filtration. Ammonium sulphate 8.57 ± 0.57 g/l was added for used as nitrogen sources. The medium was sterilized by claving at 121°C for 15 min.

3. Preseed cultivation of microbial culture in 15 ml scale

The inoculum size of 0.8 ml was used in preseed propagation in 15 ml dried longan extract medium. The cultivation of each inoculum was carried out for 24 h at 30°C (Laluce *et al*., 2009) with/without shaking in a rotary shaker at 250 rpm (Tang *et al*., 2010).

4. Seed cultivation of microbial culture in 150 ml scale

The optimum condition determined in section 2.3 was used in preseed inocula. The seed inocula were achieved by using 10%(v/v) preseed inocula for cultivation in 150 ml in the same cultivation condition and incubation period (Chaweekunlayakun *et al*., 2010).

5. Analytical determination

Five replicates were collected of 0.5 ml for preseed inocula and 5 ml for seed inocula at each time point (0, 6, 12, 18, and 24). Cells viability measurement was determined using electron microscope with the help of haemocytometer. Cells viability was checked by using trypan blue indicator. The dead cells were stained with blue indicator while viable cells remained uncolored (Arshad *et al*., 2008). Dried biomass concentration was correlated to OD600 by cubic equation as shown in Appendix D.

6. Calculation method

% Cells viability = (Number of viable cells/Total cells)*100

7. Statistical analysis

The errors were calculated from 5 replicates. The statistic analysis were made by hypothesis testing method (Skoog *et al*., 1996) and hypothesis testing program for statistically significant difference (NLST_Diff version 1.0) with symbol W 1.3281 (Leksawasdi, 2009).

Results and discussions

1. Preseed cultivation of microbial culture in 15 ml scale

The experiment investigated the suitable of preseed cultivation condition for all three microbial strains for 24 h at 30°C with/without shaking in a rotary shaker at 250 rpm. The detailed analysis of each cultivation condition and incubation period with hypothesis testing across three microbial strains is tabulated in Table F.1 – F.3.

As indicated in Table E.1 – E.3, the maximum viability was observed in a shaking system. This was significantly different ($p \leq 0.05$) from static system. The results from the current study was in agreement with Alfenore *et al*. (2004) who investigated the aeration strategy for improving ethanol production using *S. cerevisiae* in a fed batch process where aeration led to an increase in the viable cells as a result of the simultaneous increase in growth rate.

The maximum viability for *S. cerevisiae* TISTR 5606 in shaking system of 96.9 ± 0.1, and 96.8 ± 0.3% were observed at 12, and 18 h, respectively. These were significantly higher ($p \leq 0.05$) than preseed cultivation time at 0, 6, and 24 h with the corresponding viability between 88.8 – 93.8% as shown in Table F.1.

Table F.1 Viable cells (%) of *S. cerevisiae* TISTR 5606 in various modes of propagation during preseed cultivation period

Time (h)	Modes of Propagation					
	Static			Shake		
0	76.0 ± 0.2	A	I	88.8 ± 0.8	A	II
6	64.1 ± 0.2	B	I	93.8 ± 0.1	B	II
12	62.2 ± 0.7	C	I	96.9 ± 0.1	C	II
18	62.5 ± 0.6	C	I	96.8 ± 0.3	C	II
24	53.0 ± 0.3	D	I	93.2 ± 0.1	B	II

The number with the same Roman numeral (I – II) 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.

Table F.2 Viable cells (%) of *C. utilis* UNSW 709400 in various modes of propagation during preseed cultivation period

Time (h)	Modes of Propagation					
	Static			Shake		
0	58.8 ± 1.0	A	I	93.9 ± 0.7	A	II
6	63.6 ± 0.5	B	I	90.9 ± 0.2	C	II
12	61.1 ± 0.6	A	I	89.7 ± 0.3	D	II
18	53.8 ± 0.9	B	I	95.4 ± 0.5	A	II
24	57.8 ± 0.2	A	I	95.7 ± 0.5	A	II

The number with the same Roman numeral (I – II) 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.

Table F.3 Viable cells (%) of *C. utilis* UNSW 709700 in various modes of propagation during preseed cultivation period

Time (h)	Modes of Propagation					
	Static			Shake		
0	46.9 ± 0.1	A	I	93.6 ± 0.4	A	II
6	54.4 ± 0.6	B	I	93.2 ± 0.4	A	II
12	59.2 ± 0.3	B	I	95.6 ± 0.2	B	II
18	50.8 ± 0.7	B	I	95.9 ± 0.2	B	II
24	47.4 ± 0.2	A	I	96.1 ± 0.1	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 comparison of the maximum viability for *C. utilis* UNSW 709400 was observed in shaking system of 95.7 ± 0.5, 93.9 ± 0.7%, and 95.4 ± 0.5 at 0, 18, and 24 h, respectively. These were significantly higher ($p \leq 0.05$) than preseed cultivation time at 6, and 12 h of 90.9 ± 0.2, and 89.7 ± 0.3%, respectively (Table F.2).

According to Table F.3, the maximum viability for *C. utilis* UNSW 709700 was observed in shaking system of 96.1 ± 0.1, 95.9 ± 0.2, and 95.6 ± 0.2% at 24, 18, and 12 h, respectively. These were significantly higher ($p \leq 0.05$) than preseed cultivation time at 0, and 6 h of 93.6 ± 0.4 and 93.2 ± 0.4%, respectively.

The cultivation time of preseed for seed inoculation at 18 h was the best time for all three microbial strains as the viable cells was greater than 95%.

2. Seed cultivation of microbial culture in 150 ml scale

The experiment investigated the suitable of seed cultivation condition for all three microbial strains for 24 h at 30°C with/without shaking in a rotary shaker at 250 rpm. The optimum condition determined in section 2.3 was used in preseed inocula. The detailed analysis of each cultivation condition and incubation period with hypothesis testing across three microbial strains is tabulated in Table F.4 – F.6.

As indicated in Table E.4 – E.6, the maximum viability was observed in a shaking system. This was significantly different ($p \leq 0.05$) from static system. This result was similar with the preseed cultivation.

The maximum viable cells for *S. cerevisiae* TISTR 5606 in shaking system of 97.5 ± 0.2, 97.6 ± 0.1, and 97.6 ± 0.1% at 12, 18, and 24 h, respectively. These were significantly higher ($p \leq 0.05$) than seed cultivation time at 0, and 6 24 h of 81.7 ± 0.3, and 88.8 ± 0.2%, respectively as shown in Table F.4.

The comparison of the maximum viability for *C. utilis* UNSW 709400 was observed in shaking system of 96.3 ± 0.1, and 96.2 ± 0.2% at 18, and 24 h, respectively. These were significantly higher ($p \leq 0.05$) than seed cultivation time at 0, 6, and 12 h with the corresponding viability between 85.4 – 95.3% as shown in Table F.5.

According to Table F.6, the maximum viability for *C. utilis* UNSW 709700 was observed in shaking system of 92.0 ± 0.1, 95.7 ± 0.02, and 96.3 ± 0.1% at 12, 18, and 24 h, respectively. These were significantly higher ($p \leq 0.05$) than seed cultivation time at 0, and 24 h of 89.3 ± 0.4 and 88.7 ± 0.1%, respectively.

The cultivation time at 12, and 18 h for microbial strains had the viable cells greater than 95%, which was suitable for used as preseed in the seed cultivation (Saville *et al*., 1994). However, the preseed cultivation time at 12 h had the viable cells less than 95%. Therefore, the suitable preseed and seed cultivation condition is in shaking system for 18 h.

Table F.4 Viable cells (%) of *S. cerevisiae* TISTR 5606 in various modes of propagation during seed cultivation period

Time (h)	Modes of Propagation					
	Static			Shake		
0	56.2 ± 0.7	A	I	81.7 ± 0.3	A	II
6	46.6 ± 0.9	B	I	88.8 ± 0.2	B	II
12	52.4 ± 0.7	B	I	97.5 ± 0.2	C	II
18	55.7 ± 0.04	A	I	97.6 ± 0.1	C	II
24	50.0 ± 0.3	B	I	97.6 ± 0.1	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.

Table F.5 Viable cells count of *C. utilis* UNSW 709400 in various modes of propagation during seed cultivation period

Time (h)	Modes of Propagation					
	Static			Shake		
0	48.4 ± 0.4	A	I	85.4 ± 0.3	A	II
6	47.3 ± 0.6	A	I	95.0 ± 0.4	B	II
12	52.6 ± 0.4	B	I	95.3 ± 0.1	B	II
18	50.7 ± 0.6	C	I	96.3 ± 0.1	C	II
24	50.6 ± 0.9	B, C	I	96.2 ± 0.2	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.

Table F.6 Viable cells (%) of *C. utilis* UNSW 709700 in various modes of propagation during seed cultivation period

Time (h)	Modes of Propagation					
	Static			Shake		
0	45.8 ± 1.0	A	I	89.3 ± 0.4	A	II
6	43.5 ± 0.6	A	I	92.0 ± 0.1	B	II
12	45.0 ± 0.9	A	I	95.7 ± 0.02	B	II
18	48.4 ± 0.5	B	I	96.3 ± 0.1	B	II
24	44.4 ± 0.5	A	I	88.7 ± 0.1	A	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.

APPENDIX G

Determination of sugars and organic compounds concentration by HPLC

Reagent

• Mobile phase 5 mM H_2SO_4

Procedure

The concentration of sugars (sucrose, glucose, and fructose), acetic acid, ethanol, and acetoin were determined by Agilent Technologies High Performance Liquid Chromatography (HPLC) with refractive index detector and 20 min of run time. The column used was Aminex ®HPX-87H Ion Exclusive 9 μm particle size with 5 mM H_2SO_4 in distilled water. Flow rate was set at 0.75 ml/min. Temperature of column oven was set at 37°C. The refractive index was measured with Refractive Index Detector with 20 min run time. Volume of injected sample was 20 μl.

The retention time (min) of each compound is shown as follow sucrose (6.92-7.16), glucose (8.13-8.29), fructose (10.05-10.20), acetic acid (12.71-12.89), and ethanol (13.62-13.93).

Standard curve

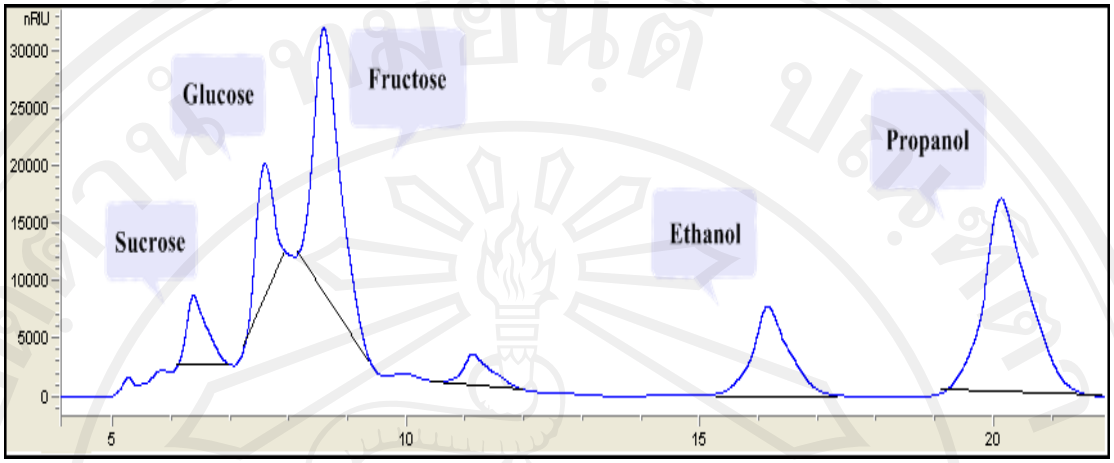

Figure G.1 Chromatogram of sucrose, glucose, fructose, acetic acid, and ethanol

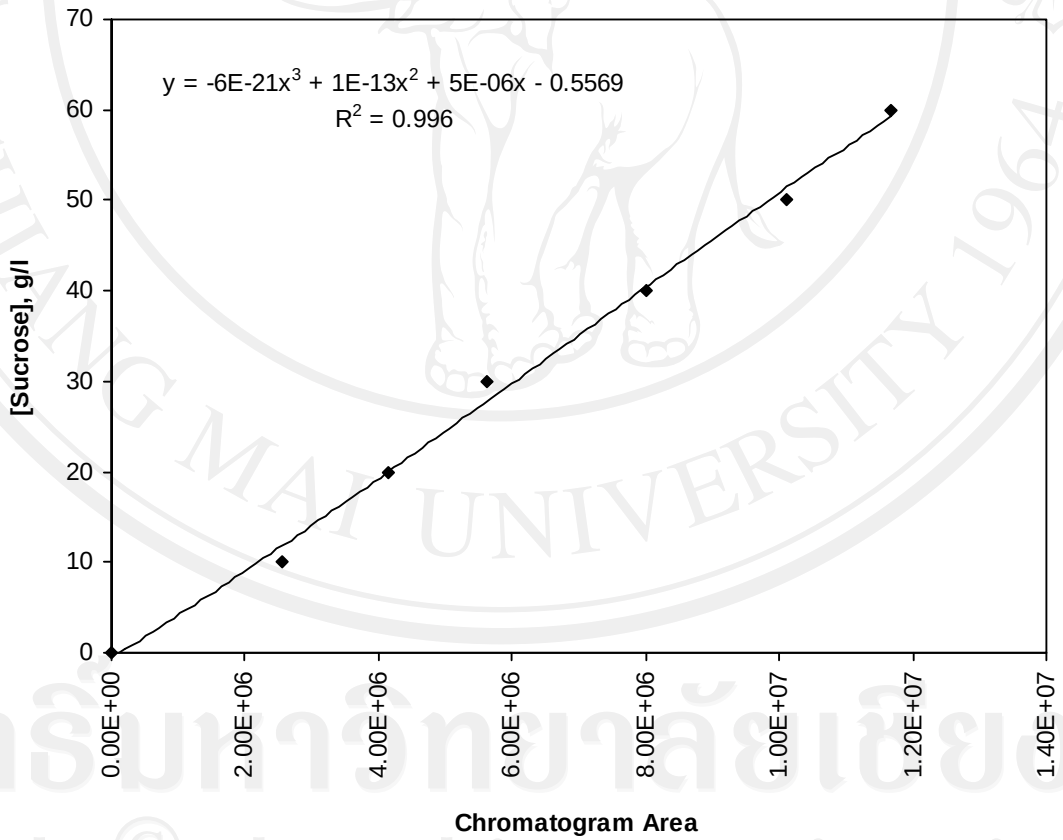

Figure G.2 Standard curve for sucrose

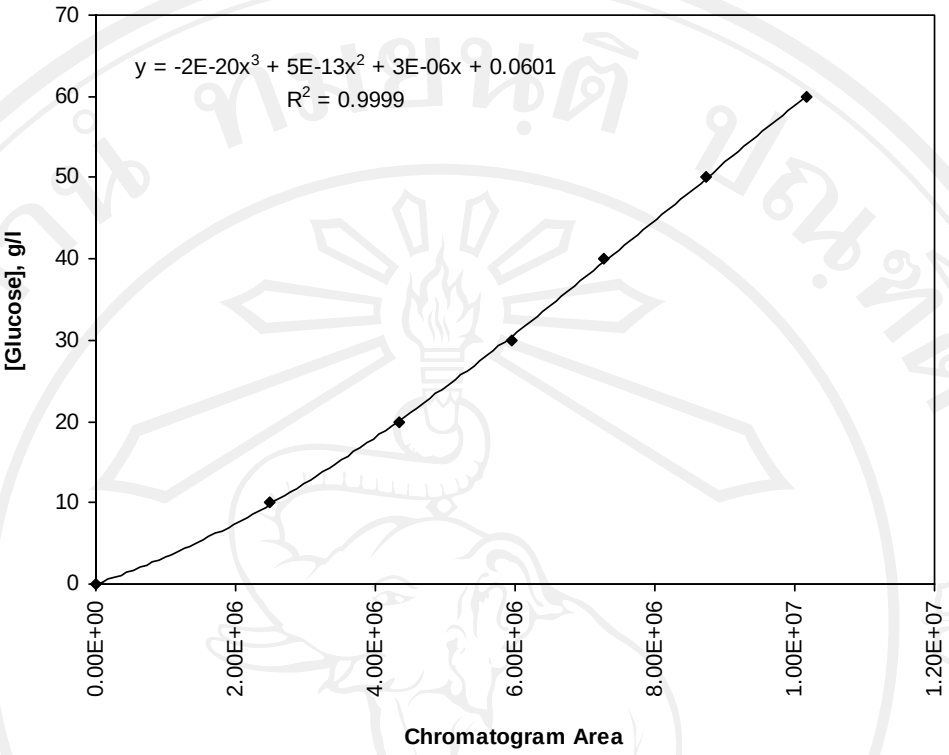

Figure G.3 Standard curve for glucose

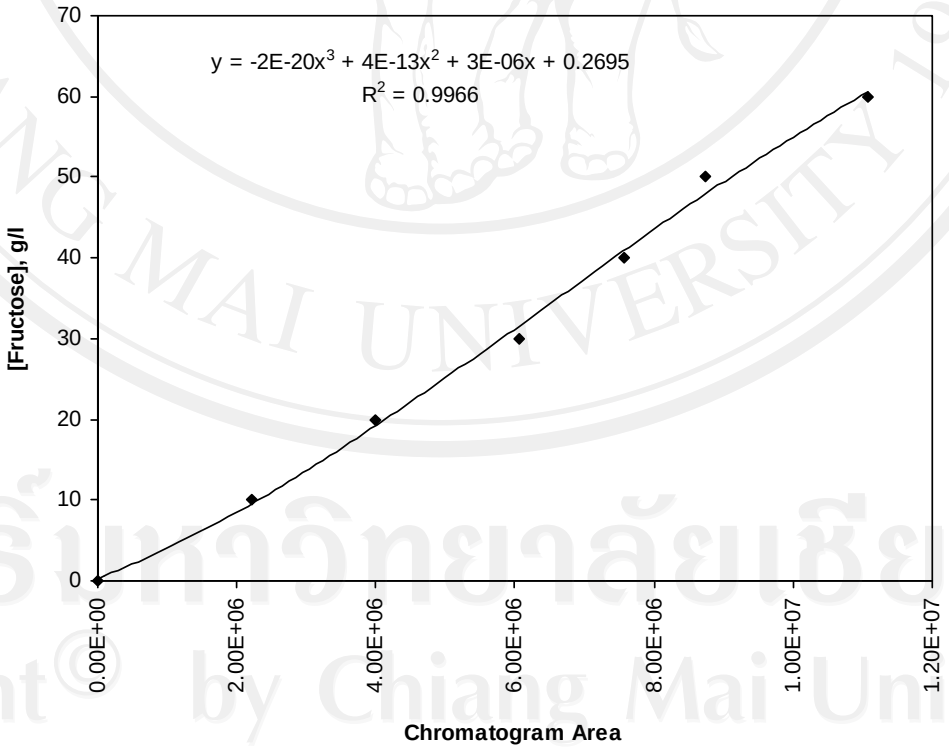

Figure G.4 Standard curve for fructose

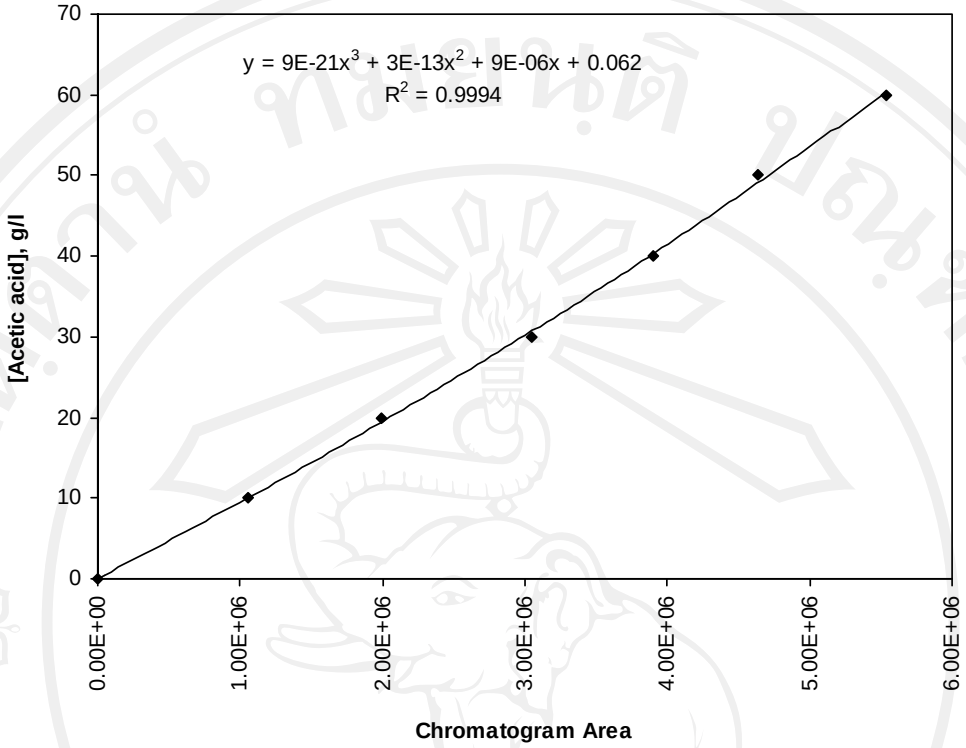

Figure G.5 Standard curve for acetic acid

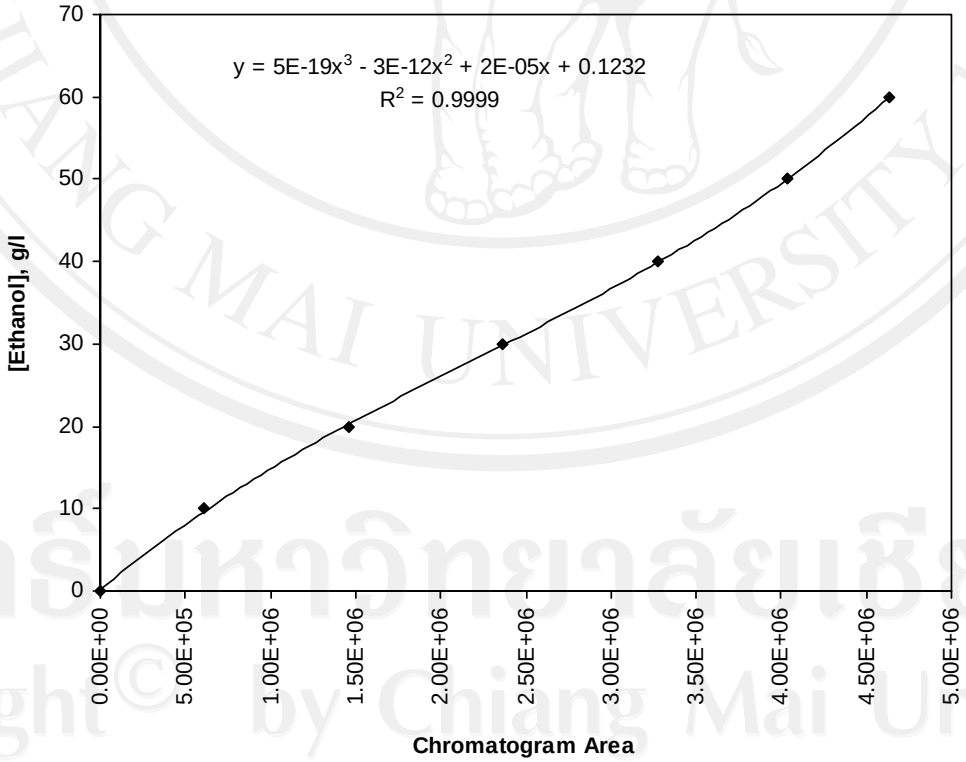

Figure G.6 Standard curve for ethanol

APPENDIX H

Determination of benzyl alcohol, PAC, benzoic acid and benzaldehyde concentration by HPLC

Reagent

- Mobile phase contained 32% (v/v) acetonitrile and 0.5% (v/v) acetic acid

Procedure

The concentrations of benzyl alcohol, PAC, benzoic acid and benzaldehyde were determined by Agilent Technologies High Performance Liquid Chromatography (HPLC) with diode array detector (DAD) and ultra violet (UV) detection wavelength at 263 nm for benzyl alcohol and 283 nm for other species with 20 min of run time. The column used was Altima™ C8 5 μm particle size with 32%(v/v) of acetonitrile and 0.5% (v/v) of acetic acid in distilled water. Flow rate was set at 1.0 ml/min. Temperature of column oven was set at room temperature. Volume of injected sample was 5μl.

The retention time (min) of each compound is shown as follow; PAC (4.00 – 4.93), benzoic acid (4.49 – 5.91), and benzaldehyde (6.87 – 10.78).

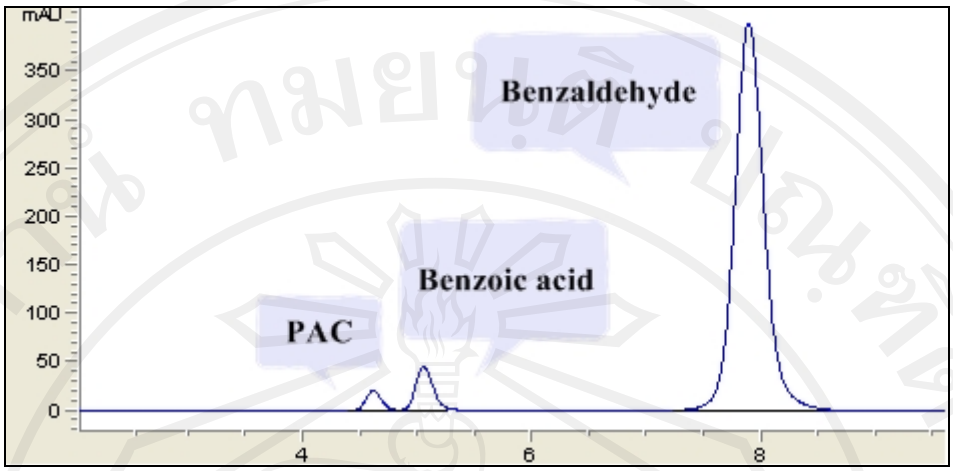

Figure H.1 Chromatogram of PAC, benzoic acid, and bemzaldehyde

APPENDIX I

Pyruvate concentration determination by modified method of Czok & Lamprecht

Reagent

- 0.25 M triethanolamine buffer pre-adjusted to pH 7.6 by 5 M KOH
- 6.35 mM NADH disodium salt in 120 mM $NaHCO_3$
- 550 U/mg LDH

Procedure

1. The 0.75 ml of 0.25 M triethanolamine buffer which was pre-adjusted to pH 7.6 by 5 M KOH which was maintained at temperature 25°C waterbath, was transferred into a cuvette.
2. The addition of 25 µl NADH disodium salt 6.35 mM in 120 mM $NaHCO_3$ was followed.
3. The 25 µl biotransformation sample was added into the cuvette.
4. The mixture was mixed well.
5. The absorbance of biotransformation sample was analyzed at the wavelength of 340 nm. This absorbance was measured as initial absorbance of pyruvate concentration determination ($A_{pyr,i}$).
6. 5 µl LDH 550 U/mg was added into the solution and mixed well.

7. The absorbance was then analyzed at the wavelength 340 nm after 8 min. This absorbance was measured as the absorbance of pyruvate concentration determination at 8 min ($A_{pyr,8}$).
8. The value of pyruvate absorbance (A_{pyr}) was obtained by subtracting $A_{pyr,i}$ and $A_{pyr,8}$.
9. The calculation of pyruvate concentration is explained below:

$A_{pyr,i}$ Cuvette initial absorption containing biotransformation sample before adding LDH.

$A_{bln,i}$ Cuvette initial absorption containing distilled water before adding LDH.

$A_{pyr,8}$ Cuvette absorption containing biotransformation sample after adding LDH for 8 min.

$A_{bln,8}$ Cuvette absorption containing distilled water after adding LDH for 8 min.

A_{pyr} $A_{pyr,i} - A_{pyr,8}$

A_{bln} $A_{bln,i} - A_{bln,8}$

Abs $A_{pyr} - A_{bln}$

V_{assay} The final volume of the total solution = 750 + 25 + 25 + 5 = 805 µl

V_{sam} The volume of sample = 25 µl

λ Cuvette path length (1 cm)

ε NADH extinction coefficient at 340 nm (6300 ml/µmol·cm)

[Pyr] Pyruvate concentration

$= (Vassay/ Vsam) \times Abs/min \times (1/ \varepsilon \lambda) \times (1000 \text{ mmol/mol})$

$= 5.111 \times Abs$ mM

APPENDIX J

Acetaldehyde concentration determination by modified method of Bernt & Bergmeyer

Reagent

- 0.25 M triethanolamine buffer pre-adjusted to pH 7.6 by 5 M KOH
- 6.35 mM NADH disodium salt in 120 mM $NaHCO_3$
- 200 U/mg ADH

Procedure

1. The 0.75 ml triethanolamine buffer 0.25 M adjusted to pH 7.6 by 5 M KOH and maintained at temperature 25°C waterbath, was transferred into a cuvette.
2. The addition of 25 µl NADH disodium salt 6.35 mM in 120 mM $NaHCO_3$ was followed.
3. The 25 µl biotransformation sample was added into the cuvette
4. The mixture was mixed well
5. The absorbance of biotransformation sample was analyzed at the wavelength of 340 nm. This absorbance was measured as initial absorbance of acetaldehyde concentration determination ($A_{ace,i}$).
6. 5 µl 200 U/mg ADH was added into the solution and mixed well.
7. The absorbance was then analyzed at the wavelength 340 nm after 8 min. This absorbance was measured as the absorbance of acetaldehyde concentration determination at 8 min ($A_{ace,8}$).

8. The value of acetaldehyde absorbance (A_{ace}) was obtained by subtracting $A_{ace,i}$ and $A_{ace,8}$.
9. The calculation of acetaldehyde concentration is explained below:

$A_{ace,i}$ Cuvette initial absorption containing biotransformation sample before adding ADH.

$A_{bln,i}$ Cuvette initial absorption containing distilled water before adding ADH.

$A_{ace,8}$ Cuvette absorption containing biotransformation sample after adding ADH for 8 min.

$A_{bln,8}$ Cuvette absorption containing distilled water after adding ADH for 8 min.

A_{ace} $A_{ace,i} - A_{ace,8}$

A_{bln} $A_{bln,i} - A_{bln,8}$

Abs $A_{ace} - A_{bln}$

V_{assay} The final volume of the total solution = 750 + 25 + 25 + 5 = 805 µl

V_{sam} The volume of sample = 25 µl

λ Cuvette path length (1 cm)

ε NADH extinction coefficient at 340 nm (6300 ml/µmol·cm)

[Ace] Acetaldehyde concentration

$= (Vassay/ Vsam) \times Abs/min \times (1/ \varepsilon \lambda) \times (1000 \text{ mmol/mol})$

$= 5.111 \times Abs$ mM

APPENDIX K

Biotransformation of PAC in two phase emulsion system

Preparation of wet biomass

1. The ethanol production was carried out in 5,000 ml scale with *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400, and 709700 in the condition of
   (a) batch cultivation with DLE at 48th h fermentation period,
   (b) fed batch cultivation with DLE at 60th h fermentation period,
   (c) fed batch cultivation with DDLFH at 60th h fermentation period.

   The cultivation with *S. cerevisiae* TISTR 5606
   - In condition (a) resulted in the dried biomass of 7.73 ± 0.05 g/l.
   - In condition (b) resulted in the dried biomass of 9.11 ± 0.05 g/l.
   - In condition (c) resulted in the dried biomass of 6.84 ± 0.05 g/l.

   The cultivation with *C. utilis* UNSW 709400
   - In condition (a) resulted in the dried biomass of 3.72 ± 0.06 g/l.
   - In condition (b) resulted in the dried biomass of 3.71 ± 0.05 g/l.
   - In condition (c) resulted in the dried biomass of 3.41 ± 0.06 g/l.

The cultivation with *C. utilis* UNSW 709700

- In condition (a) resulted in the dried biomass of 3.93 ± 0.05 g/l.
- In condition (b) resulted in the dried biomass of 3.86 ± 0.05 g/l.
- In condition (c) resulted in the dried biomass of 3.63 ± 0.05 g/l.

2. The cells culture was centrifuged at 6,000 rpm for 15 min at 4°C by temperature controlled centrifuge machine to separate cells pellet from the supernatant.
3. Addition of distilled water to the centrifuge tubes was performed to wash cells pellet.
4. The centrifugation was performed once more and the cells pellet was collected in the clear bottles.
5. The wet biomass was kept in the freezer at -20^{o}C.
6. The concentration of wet biomass in each experiment was pre-adjusted prior to the addition of 1,304 µl pre-adjusted wet biomass to the 10 ml biotransformation system to initiate the reaction.

Biomass concentration adjustment

1. The initial volume of cells culture before centrifugation were 400 ml.
2. The cells pellets were dissolved with 1.2 M phosphate buffer (pH level pre-adjusted to 6.0) to reach the dried biomass equivalent concentration of 100 g/l.

***S. cerevisiae* TISTR 5606:**

In condition (a), (7.7 g/l)(400 ml)/(100 g/l) = 30.8 ml

In condition (b), (9.1 g/l)(400 ml)/(100 g/l) = 36.4 ml

In condition (c), (6.8 g/l)(400 ml)/(100 g/l) = 27.2 ml

***C. utilis* UNSW 709400:**

In condition (a), (3.7 g/l)(400 ml)/(100 g/l) = 14.8 ml

In condition (b), (3.7 g/l)(400 ml)/(100 g/l) = 14.8 ml

In condition (c), (3.4 g/l)(400 ml)/(100 g/l) = 13.6 ml

***C. utilis* UNSW 709700:**

In condition (a), (3.9 g/l)(400 ml)/(100 g/l) = 15.6 ml

In condition (b), (3.9 g/l)(400 ml)/(100 g/l) = 15.6 ml

In condition (c), (3.6 g/l)(400 ml)/(100 g/l) = 14.4 ml

Recheck the concentration of newly adjusted wet biomass in each set of experiment.

***S. cerevisiae* TISTR 5606:**

In condition (a), (7.73 g/l)(400 ml)/(30.8 g/l) = 100 ml

In condition (b), (9.11 g/l)(400 ml)/(36.4 g/l) = 100 ml

In condition (c), (6.84 g/l)(400 ml)/(27.2 g/l) = 100 ml

***C. utilis* UNSW 709400:**

In condition (a), (3.72 g/l)(400 ml)/(14.8 g/l) = 100 ml

In condition (b), (3.71 g/l)(400 ml)/(14.8 g/l) = 100 ml

In condition (c), (3.41 g/l)(400 ml)/(13.6 g/l) = 100 ml

***C. utilis* UNSW 709700:**

In condition (a), (3.93 g/l)(400 ml)/(15.6 g/l) = 100 ml

In condition (b), (3.86 g/l)(400 ml)/(15.4 g/l) = 100 ml

In condition (c), (3.63 g/l)(400 ml)/(14.4 g/l) = 100 ml

3. In each biotransformation bottle, the volume of wet biomass was added which was equal to V_2 µl from step 2 to initiate the experiment.

From; $C_1V_1 = C_2V_2$

When C_1 = Initial wet biomass concentration (g/l)

C_2 = Wet biomass concentration in biotransformation bottle (g/l)

V_1 = Volume of wet biomass is added (ml)

V_2 = Volume of solvent in biotransformation bottle = 10.652 ml

$(100 \text{ g/l})(V_1) = (12.24)(10.652)$

$V_1 = 1.304$ ml

Recheck: $(100 \text{ g/l})(1.304 \text{ ml}) = (C_2)(10.652)$

$C_2 = 12.24$ g/l

Preparation of biotransformation bottle for two-phase system (The influence of three carbon source types and three microbial strains for microbial cultivation at 12.24 g/l dried biomass equivalent)

1. The effect of three carbon source types to the production level of PAC used in cultivation of three microbial strains in octanol with benzaldehyde concentration of 1.75 M in the organic phase (5 ml volume) and sodium pyruvate at the concentration level of 300 mM, 1 mM TPP, and 1 mM $MgSO_4.7H_2O$ in the solution containing 1.2 M phosphate buffer at 5 ml for 72 h.

2. Preparation of 500 ml of 1.2 M phosphate buffer
   - 2.1 KH_2PO_4 was weighed for 81.7 g
   - 2.2 Distilled water was added to make up volume of 300 ml
   - 2.3 The pH was adjusted to 6.0 with 5 M KOH and/or 5x diluted H_3PO_4 (**Preparation of KOH:** add 50 ml of 10 M KOH to distilled water of equivalent volume **Preparation of 5x diluted H_3PO_4:** add 20 ml of Conc. H_3PO_4 to distilled water of 80 ml volume)
   - 2.4 The final volume was adjusted to 500 ml

     Check the prepared phosphate buffer

     (81.7 g)(1/136.09 mol/g)(1/0.5 /l) = 1.2 mol/l = 1.2 M

3. Preparation of 1.75 M benzaldehyde in octanol

Benzaldehyde was weighed into each bottle inside the fume cupboard for 0.93 g before adding octanol to reach the final volume of 5 ml.

Check the concentration of prepared benzaldehyde

(0.93 g)(1/106.13 mol/g)(1/0.005 /l) = 1.752 mol/l = 1.75 M

4. The weighing in of $MgSO_4.7H_2O$ and TPP in each biotransformation bottle was relatively inconvenient. The preparation of each chemical was thus performed in the concentrated form and followed by the addition of 50 µl volume in each bottle.
   - 4.1 TPP was weighed for 0.125 g before adding in 1.25 ml of phosphate buffer 1.2 M pH 6.0 (step 2).

4.2 $MgSO_4.7H_2O$ was weighed for 0.065 g before adding in 1.25 ml of phosphate buffer 1.2 M pH 6.0 (step 2).

4.3 Combined the volume from step 4.1 and 4.2 to attain the overall volume of 2.5 ml.

Recheck

Recheck for [TPP] = (0.125 g)(1/460.8 mol/g)(1/2.5 /ml)(1000 ml/l)

= 0.108 mol/l

Recheck for [Mg2+] = (0.065 g)(1/246.47 mol/g)(1/2.5 /ml)(1000ml/l)

= 0.105 mol/l

The addition 50 µl mixture of TPP & Mg^{2+} should be performed besides pyruvate powder before addition of the aqueous phase.

5. Preparation of 300 mM pyruvate

5.1 Sodium pyruvate was weighed 0.180 g in empty biotransformation bottle.

5.2 The biotransformation bottle was kept in 4°C

Check for pyruvate concentration

(0.180 g)(1/110.04 mol/g)(1/0.005 /l) = 0.327 mol/l

Taking into account dilution factor (0.327 mol/l)(5/5.376) = 304 mM #Correct

Experimental sequence

- Preparation of 1.75 M of benzaldehyde in octanol as organic phase, in the first set of biotransformation bottle. The bottles were kept in -20°C for long storage or 4°C for short storage.
- Preparation of concentrated cofactor solution between TPP and $MgSO_4.7H_2O$. The solution was kept at 4°C.
- Preparation of 300 mM of sodium pyruvate in second set of biotransformation bottle.
- Aqueous phase was made by adding concentrated cofactor solution to the second set of biotransformation bottle containing sodium pyruvate. Phosphate buffer 1.2 M pH 6.0 was added into these bottles to dissolve the content.
- Aqueous phase was poured into the first set of biotransformation bottles containing organic phase. Each bottle was swirled horizontally with upright cap.
- The preweighed biomass was added into the aqueous phase bottle, mix well. The liquid in aqueous phase bottle was transferred into organic phase bottle.
- The biotransformation bottles were put in the tumbler mixer at 10 rpm at 4°C for 72 h.

APPENDIX L

Biotransformation calculation

Substrate balance is defined as a percentage ratio of summation of produced product/by-products concentration and remnant substrates concentration over initial substrate concentration in which the combined concentration of all involved species in aqueous and organic phases are accounted for. The decarboxylation of a pyruvate molecule by PDC formed an active acetaldehyde which reacted with benzaldehyde to produce PAC. The other associated by-products were also formed in the related reactions. Substrate benzaldehyde could be reduced to benzyl alcohol and/or oxidized into benzoic acid while substrate pyruvate resulted in by - products such as acetaldehyde and acetoin through a common dissociation process of enzyme-substrate complex and condensation with another active acetaldehyde, respectively.

The overall remnant substrate concentration in aqueous phase and organic phase is calculated by equation;

Overall remnant substrate concentration =

(concentration of remnant substrate in organic phase*volume ratio + concentration of remnant substrate in aqueous phase*1)/ (1 + volume ratio)

The overall produced product or by-products concentrations in aqueous and organic phases could be calculated by the following equation;

Overall produced product or by-products concentrations =

(concentrations of produced product or by-products in organic phase*volume ratio + concentration of produced product or by-products in aqueous phase*1)/ (1 + volume ratio)

where volume ratio was the measured volume after centrifugation process in organic phase/the measured volume after centrifugation process in aqueous phase.

Calculation of substrate balance percentage

Percentage of benzaldehyde balance =

(overall produced PAC concentration + overall produced benzoic acid concentration + overall remnant benzaldehyde concentration)*100/ overall initial benzaldehyde concentration

Percentage of pyruvate balance =

(overall produced PAC concentration + overall produced acetaldehyde concentration + 2*overall produced acetoin concentration + overall remnant pyruvate concentration)*100/ overall initial pyruvate concentration

* Formation of 1 mole acetoin requires 2 moles of pyruvate

CURRICULUM VITAE

Full name	Pornpun Wiruch
Date of birth	04 November 1985

Education background

2008 – Present	Master of Science in Food Engineering Chiang Mai University
2003 – 2007	Bachelor of Science in Food Science and Technology Maejo University
2000 – 2002	Senior High School Certificate of Science and Math program Suanboon Yophathum School

Work experience/Training history

May 2010	Participated in the International APEC Symposium on "Biofuels from Agricultural and Agro-Industrial Wastes" APEC, and Office of Agricultural Economics, Minister of Agricultural and Cooperatives
March – May 2009	Participated in The Program of Industrial Production Process Improvement with Cleaner Technology Chiang Mai University, National Metal and Materials Technology Center, National Science and Technology Development Agency, and Ministry of Science and Technology
2007 – 2008	Quality Assurance Supervisor at Chang Award (1959) Company. Chiang Mai, Thailand

January 2007	Completed a workshop on the Integrate of ISO 14001 : 2004/OHSAS 18001 : 1999 Faculty of Economics, Maejo University
November 2006	Attended a training course on GMP, HACCP Inspection of Product Quality and Standard of Product Maejo University
April – May 2006	Intern Production Supervisor Assistant at CPF Food Products Co., Ltd. Minburi, Bangkok - Worked in Production and Quality Control

Publication

Review Articles

Pornpun Wiruch. 2009. Replenish Energy from Agricultural Materials. Agro-Industry Review Article, Faculty of Agro-Industry, Chiang Mai University 1(4, September): 2.

National presentation

Pornpun Wiruch, Ronachai Pratanaphon, Charin Techapun and Noppol Leksawasdi. 2011. Selection of the Suitable Commercial Enzyme Addition Strategy for Digestion of Dried Longan Flesh with Low Sugar Level. The 12th Khon Kaen University 2011 Graduate Research Conference, 28 January 2011, Khon Kaen University, Khon Kaen, Thailand, Oral Presentration Number BM03, Page 518 – 529, Full paper in CD.

Pornpun Wiruch and Noppol Leksawasdi. 2010. The application of clean technology for swine feed production process and swine slaughter house. The Second Agro-Industry National Symposium, Faculty of Agro-Industry, Chiang Mai University November 18–24, 2010. Poster presentation.

Pornpun Wiruch, Pannipa Tasak, Netnapa Makhamrueang, Noppol Leksawasdi. 2009. The Application of Cleaner Technology in The Survey and Preliminary Assessment of Swine Feed Production Process and Swine Slaughter House, 35th Congress on Science and Technology of Thailand, 15-17 October 2009, The Tide Resort (Bangsaen Beach), Chonburi, Thailand, Poster Presentation Number G_G0001, Page 239-239, Full paper in CD, ISBN 978-616-12-0024-4.

Research report

Noppol Leksawasdi, Ronachai Pratanaphon, Krit Sittivangkul, Nicha Krutklom, Peerawat Jaiwunglok, **Pornpun Wiruch**, Tatiya Kumtip. 2010. The Utilization of Expired Dried Longan in the Production of Commercial Chemicals. Final Report Submitted for TTSF (Thailand Toray Science Foundation). Faculty of Agro-Industry, Chiang Mai University, Thailand, 110 pp.

Noppol Leksawasdi, Chartchai Khanongnuch, Nopphorn Leksawasdi, Taweeyot Kunyotying, Warayut Netikan, **Pornpun Wiruch**. 2010. The Improvement of Anthraquinone Extraction Efficiency from *Morinda* sp. Root Using Pressurized Steamer and Solid-Liquid Extraction Unit. Faculty of Agro-Industry, Chiang Mai University. Final report. 119 pages.

Noppol Leksawasdi, Ronachai Pratanaphon, Titiporn Ganbua, Nicha Krutklom, Tatiya Kumtip, Nopphorn Leksawasdi, Nisakorn Tadkaew, Punyapa Sukhapunnaphan, **Pornpun Wiruch**, Panitnan Sittimoon, Ratikorn Buakham, Warayut Netikan, Suree Achawasamit, Agustina A. S. 2009. The Utilization of Expired Dried Longan in the Production of Commercial Chemical. Faculty of Agro-Industry, Chiang Mai University. Final report. 432 pages.