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APPENDICES

APPENDIX A

Reducing sugar concentration analysis

In this study, the sugar concentration analysis is modified from Dinitrosalicylic acid reagent Method as following.

Preparation the DNS reagent

1. Dissolve 1.6 gram of NaOH in 20 mL of distilled water.
2. Slowly add 1 gram of 3,5-dinitrosalicylic acid and stir until the solution is homogeneous.
3. Dilute the solution by adding 50 mL of distilled water.
4. Add 30 gram of sodium potassium tartrate and mix thoroughly.
5. Adjust the volume to 100 mL by adding distilled water.
6. Keep the prepared DNS solution in a brown bottle for 3 days.

Standard curve for DNS method

1. Dry sucrose in an oven at 100-105°C for 2 hours and then cool down the dried sucrose in a desiccator.
2. Dissolve 25 gram of dried sucrose in 100 mL of distilled water by using a volumetric flask.
3. Prepare the sucrose solution in the different concentration as shown in Table A.1.

Table A.1 Standard sucrose dilution

No.	Standard sucrose solution (mL)	Distilled water (mL)	Sucrose concentration (g/5mL)
1	0	5	0
2	1	4	5
3	2	3	10
4	3	2	15
5	4	1	20
6	5	0	25

4. Mix 0.2 mL of the prepared sucrose solution with 0.8 mL of distilled water in 25 mL tube.
5. Blend the prepared standard sample with 0.5 mL of conc. HCl (33%v/v) and hydrolyze sucrose in boil water for 10 minutes
6. Stop the reaction by placing in ice bath.
7. Add 0.5 mL of 20%v/v NaOH and mix.

8. Centrifuge the sample at 3000 rpm for 10 minutes to separate undissolved solid.
9. Mix 0.2 mL of the supernatant with 1 mL DNS reagent in 25 mL tubes.
10. Boil the sample in water bath for 10 minutes and place in ice bath to stop the reaction.
11. Add 10 mL of distilled water and mix thoroughly.
12. Measure absorbance at 520 nm by using sample no. 1 in the table A.1.
13. Plot the absorbance versus sucrose concentration.

Residual sugars concentration

The samples obtained from experiments are analyzed as the same procedure as the standard sample and quantify base on the standard curve. The calculation of sugars concentration is as shown in equation A.1.

$$\text{Sample sugar concentration (g/100ml.)} = \frac{\text{Absorbance}_{520}}{\text{Standard RDS slope}} \quad \text{----- (A.1)}$$

APPENDIX B

Mathematic method

Initial rate method

The initial rate method is a mode of measurement in a kinetic method of analysis, in which the initial reaction rate is determined (usually by the slope method).

The example of initial rate method is illustrated as follow:

From Monod equation

$$\frac{dC_x}{dt} = \mu = \frac{\mu_m C_s}{K_s + C_s} C_x \quad \text{----- (B.1)}$$

It can be transform to slope method for finding parameter.

$$\frac{1}{C_x} \frac{dC_x}{dt} = \mu = \frac{\mu_m C_s}{K_s + C_s} \quad \text{----- (B.2)}$$

$$\frac{1}{\mu} = \frac{K_s + C_s}{\mu_m C_s} \quad \text{----- (B.3)}$$

$$\frac{1}{\mu} = \frac{K_s}{\mu_m} \frac{1}{C_s} + \frac{1}{\mu_m} \quad \text{----- (B.4)}$$

From curve fitting between $1/\mu$ and $1/C_s$ data, K_s/μ_m and $1/\mu_m$ can be estimated from the slope and the intersection respectively.

For example, from 3% reducing sugar concentration, the specific growth rate can be calculated from experimental data by equation B.2.

$$\frac{1}{C_x} \frac{dC_x}{dt} = \mu \quad \text{----- (B.5)}$$

Then, the kinetic parameters can be determined by the initial rate method as shown in Table B.1.

Table B.1 Parameters for initial rate method calculation

Time	C_x	C_s	μ	$1/\mu$	$1/C_s$
6	2	25	0.1974	5.0667	0.04
12	4	10	0.0938	10.6667	0.10
18	7	5	0.0536	18.6667	0.20

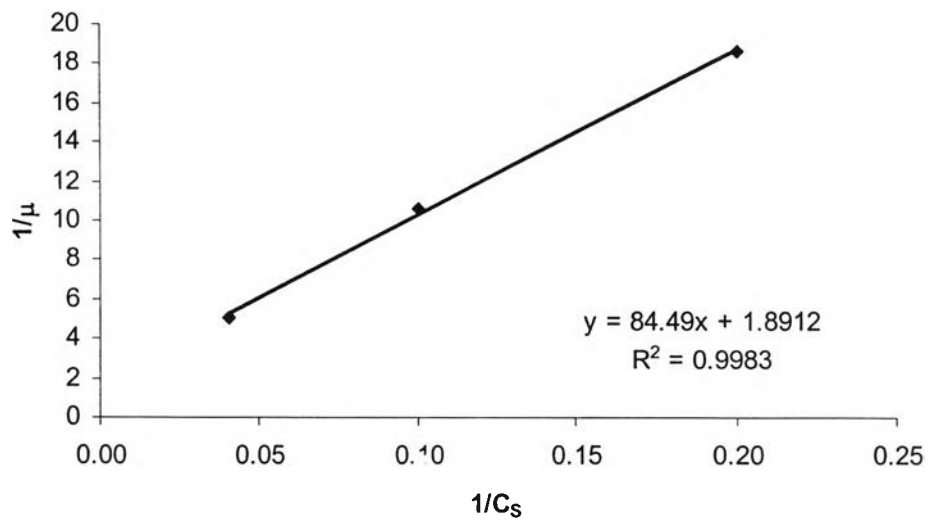


Figure B.1 Curve fitting between $1/\mu$ and $1/C_s$. Line corresponds linear equation whose slope and intersection of Y axis are K_S/μ_m and $1/\mu_m$.

where, slope = $\frac{K_S}{\mu_m}$ and Y axis intersection = $\frac{1}{\mu_m}$

$$\mu_m = 1/1.839 = 0.53 \text{ h}^{-1}$$

$$K_S = 84.49 \times 0.53 = 44.68 \text{ g/l}$$

Common Numerical Methods for Solving ODE's

The numerical methods for solving ordinary differential equations are methods of *integrating a system of first order differential equations*, since higher order ordinary differential equations can be reduced to a set of first order ODE's. For example,

$$p(x) \frac{d^2 y}{dx^2} + q(x) \frac{dy}{dx} = r(x)$$

$$\text{Let } y(x) = y_1(x) \text{ and } \frac{dy_1}{dx} = y_2(x)$$

$$\Rightarrow \begin{cases} \frac{dy_1}{dx} = y_2(x) \\ \frac{dy_2}{dx} = [r(x) - q(x)y_2(x)]/p(x) \end{cases}$$

An n th order ordinary differential can be similarly reduced to

$$\frac{dy_k(x)}{dx} = f_k(x, y_1, y_2, \dots, y_n) \text{ where } k = 1, 2, \dots, n$$

Common numerical methods for solving *initial value problems* of ordinary differential equations are summarized in three methods, Euler Method, Midpoint Method, and Runge-Kutta Method.

Euler Method

The Euler method is important in concept for it points the way of solving ODE by marching a small step at a time on the right-hand-side to approximate the "derivative" on the left-hand-side.

$$\text{To solve a first order ODE } \frac{dy}{dx} = f(x, y)$$

Given the initial condition $y(x_0) = y_0$ and pick the marching step h ,

$$k_1 = hf(x_n, y_n)$$

$$\Rightarrow y_{n+1} = y_n + k_1 + O(h^2)$$

However, the Euler method has *limited value in practical usage*.

Midpoint Method

The midpoint method, also known as the second-order Runge-Kutta method, improves the Euler method by adding a midpoint in the step which increases the accuracy by one order.

To solve a first order ODE $\frac{dy}{dx} = f(x, y)$

Given the initial condition $y(x_0) = y_0$ and pick the marching step h ,

$$k_1 = hf(x_n, y_n)$$

$$k_2 = hf\left(x_n + \frac{h}{2}, y_n + \frac{k_1}{2}\right)$$

$$\Rightarrow y_{n+1} = y_n + k_2 + O(h^3)$$

Runge-Kutta Method

The fourth-order Runge-Kutta method is by far the ODE solving method *most often used*. It can be summarized as follows:

To solve a first order ODE $\frac{dy}{dx} = f(x, y)$

Given the initial condition $y(x_0) = y_0$ and pick the marching step h ,

$$k_1 = hf(x_n, y_n)$$

$$k_2 = hf\left(x_n + \frac{h}{2}, y_n + \frac{k_1}{2}\right)$$

$$k_3 = hf\left(x_n + \frac{h}{2}, y_n + \frac{k_2}{2}\right)$$

$$k_4 = hf(x_n + h, y_n + k_3)$$

$$\Rightarrow y_{n+1} = y_n + \frac{k_1}{6} + \frac{k_2}{3} + \frac{k_3}{3} + \frac{k_4}{6} + O(h^5)$$

Numerical computing with MATLAB

Solver for stiff and non-stiff problems

There are many solvers in MATLAB as shown in Table B.2. In this study, ode45 and ode15s are chosen to be ode functions in programming.

Table B.2 ODE function summary in MATLAB

Solver	Solves These Kinds of Problems	Method
<u>ode45</u>	Nonstiff differential equations	Runge-Kutta
<u>ode23</u>	Nonstiff differential equations	Runge-Kutta
<u>ode113</u>	Nonstiff differential equations	Adams
<u>ode15s</u>	Stiff differential equations and DAEs	NDFs (BDFs)
<u>ode23s</u>	Stiff differential equations	Rosenbrock
<u>ode23t</u>	Moderately stiff differential equations and DAEs	Trapezoidal rule
<u>ode23tb</u>	Stiff differential equations	TR-BDF2

Algorithms

ode45

Ode45 is based on an explicit Runge-Kutta (4,5) formula, the Dormand-Prince pair. It is a one-step solver – in computing $y(t_n)$, it needs only the solution at the immediately preceding time point, $y(t_{n-1})$. In general, ode45 is the best function to apply as a "first try" for most problems.

ode15s

Variable-order solver based on the numerical differentiation formulas (NDFs). Optionally it uses the backward differentiation formulas, BDFs, (also known as Gear's method). Like ode113, ode15s is a multistep solver. If you suspect that a problem is stiff or if ode45 failed or was very inefficient, tries ode15s.

ode15s Properties

The ode15s solver is a variable-order stiff solver based on the numerical differentiation formulas (NDFs). The NDFs are generally more efficient than the closely related family of backward differentiation formulas (BDFs), also known as Gear's methods. The ode15s properties let you choose between these formulas, as well as specifying the maximum order for the solver.

Table B.3 ode15s Properties

Property	Value	Description
MaxOrder	1 2 3 4 {5}	The maximum order formula used.
BDF	on {off}	Specifies whether the backward differentiation formulas are to be used instead of the default numerical differentiation formulas.

MaxOrder

MaxOrder is an integer 1 through 5 used to set an upper bound on the order of the formula that computes the solution. By default, the maximum order is 5.

BDF

Set BDF on to have ode15s use the BDFs. By default, BDF is off, and the solver uses the NDFs.

For both the NDFs and BDFs, the formulas of orders 1 and 2 are A-stable (the stability region includes the entire left half complex plane). The higher order formulas are not as stable and the higher the order the worse the stability. There is a class of stiff problems (stiff oscillatory) that is solved more efficiently if MaxOrder is reduced (for example to 2) so that only the most stable formulas are used.

The Method of Least Squares

The method of least squares assumes that the best-fit curve of a given type is the curve that has the minimal sum of the deviations squared (*least square error*) from a given set of data.

Suppose that the data points are (x_1, y_1) , (x_2, y_2) , ..., (x_n, y_n) where x is the independent variable and y is the dependent variable. The fitting curve $f(x)$ has the deviation (error) d from each data point, i.e., $d_1 = y_1 - f(x_1)$, $d_2 = y_2 - f(x_2)$, ..., $d_n = y_n - f(x_n)$. According to the method of least squares, the best fitting curve has the property that:

$$\Pi = d_1^2 + d_2^2 + \dots + d_n^2 = \sum_{i=1}^n d_i^2 = \sum_{i=1}^n [y_i - f(x_i)]^2 = \text{a minimum}$$

The Least-Squares m th Degree Polynomials

When using an m^{th} degree polynomial

$$y = a_0 + a_1x + a_2x^2 + \dots + a_mx^m$$

to approximate the given set of data, (x_1, y_1) , (x_2, y_2) , ..., (x_n, y_n) , where $n \geq m + 1$, the best fitting curve $f(x)$ has the least square error, i.e.,

$$\Pi = \sum_{i=1}^n [y_i - f(x_i)]^2 = \sum_{i=1}^n \left[y_i - (a_0 + a_1x_i + a_2x_i^2 + \dots + a_mx_i^m) \right]^2 = \min$$

Please note that a_0 , a_1 , a_2 , ..., and a_m are unknown coefficients while all x_i and y_i are given. To obtain the least square error, the unknown coefficients a_0 , a_1 , a_2 , ..., and a_m must yield zero first derivatives.

$$\left. \begin{aligned}
 \frac{\partial \Pi}{\partial a_0} &= 2 \sum_{i=1}^n \left[y_i - (a_0 + a_1 x_i + a_2 x_i^2 + \dots + a_m x_i^m) \right] = 0 \\
 \frac{\partial \Pi}{\partial a_1} &= 2 \sum_{i=1}^n x_i \left[y_i - (a_0 + a_1 x_i + a_2 x_i^2 + \dots + a_m x_i^m) \right] = 0 \\
 \frac{\partial \Pi}{\partial a_2} &= 2 \sum_{i=1}^n x_i^2 \left[y_i - (a_0 + a_1 x_i + a_2 x_i^2 + \dots + a_m x_i^m) \right] = 0 \\
 &\vdots \\
 &\vdots \\
 \frac{\partial \Pi}{\partial a_m} &= 2 \sum_{i=1}^n x_i^m \left[y_i - (a_0 + a_1 x_i + a_2 x_i^2 + \dots + a_m x_i^m) \right] = 0
 \end{aligned} \right\}$$

Expanding the above equations, we have

$$\left. \begin{aligned}
 \sum_{i=1}^n y_i &= a_0 \sum_{i=1}^n 1 + a_1 \sum_{i=1}^n x_i + a_2 \sum_{i=1}^n x_i^2 + \dots + a_m \sum_{i=1}^n x_i^m \\
 \sum_{i=1}^n x_i y_i &= a_0 \sum_{i=1}^n x_i + a_1 \sum_{i=1}^n x_i^2 + a_2 \sum_{i=1}^n x_i^3 + \dots + a_m \sum_{i=1}^n x_i^{m+1} \\
 \sum_{i=1}^n x_i^2 y_i &= a_0 \sum_{i=1}^n x_i^2 + a_1 \sum_{i=1}^n x_i^3 + a_2 \sum_{i=1}^n x_i^4 + \dots + a_m \sum_{i=1}^n x_i^{m+2} \\
 &\vdots \\
 &\vdots \\
 \sum_{i=1}^n x_i^m y_i &= a_0 \sum_{i=1}^n x_i^m + a_1 \sum_{i=1}^n x_i^{m+1} + a_2 \sum_{i=1}^n x_i^{m+2} + \dots + a_m \sum_{i=1}^n x_i^{2m}
 \end{aligned} \right\}$$

The unknown coefficients a_0 , a_1 , a_2 , ..., and a_m can hence be obtained by solving the above linear equations.

APPENDIX C

Standard curve and experiment data

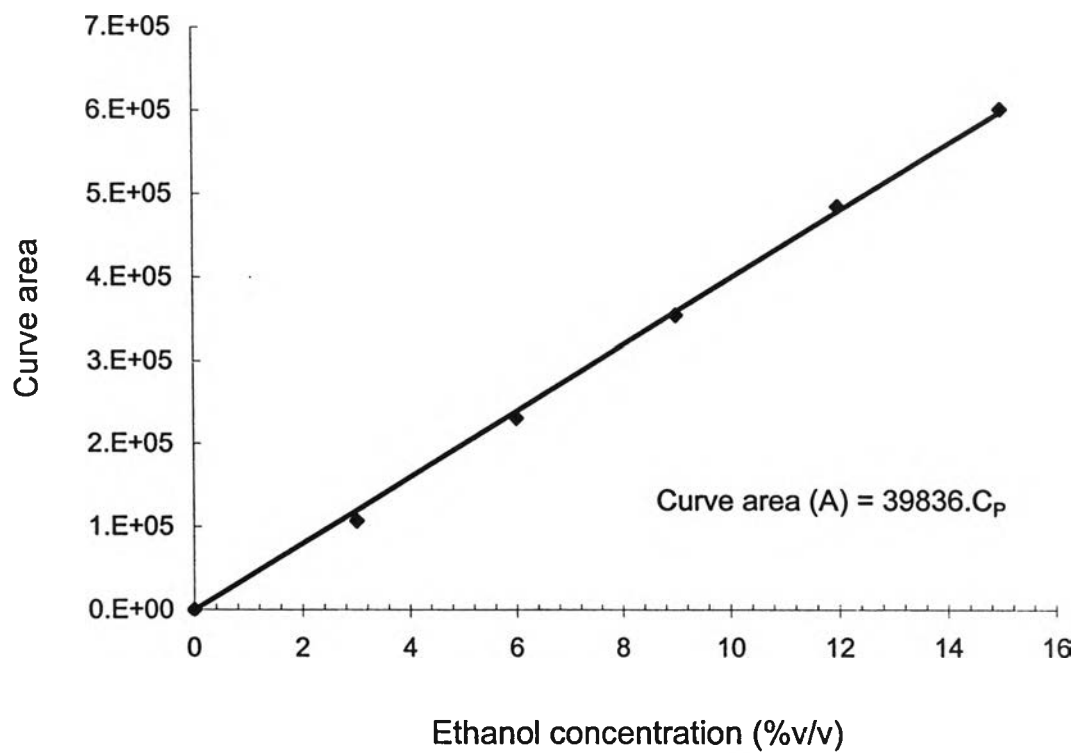


Figure C.1 Example of standard curve for ethanol concentration analysis

Table C.1 Experimental data with time and cell concentration of ethanol batch fermentation in shaking flasks with ranging from 3 to 25%w/v reducing sugar at 33°C

Time (hr)	Cell concentration (g/l)									
	3%	5%	8%	11%	13%	15%	17%	20%	22%	25%
0	0.21	0.15	0.15	0.18	0.18	0.21	0.27	0.27	0.24	0.24
6	2.58	2.64	3.67	4.70	2.52	2.36	1.48	1.45	1.82	1.03
12	4.55	3.51	5.45	6.06	5.09	4.18	4.58	4.48	3.97	3.61
18	4.24	5.42	5.30	5.91	5.24	4.97	4.70	6.42	7.03	4.21
24	5.30	5.00	5.42	5.58	5.70	6.88	5.06	5.45	6.09	6.30
30	5.54	5.70	5.76	5.61	5.97	7.15	6.64	5.97	5.21	5.58
36	6.00	6.24	5.82	6.15	6.88	5.61	6.82	5.79	5.76	4.79
42	5.73	6.15	5.91	6.06	5.58	6.70	7.61	5.00	5.51	5.12
48	5.76	5.61	5.09	5.76	6.18	5.48	6.91	4.85	5.67	4.97
54	6.00	5.70	5.82	5.61	5.73	5.61	7.73	5.09	5.85	4.55
60	4.97	5.61	5.70	6.42	6.42	6.48	6.51	6.21	6.45	4.39
66	5.00	5.42	5.45	5.54	6.45	6.82	6.48	5.45	5.64	4.64
72	3.79	4.15	5.42	5.91	6.52	6.00	6.51	5.00	5.15	4.09

Table C.2 Experimental data with time and substrate concentration of ethanol batch fermentation in shaking flasks with ranging from 3 to 25%w/v reducing sugar at 33°C

Time (hr)	Substrate concentration (g/l)									
	3%	5%	8%	11%	13%	15%	17%	20%	22%	25%
0	31.30	53.80	82.00	115.00	132.62	150.61	165.00	198.60	219.10	256.00
6	6.00	17.00	53.00	82.00	102.74	132.93	135.80	182.40	188.90	248.30
12	9.00	15.00	13.00	36.00	90.55	110.98	48.50	97.70	117.60	208.50
18	3.60	8.20	18.00	24.00	78.05	101.83	36.50	66.80	90.00	83.00
24	1.00	1.50	16.00	29.00	50.91	70.43	53.00	41.40	55.20	79.80
30	2.00	4.00	9.00	25.00	30.79	35.67	48.30	37.90	48.70	72.30
36	1.50	5.60	12.40	17.20	21.79	26.43	49.30	42.90	51.80	75.30
42	2.00	4.80	7.80	10.50	18.93	26.43	47.80	41.30	42.00	77.00
48	1.50	3.90	4.70	15.10	21.43	25.71	51.30	42.40	41.00	69.00
54	2.00	7.00	5.70	9.80	20.00	26.07	45.30	39.80	42.30	71.00
60	1.30	6.70	7.00	9.20	20.18	25.54	44.50	40.60	43.20	68.90
66	1.60	5.80	7.20	9.40	20.36	25.00	52.00	39.80	44.60	65.00
72	1.50	6.00	5.80	8.80	21.07	27.50	38.00	40.50	40.90	58.30

Table C.3 Experimental data with time and ethanol concentration of ethanol batch fermentation in shaking flasks with ranging from 3 to 25%w/v reducing sugar at 33°C

Time (hr)	Ethanol concentration (g/l)									
	3%	5%	8%	11%	13%	15%	17%	20%	22%	25%
0	1.19	1.43	2.94	3.57	4.05	4.40	4.05	1.19	4.13	4.13
6	4.37	8.02	8.02	11.75	13.91	13.06	26.20	3.18	9.53	13.65
12	6.83	11.99	16.04	24.69	29.72	23.05	52.20	21.28	29.85	27.15
18	6.91	12.70	17.62	26.12	35.62	37.54	54.90	26.36	59.06	42.23
24	6.43	14.60	20.72	32.71	44.81	37.88	62.40	67.64	66.60	76.13
30	6.19	14.69	20.48	29.53	42.93	43.60	72.30	69.70	79.94	78.04
36	7.38	14.61	22.23	30.80	37.95	47.66	72.80	70.97	76.45	91.21
42	5.64	14.30	23.74	36.68	42.42	45.24	65.90	69.22	74.62	88.43
48	6.11	14.45	22.62	31.52	40.32	46.95	69.00	63.83	80.34	78.35
54	7.18	14.60	21.91	31.44	35.65	44.01	61.40	70.49	72.64	91.05
60	7.78	14.84	18.50	31.04	36.97	43.79	61.90	68.43	75.89	75.89
66	7.19	15.08	16.99	30.48	35.58	42.60	66.00	67.79	79.07	80.26
72	7.67	15.32	18.18	28.74	36.00	42.02	71.80	68.19	76.37	77.88



Table C.4 Experimental data with time and cell concentration of ethanol batch fermentation in shaking flasks with 22%w/v reducing sugar at operating temperature ranging from 30 to 42°C.

Time (hr)	Temperature (Degree celcius)				
	30	33	35	38	42
0	0.20	0.29	0.18	0.23	0.18
4	0.77	0.63	0.36	0.47	0.30
8	1.57	1.55	0.58	1.01	0.60
12	4.00	4.99	1.60	1.37	0.87
16	5.17	7.31	2.44	1.80	1.10
20	6.43	6.69	3.94	2.33	1.13
24	7.30	7.03	5.52	2.57	1.17
30	7.80	6.79	7.00	2.67	1.40
36	8.15	7.22	8.02	2.62	1.13
42	7.81	6.51	7.50	2.80	1.40
48	7.90	8.19	7.49	2.45	1.30
56	7.53	6.86	6.53	2.90	0.97
64	6.90	5.83	7.38	2.90	1.17
72	6.43	5.83	6.98	2.93	1.03

Table C.5 Experimental data with time and substrate concentration of ethanol batch fermentation in shaking flasks with 22%w/v reducing sugar at operating temperature ranging from 30 to 42°C

Time (hr)	Temperature (Degree celcius)				
	30	33	35	38	42
0	221.86	222.01	220.47	229.13	223.70
4	212.79	215.95	206.93	219.97	209.42
8	178.95	177.40	187.17	205.56	213.96
12	144.33	105.50	157.09	203.27	191.23
16	119.63	63.79	130.08	182.32	170.45
20	103.17	43.01	115.28	180.03	167.86
24	75.23	51.91	86.61	163.01	145.13
30	55.01	37.71	62.36	137.48	137.89
36	52.03	39.78	51.97	136.17	141.23
42	46.73	43.38	47.56	129.62	148.05
48	46.06	42.43	51.34	130.28	146.85
56	49.71	41.72	51.02	127.66	135.39
64	50.04	40.65	53.23	130.28	149.35
72	50.37	39.83	55.43	132.90	144.81

Table C.6 Experimental data with time and ethanol concentration of ethanol batch fermentation in shaking flasks with 22%w/v reducing sugar at operating temperature ranging from 30 to 42°C

Time (hr)	Temperature (Degree celcius)				
	30	33	35	38	42
0	0.91	4.16	4.04	2.69	6.52
4	2.37	5.27	6.05	3.12	5.17
8	7.39	8.36	8.14	8.28	5.39
12	19.65	32.88	21.83	9.29	11.53
16	31.51	55.15	33.98	11.88	16.71
20	42.61	83.79	37.30	16.61	20.79
24	59.68	82.13	53.08	23.65	27.67
30	71.60	83.22	61.92	31.64	31.51
36	82.18	84.17	57.71	33.53	30.13
42	88.55	80.36	64.14	32.05	29.79
48	83.85	78.16	64.18	31.85	28.02
56	86.22	81.13	62.84	31.50	24.71
64	83.96	78.88	62.89	30.84	23.97
72	81.11	80.11	58.12	30.65	21.38

BIOGRAPHY

Mr. Nuttapan Srirattana was born on 14th November, 1980 in Bangkok. He finished his secondary course from Saint Gabriele's College in March, 1998. After that, he studied in the major of Chemical Engineering in Faculty of Engineering at Chulalongkorn University. He continued his further study for Master's degree in Chemical Engineering at Chulalongkorn University. He participated in the Biochemical Engineering Research Group and achieved his Master's degree in April, 2004.