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APPENDIX A

A.1 Specification of TAKA-THERM® L-340

THERMAL STABLE BACTERIAL ALPHA-AMYLASES FOR STARCH LIQUEFACTION

DESCRIPTION

TAKA-THERM bacterial alpha-amylase (E.C.3.2.1.1 1,4α-D-Glucan glucanohydrolase) is obtained by the controlled fermentation of Bacillus licheniformis var. TAKA-THERM complies with FCC and FAO/WHO (JECFA) recommended specifications for food grade enzymes. The alpha-amylase is characterized by dextrinizing actions on starch. The enzyme is an endoamylase capable of randomly hydrolyzing the interior alpha-1,4-glucosidic linkages of starch, glycogen and their degradation products. Consequently, the viscosity of gelatinous starch solutions is rapidly reduced. TAKA-THERM alpha-amylase initially hydrolyzes starch solutions yielding soluble dextrins and oligosaccharides. Prolonged hydrolysis with TAKA-THERM results in the formation of small quantities of glucose and moltose.

TAKA-THERM alpha-amylase demonstrates exceptional thermal stability. The alpha-amylase has a temperature optimum of 90 °C. The half-life of the alpha-amylase is such that the enzyme is active an stable at temperature above 100 °C., permitting the efficient liquefaction of starch slurries in both batch and continuous starch liquefaction systems.

TAKA-THERM is essentially free of protease activity. Problems caused by protein hydrolysis during starch liquefaction are thus avoided. An additional benefit of this low protease activity is prolonged storage stability.

Product	**Activity**	**Comment**
TAKA-THERM L340	340,000 MWU/ml	Stabilized and standardized Liquid

PROPERTIES

Form	Non-viscous Liquid
Color	Golden to Light Brown
Odor	Free of Offensive Odor
Taste	Free of Offensive Taste
Solubility	Miscible with Water
Density	1.15 to 1.25 g/ml
pH	5.18-5.20

Activators and Cofactors

No activators are necessary for the complete activity of TAKA-THERM. The alpha-amylase is a calcium metalloprotein and requires calcium ions as cofactor for maximum stability. Calcium ions are not involved in the starch hydrolysis step but stabilize the structure of the protein molecule to maintain an enzymatically active configuration. In the presence of sufficient calcium ions, TAKA-THERM tightly binds the calcium ions and is subsequently resistant to denaturation at extreme of temperature and pH. The addition of calcium salts to the starch slurry is recommended to ensured that sufficient calcium is available during liquefaction. For liquefaction of starch slurries, the addition of 100 to 200 mg/l (ppm) calcium ion is recommended. Calcium sources such as calcium oxide and calcium chloride are acceptable.

At 70 °C , maximum stability is observed when 5 to 10 mg/l (ppm) calcium is present in aqueous solution. Some whole cereal grains contain organic acids, phosphates and other calcium complexing compounds. These compounds complex calcium ions in solution, thereby lowering the available calcium ion level below that required for maximum enzyme stability. When liquefying such whole cereal grains (corn, rice, malt and barley),the addition of 100 to 200 mg/l (ppm) calcium ion is recommended. This ensures that sufficient calcium ion is available for enzyme stability.

Activity

One Modifies Wohlgemuth Unit (MWU) is that activity which will dextrinize one milligram of soluble starch to a defined size dextrin in thirty minutes under the conditions of assay. A copy of the Determination of Liquefying Amylase (Modified Wholgemuth Method) is available upon request.

The effect of pH

TAKA-THERM bacterial alpha-amylase has an optimum pH range of pH 5.0 to 7.0. In the presence of 35% refined corn starch on a dry solids basis (DBS), the enzyme demonstrates maximum hydrolysis over the range of pH 6.0 to 6.5 at 95 °C. Although the rates of hydrolysis are diminished at the extremes, TAKA-THERM is stable in aqueous solution over the pH range of pH 5.0 to 11.0 at 25 °C.

Optimum pH range	pH 5.5 to 7.0
Effective pH range	pH 5.0 to 8.0
pH stability	pH 5.0 to 11.0

The effect of temperature

TAKA-THERM bacterial alpha-amylase has a temperature optimum of 90 °C at pH 6.0 in the presence of 10 mg/l (ppm) calcium ion. In the presence of higher starch concentrations (10% - 50%DBS) and sufficient soluble calcium ion (50-70 mg/l), the alpha-amylase demonstrates exceptional thermal stability, effectively hydrolyzing starch at elevated temperatures. Temperatures below 95 °C at pH 6.0 to 6.8 result in extended enzyme stability. Temperatures below 95 °C result in an accelerated rate of starch hydrolysis with an increase in the rate of enzyme inactivation.

Optimum temperature	90 °C
Optimum temperature range	90 °C
Effective temperature range	Up to 100 °C

Inhibitors

Several metal ions inhibit TAKA-THERM. Copper, ferrous and cobalt ions are moderate inhibitors and aluminum, lead and zinc ions are strong inhibitors. Being a calcium metalloprotein, TAKA-THERM is negatively affected by strong oxidizing and sequestering agents (EDTA).

Other Enzyme Components

In addition to alpha amylase activity, TAKA-THERM is characterized by a low level of protease activity.

Solubility

TAKA-THERM liquid is completely miscible with water. The enzyme can be manually added to the starch slurry or can be added directly by an appropriate metering system.

Substrate

TAKA-THERM may be used to liquefy malt, barley, corn, milo, potato, wheat and other starches or whole cereal grains.

Use Level

The exact use level of TAKA-THERM alpha-amylase in starch liquefaction varies with the starch substrate, liquefaction equipment and processing conditions. To optimize the use of TAKA-THERM alpha-amylase, we recommend a number of liquefaction trials prior to routine use of the enzyme. This assures an optimum liquefaction process and an uniform hydrolysate. Evaluate TAKA-THERM L-340 at 0.25 to 0.75 l/1000 kg starch DBS (equivalent to 85,000 to 255,000 MWU/kg starch DBS). Under standard conditions, this will adequately liquefy a 30 to 38 % DSB starch slurry .

APPLICATIONS

Food industry

Brewing	Liquefaction of adjuncts and removal of starch haze.
Candy	Scrap candy recovery.
Cane Sugar	Starch haze removal
Cereal	Production of precooked cereals. Reduce the viscosity of the cereal prior to roll drying.
Cocoa	Reduces the viscosity of chocolate syrups.
Distilling	Mash liquefaction.
Fermentation	Liquefaction of starch fermentation media.
Starch	Reduce of high viscosity starch slurries. Production of maltodextrins and low DE syrups. Production of starch hydrolysates for crystalline glucose, glucose and high fructose syrup production. Production of starch hydrolysates for further enzyme conversions. Removal of starch hazes from syrup.

Other industries

Paper	Preparation of starch sizings and coatings.
Starch	Preparation of starch adhesives.
Textile	Desizing.
Other	Cleaning starch processing equipment (extruders, filters, etc.)

A.2 Specification of OPTIDEX

Glucoamylase

INTRODUCTION

OPTIDEX-L is a glucoamylase (amyloglucosidase) of food grade quality produced by a selected strain of Aspergillus niger. Being an exoenzyme it cleaves glucose molecules from the non reducing end of liquefied starch, amylase, amylopectins or maltodextrins. The enzyme hydrolyses not only the 1,4-∝-glycosidic linkages but also attacks the 1,6-∝-glycosidic branching points thus preventing the formation of limit dextrins.

OPTIDEX-L contains an acid ∝-amylase, only a slight amount of protease and is almost free of transglucosidase. The an efficient saccharification without the development of undesired color is ensured.

Besides this OPTIDEX-LW contains a high content of phospholipase and pentosanase (arabinoxylanase) which especially after saccharification of wheat starch results in better filtration rates.

CHARACTERISTIC

Appearance

OPTIDEX-L and -LW are offered in liquid formulations, where the addition of preservatives - of food grade quality - guarantees an excellent shelf life.

Properties

Microorganism :	Aspergillus niger
Form:	liquid
Color:	light brown
Odor:	slight, typical for fermentation

Density:	1.1 - 1.3
pH	4.5

Activity

One Glucoamylase Unit (GAU) is defined as the amount of enzyme that under standardized conditions (pH 4.2; 60 °C; 60 min) liberates 1g. of reducing sugars determined as glucose per hour from a 4% soluble starch solution.

Activity:

L300	300GAU/ml min
LW300	300GAU/ml min

Optimum:

pH	3.8 - 4.5
Temperature	55 - 60 °C

Use Level:

L300	0.5 - 0.6 l/ton d.s.
LW300	0.5 - 0.6 l/ton d.s.

Effect of pH

The enzymatic of OPTIDEX is stable over a broad pH range. To reach maximum DE (dextrose equivalent, method: Lane Eynon) with minimum of color formation the pH of the substrate should be within 3.8-4.2.

Effect of temperature

OPTIDEX has an optimum temperature between 55-60 °C; in order to avoid any hazard of microbial contamination, 60 °C has proved to be the appropriate temperature.

Effect of substrate

To achieve an efficient saccharification we recommend to use a starch substrate of 14-18 DE and a dry substance between 30-35 %.

Effect of dosage

The time necessary to reach the desired DE is influenced by the dosage of the enzyme.

Inactivation

OPTIDEX is inactivated by increasing the temperature to 85 °C and holding for 10 minutes.

STORAGE

OPTIDEX formulations are stable for several months when stored at room temperature (~22 °C).

TECHNICAL SERVICE

Solvey Enzyme maintain an experienced technical staff and well equipped laboratories to assist the customer with the use of our products.

Table A-1 Data of Bacillus subtilis TISTR 25 standard curve

O.D.420 nm.	Volume (ml)	Biomass (g)	Biomass Concentration (g/l)
1.000	5	0.0034	0.6800
1.000	5	0.0032	0.6400
1.000	5	0.0034	0.6800
0.758	5	0.0022	0.4400
0.758	5	0.0023	0.4600
0.758	5	0.0023	0.4600
0.554	5	0.0019	0.3800
0.554	5	0.0018	0.3600
0.554	5	0.0016	0.3200
0.456	5	0.0012	0.2400
0.456	5	0.0013	0.2600
0.456	5	0.0014	0.2800
0.382	5	0.0009	0.1800
0.382	5	0.0009	0.1800
0.382	5	0.0008	0.1600
0.287	5	0.0008	0.1600
0.287	5	0.0008	0.1600
0.287	5	0.0008	0.1600
0.190	5	0.0004	0.0800
0.190	5	0.0005	0.1000
0.190	5	0.0005	0.1000
0.100	5	0.0004	0.0800
0.100	5	0.0004	0.0800
0.100	5	0.0003	0.0700

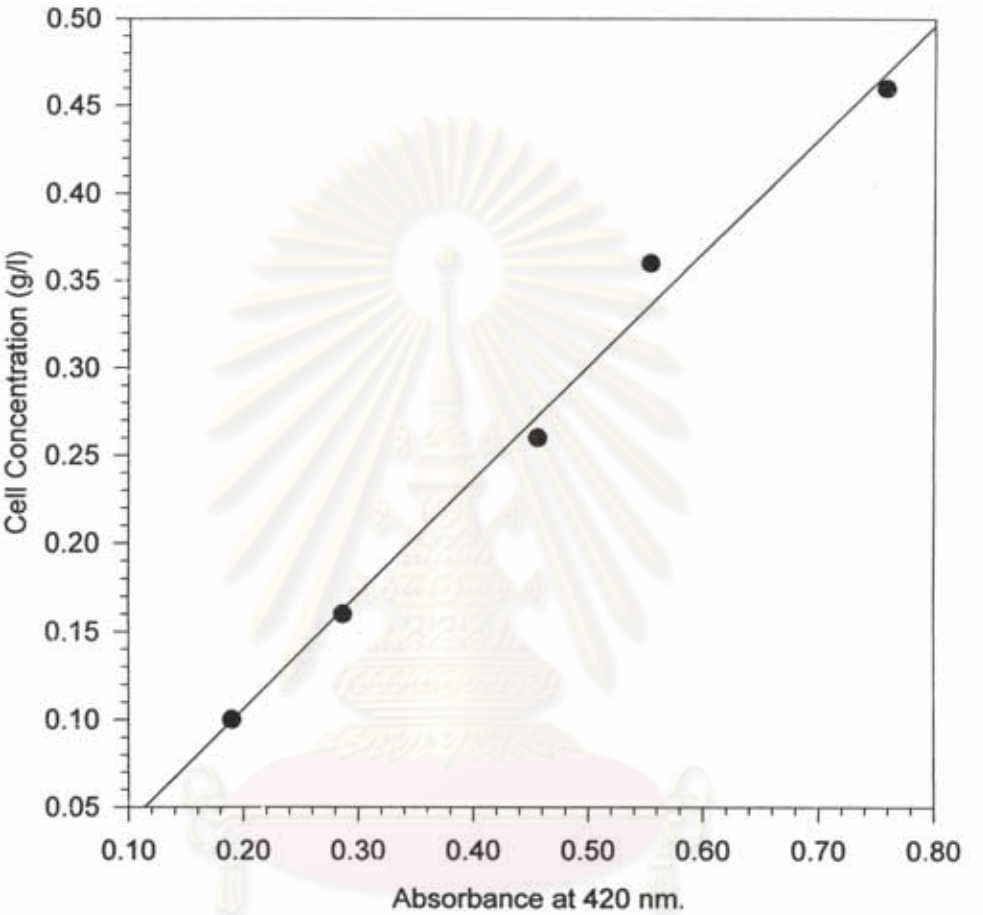

Figure A-1 Standard curve of Bacillus subtilis TISTR 25

Table A-2 Data of tyrosine standard curve

Tyrosine concentration (µg/ml)	Absorbance at 280 nm.		
	I	II	Average
20	0.1080	0.1130	0.1105
40	0.2300	0.2150	0.2225
60	0.3300	0.3430	0.3365
80	0.4300	0.4325	0.4313
100	0.5260	0.5260	0.5260
120	0.6080	0.6110	0.6095
140	0.6830	0.6845	0.6838

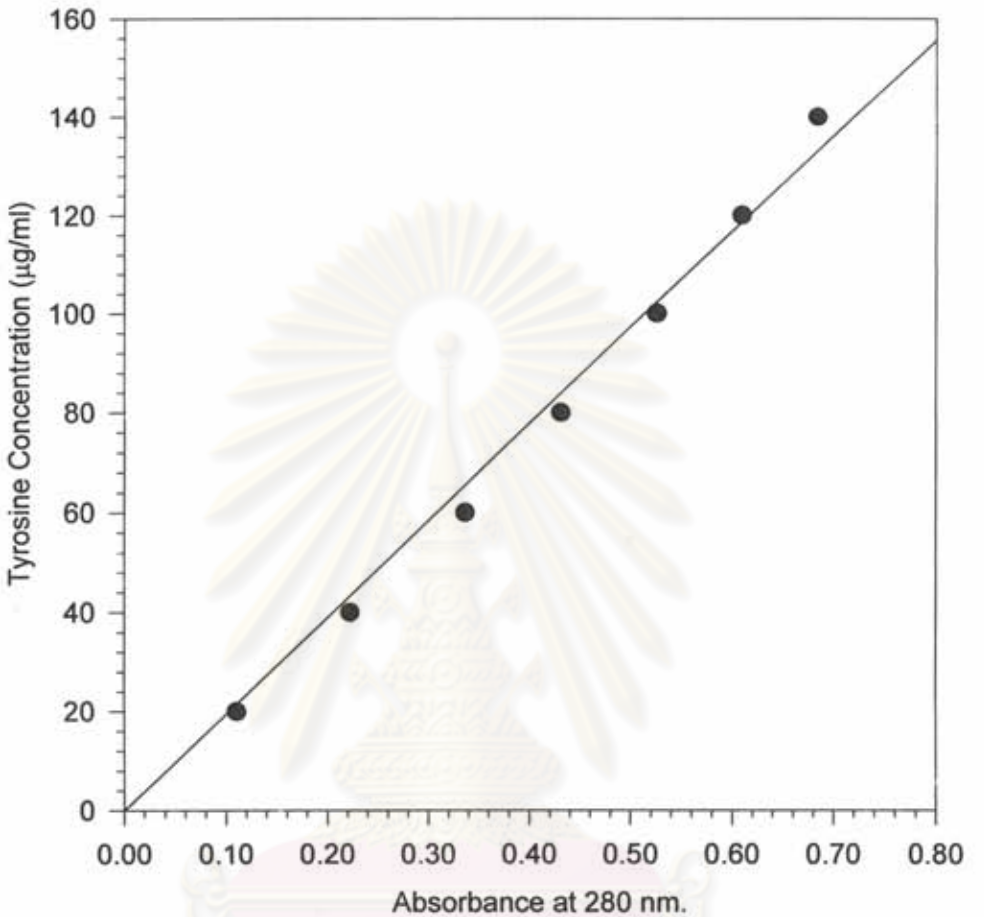

Figure A-2 Standard curve of tyrosine

Table A-3 Experimental data of controlled pH of 6.0 in batch fermentation.

Time (hr)	Acid (ml)	Base (ml)	Cell (g/l)	Glucose (g/l)	Product (unit/ml)	Cell Prod. (g-cell/hr)	Prod. Prod. (unit/ml-hr)
0	0.0	0.0	0.0691	1.75	0.0548	no	no
2	0.0	0.0	0.1165	1.55	0.0526	0.058	0.026
4	0.0	1.0	0.2765	1.35	0.2491	0.069	0.062
6	0.0	4.3	0.3415	1.17	0.3865	0.057	0.064
8	0.8	4.0	0.3903	0.93	0.5132	0.049	0.064
10	1.2	6.2	0.4595	0.71	0.5035	0.046	0.050
14	2.0	7.4	0.5496	0.52	0.5293	0.039	0.038
18	1.0	6.0	0.5767	0.39	0.5293	0.032	0.029
22	1.5	2.0	0.6499	0.22	0.5991	0.030	0.027
26	2.5	1.0	0.6520	0.08	0.6270	0.025	0.024
30	2.4	1.5	0.6628	0.00	0.6796	0.022	0.023
34	2.6	1.6	0.6554	0.00	0.6807	0.019	0.020
38	3.2	1.4	0.6364	0.00	0.6807	0.017	0.018
42	3.5	0.8	0.6567	0.00	0.6667	0.016	0.016
46	2.0	1.0	0.6472	0.00	0.6732	0.014	0.015

Kinetic parameters at the hour of 0-34

$Y_{x/s}$ (g-cell/ g-glucose)	0.335
$Y_{p/s}$ (unit/mg-glucose)	0.358
$Y_{p/x}$ (unit/mg-cell)	1.068
Cell Productivity (g-cell/l-hr)	0.019
Product Productivity (unit/ml-hr)	0.020

Table A-4 Experimental data of controlled pH of 6.5 in batch fermentation.

Time (hr)	Acid (ml)	Base (ml)	Cell (g/l)	Glucose (g/l)	Product (unit/ml)	Cell Prod. (g-cell/hr)	Prod. Prod. (unit/ml-hr)
0	0.0	0.0	0.0697	1.86	0.0784	no	no
2	0.0	1.0	0.1043	1.65	0.0784	0.052	0.036
4	0.0	9.0	0.3653	1.42	0.0623	0.091	0.016
6	2.2	3.5	0.5510	1.25	0.1428	0.092	0.024
8	0.8	0.0	0.6228	1.10	0.3586	0.078	0.045
10	1.0	2.0	0.6689	0.91	0.5723	0.067	0.057
14	0.0	0.4	0.6635	0.61	0.7000	0.047	0.050
18	0.0	7.0	0.6703	0.36	0.7397	0.037	0.041
22	0.0	7.0	0.7082	0.15	0.7644	0.032	0.035
26	0.0	1.0	0.6689	0.00	0.7494	0.026	0.029
30	0.0	4.8	0.6174	0.00	0.7161	0.021	0.024
34	0.0	1.0	0.6120	0.00	0.7752	0.018	0.023
38	0.0	1.0	0.6554	0.00	0.7365	0.017	0.019
42	0.0	1.0	0.6093	0.00	0.6925	0.015	0.016
46	3.0	2.0	0.5808	0.00	0.6522	0.013	0.014

Kinetic parameters at the hour of 0-22

$Y_{x/s}$ (g-cell/ g-glucose)	0.373
$Y_{p/s}$ (unit/mg-glucose)	0.401
$Y_{p/x}$ (unit/mg-cell)	1.074
Cell Productivity (g-cell/l-hr)	0.032
Product Productivity (unit/ml-hr)	0.035

Table A-5 Experimental data of controlled pH of 7.0 in batch fermentation.

Time (hr)	Acid (ml)	Base (ml)	Cell (g/l)	Glucose (g/l)	Product (unit/ml)	Cell Prod. (g-cell/hr)	Prod. Prod. (unit/ml-hr)
0	0.0	0.0	0.0616	1.76	0.0150	no	no
2	0.8	3.0	0.0928	1.59	0.0150	0.046	0.008
4	0.0	8.8	0.1809	1.35	0.0236	0.045	0.006
6	0.0	2.8	0.3517	1.19	0.1492	0.059	0.025
8	0.0	1.2	0.6323	1.01	0.4606	0.079	0.058
10	0.0	1.0	0.6323	0.88	0.6839	0.073	0.068
14	0.0	3.0	0.7889	0.58	0.7977	0.056	0.057
18	0.0	4.0	0.7570	0.30	0.7881	0.042	0.044
22	0.0	3.0	0.7387	0.10	0.7773	0.034	0.035
26	0.0	4.8	0.7326	0.03	0.7655	0.028	0.029
30	0.0	2.2	0.7218	0.00	0.7344	0.024	0.024
34	3.0	0.0	0.6987	0.00	0.7322	0.021	0.022
38	3.0	0.0	0.6947	0.00	0.7312	0.018	0.019
42	3.5	0.0	0.7042	0.00	0.7290	0.017	0.017
46	2.0	0.0	0.7015	0.00	0.7312	0.015	0.016

Kinetic parameters at the hour of 0-14

$Y_{x/s}$ (g-cell/ g-glucose)	0.616
$Y_{p/s}$ (unit/mg-glucose)	0.663
$Y_{p/x}$ (unit/mg-cell)	1.076
Cell Productivity (g-cell/l-hr)	0.056
Product Productivity (unit/ml-hr)	0.057

Table A-6 Experimental data of controlled pH of 7.5 in batch fermentation.

Time (hr)	Acid (ml)	Base (ml)	Cell (g/l)	Glucose (g/l)	Product (unit/ml)	Cell Prod. (g-cell/hr)	Prod. Prod. (unit/ml-hr)
0	0.0	0.0	0.0867	1.79	0.0633	no	no
2	0.0	2.0	0.1104	1.70	0.0633	0.055	0.032
4	3.5	5.0	0.4134	1.44	0.2158	0.103	0.054
6	0.0	7.0	0.5903	1.28	0.3242	0.098	0.054
8	0.0	6.5	0.6459	1.16	0.4778	0.081	0.060
10	0.0	9.0	0.6689	0.98	0.5486	0.067	0.055
14	0.0	6.0	0.6628	0.74	0.6066	0.047	0.043
18	0.0	4.0	0.6594	0.45	0.6592	0.037	0.037
22	0.0	4.0	0.6635	0.09	0.6710	0.030	0.031
26	0.0	4.6	0.5889	0.03	0.6689	0.023	0.026
30	1.6	0.6	0.5537	0.00	0.6657	0.018	0.022
34	0.0	0.6	0.4846	0.00	0.6657	0.014	0.020
38	0.0	0.0	0.4412	0.00	0.6624	0.012	0.017
42	2.4	0.6	0.4873	0.00	0.6646	0.012	0.016
46	7.8	0.0	0.4344	0.00	0.6657	0.009	0.014

Kinetic parameters at the hour of 0-22

$Y_{x/s}$ (g-cell/ g-glucose)	0.339
$Y_{p/s}$ (unit/mg-glucose)	0.357
$Y_{p/x}$ (unit/mg-cell)	1.053
Cell Productivity (g-cell/l-hr)	0.030
Product Productivity (unit/ml-hr)	0.031

Table A-7 Experimental data of controlled pH of 8.0 in batch fermentation.

Time (hr)	Acid (ml)	Base (ml)	Cell (g/l)	Glucose (g/l)	Product (unit/ml)	Cell Prod. (g-ceil/hr)	Prod. Prod. (unit/ml-hr)
0	0.0	0.0	0.0521	1.77	0.0440	no	no
2	0.0	2.0	0.1063	1.65	0.0354	0.053	0.018
4	0.0	6.5	0.3442	1.41	0.1965	0.086	0.049
6	0.0	7.6	0.4391	1.15	0.3511	0.073	0.059
8	0.0	7.2	0.5442	0.85	0.4756	0.068	0.059
10	0.0	8.5	0.5815	0.68	0.5025	0.058	0.050
14	0.0	6.4	0.5828	0.46	0.5454	0.042	0.037
18	0.0	4.2	0.5977	0.20	0.5798	0.033	0.030
22	0.0	4.5	0.5883	0.08	0.5604	0.027	0.026
26	0.0	4.0	0.5944	0.03	0.6141	0.023	0.024
30	0.0	4.6	0.5266	0.00	0.6012	0.018	0.020
34	0.0	1.8	0.4818	0.00	0.5991	0.014	0.018
38	0.0	2.0	0.4358	0.00	0.5969	0.011	0.016
42	0.0	1.6	0.4595	0.00	0.5808	0.011	0.014
46	0.0	1.8	0.4385	0.00	0.5776	0.010	0.013

Kinetic parameters at the hour of 0-26

$Y_{x/s}$ (g-ceil/ g-glucose)	0.312
$Y_{p/s}$ (unit/mg-glucose)	0.328
$Y_{p/x}$ (unit/mg-cell)	1.051
Cell Productivity (g-cell/l-hr)	0.023
Product Productivity (unit/ml-hr)	0.024

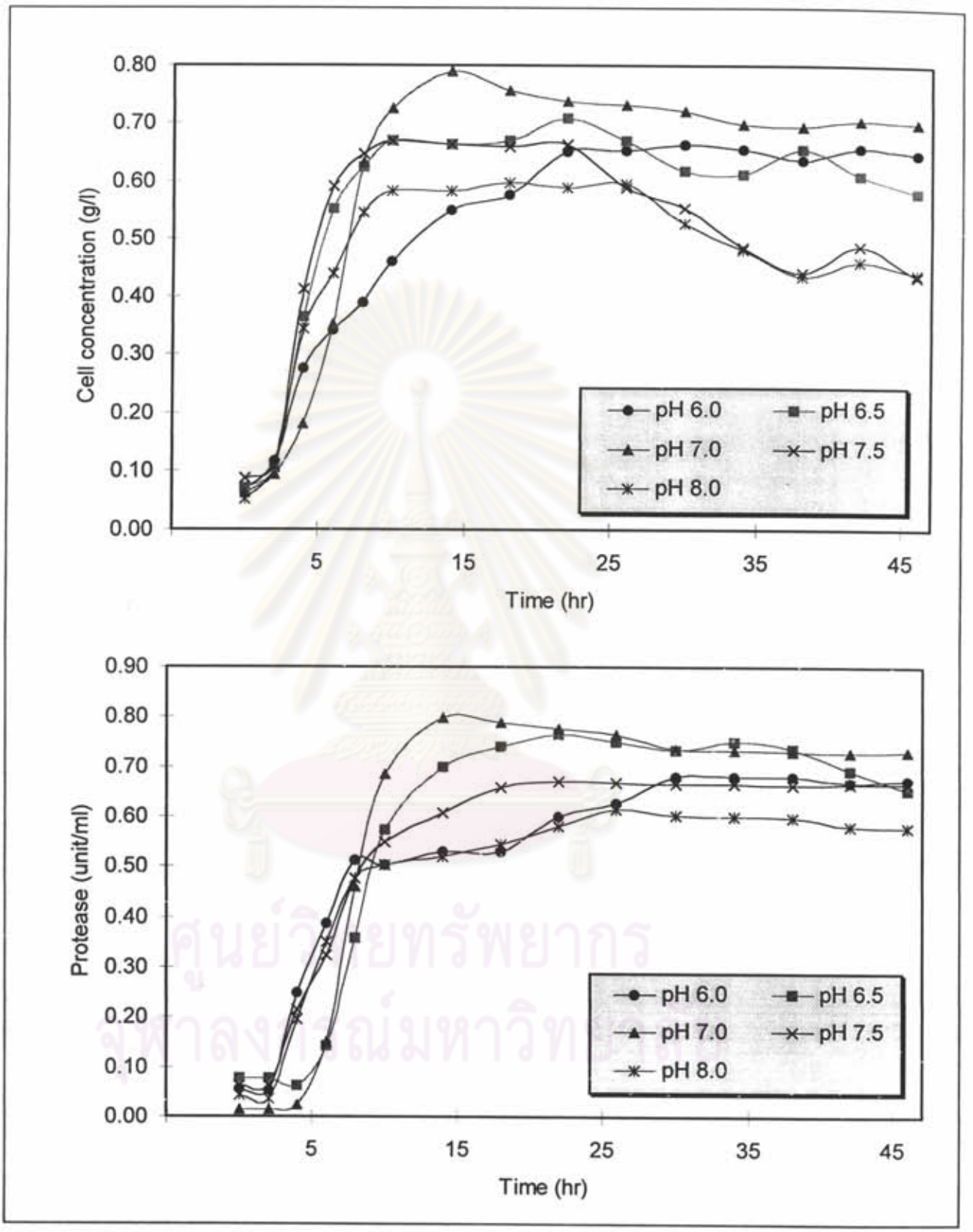

Figure A-3 Comparison of controlled pH on growth and protease production

Table A-8 Experimental data of initial glucose concentration of 1.0 g/l in batch fermentation.

Time (hr)	Acid (ml)	Base (ml)	Cell (g/l)	Glucose (g/l)	Product (unit/ml)
0	0.0	0.0	0.0623	1.02	0.0279
2	0.0	2.0	0.0779	0.95	0.0268
4	0.0	5.6	0.1341	0.78	0.0419
6	0.0	2.0	0.2243	0.54	0.1471
8	0.0	1.0	0.3863	0.37	0.2942
10	0.0	1.5	0.5171	0.26	0.4681
14	0.0	1.7	0.6391	0.08	0.6077
18	0.0	1.8	0.6344	0.00	0.6249
22	0.0	2.0	0.6337	0.00	0.6012
26	0.0	1.0	0.6194	0.00	0.6002
30	0.0	1.7	0.6052	0.00	0.5690
34	0.0	0.0	0.6194	0.00	0.5841
38	1.2	0.0	0.5984	0.00	0.5959
42	1.6	0.0	0.6113	0.00	0.6120
46	2.8	0.0	0.6208	0.00	0.5937

Kinetic parameters at the maximum protease production

Yx/s (g-cell/ g-glucose)	0.561
Yp/s (unit/mg-glucose)	0.585
Yp/x (unit/mg-cell)	1.043
Cell Productivity (g-cell/l-hr)	0.035
Prod. Productivity (unit/ml-hr)	0.035

Table A-9 Experimental data of initial glucose concentration of 1.8 g/l in batch fermentation.

Time (hr)	Acid (ml)	Base (ml)	Cell (g/l)	Glucose (g/l)	Product (unit/ml)
0	0.0	0.0	0.0616	1.76	0.0150
2	0.8	3.0	0.0928	1.59	0.0150
4	0.0	8.8	0.1809	1.35	0.236
6	0.0	2.8	0.3517	1.19	0.1492
8	0.0	1.2	0.6323	1.01	0.4606
10	0.0	1.0	0.7259	0.88	0.6839
14	0.0	3.0	0.7889	0.58	0.7977
18	0.0	4.0	0.7570	0.30	0.7881
22	0.0	3.0	0.7387	0.10	0.7773
26	0.0	4.8	0.7326	0.03	0.7655
30	0.0	2.2	0.7218	0.00	0.7344
34	3.0	0.0	0.6987	0.00	0.7322
38	3.0	0.0	0.6947	0.00	0.7312
42	3.5	0.0	0.7042	0.00	0.7290
46	2.0	0.0	0.7015	0.00	0.7312

Kinetic parameters at the maximum protease production

Yx/s (g-cell/ g-glucose)	0.616
Yp/s (unit/mg-glucose)	0.663
Yp/x (unit/mg-cell)	1.076
Cell Productivity (g-cell/l-hr)	0.056
Prod. Productivity (unit/ml-hr)	0.057

Table A-10 Experimental data of initial glucose concentration of 3.2 g/l in batch fermentation.

Time (hr)	Acid (ml)	Base (ml)	Cell (g/l)	Glucose (g/l)	Product (unit/ml)
0	0.0	0.0	0.0900	3.18	0.0172
2	0.0	0.0	0.1750	2.91	0.0172
4	0.0	2.4	0.4886	2.76	0.0204
6	0.0	10.0	0.6513	2.50	0.1194
8	0.0	3.8	0.7530	2.30	0.2759
10	0.0	2.0	0.8485	2.18	0.4488
14	0.0	2.2	0.8914	1.98	0.6120
18	0.0	3.8	0.9400	1.80	0.6657
22	0.0	1.0	0.9238	1.56	0.6517
26	0.0	1.0	0.9123	1.30	0.6549
30	0.0	1.0	0.9014	1.18	0.6581
34	0.0	0.8	0.9007	1.13	0.6334
38	0.0	1.0	0.8872	0.95	0.6463
42	0.0	1.0	0.8702	0.90	0.6528
46	0.0	1.0	0.8533	0.84	0.6324

Kinetic parameters at the maximum protease production

Yx/s (g-cell/ g-glucose)	0.616
Yp/s (unit/mg-glucose)	0.470
Yp/x (unit/mg-cell)	0.763
Cell Productivity (g-cell/l-hr)	0.052
Prod. Productivity (unit/ml-hr)	0.037

Table A-11 Experimental data of initial glucose concentration of 4.5 g/l in batch fermentation.

Time (hr)	Acid (ml)	Base (ml)	Cell (g/l)	Glucose (g/l)	Product (unit/ml)
0	0.0	0.0	0.0715	4.50	0.0172
2	0.0	1.6	0.1456	4.32	0.0172
4	0.0	8.0	0.4900	4.05	0.0365
6	0.0	3.8	0.7055	3.90	0.1192
8	0.0	2.2	0.7882	3.75	0.3146
10	0.0	2.2	0.8885	3.57	0.4552
14	0.0	3.6	0.9711	3.30	0.5905
18	0.0	4.0	1.0600	3.10	0.6581
22	0.0	4.2	1.0200	2.75	0.6410
26	0.0	3.0	1.0000	2.37	0.6302
30	0.0	5.2	0.9800	2.04	0.6120
34	0.0	4.2	0.9972	1.17	0.6442
38	0.0	6.6	0.9812	1.50	0.6356
42	0.0	4.2	0.9754	1.29	0.6088
46	0.0	5.0	0.9715	1.14	0.6324

Kinetic parameters at the maximum protease production

Yx/s (g-cell/ g-glucose)	0.706
Yp/s (unit/mg-glucose)	0.458
Yp/x (unit/mg-cell)	0.648
Cell Productivity (g-cell/l-hr)	0.059
Prod. Productivity (unit/ml-hr)	0.037

Table A-12 Experimental data of initial glucose concentration of 7.5 g/l in batch fermentation.

Time (hr)	Acid (ml)	Base (ml)	Cell (g/l)	Glucose (g/l)	Product (unit/ml)
0	0.0	0.0	0.0800	7.52	0.0064
2	0.0	1.8	0.1951	7.24	0.0064
4	0.0	9.2	0.5510	7.04	0.0333
6	0.0	4.0	0.7150	6.90	0.1836
8	0.0	4.0	0.7916	6.72	0.3414
10	0.0	1.8	0.8980	6.50	0.4649
14	0.0	3.0	0.9794	6.20	0.5508
18	0.0	4.0	1.0700	6.00	0.6337
22	0.0	4.0	1.0412	5.70	0.6120
26	0.0	3.6	1.0530	5.36	0.6167
30	1.2	5.0	0.9945	5.20	0.6163
34	1.0	1.6	0.9994	5.04	0.6324
38	0.0	6.2	0.9872	4.92	0.6238
42	1.2	3.8	0.9289	4.76	0.6206
46	1.2	4.0	0.9217	4.56	0.6216

Kinetic parameters at the maximum protease production

Yx/s (g-cell/ g-glucose)	0.651
Yp/s (unit/mg-glucose)	0.413
Yp/x (unit/mg-cell)	0.634
Cell Productivity (g-cell/l-hr)	0.059
Prod. Productivity (unit/ml-hr)	0.035

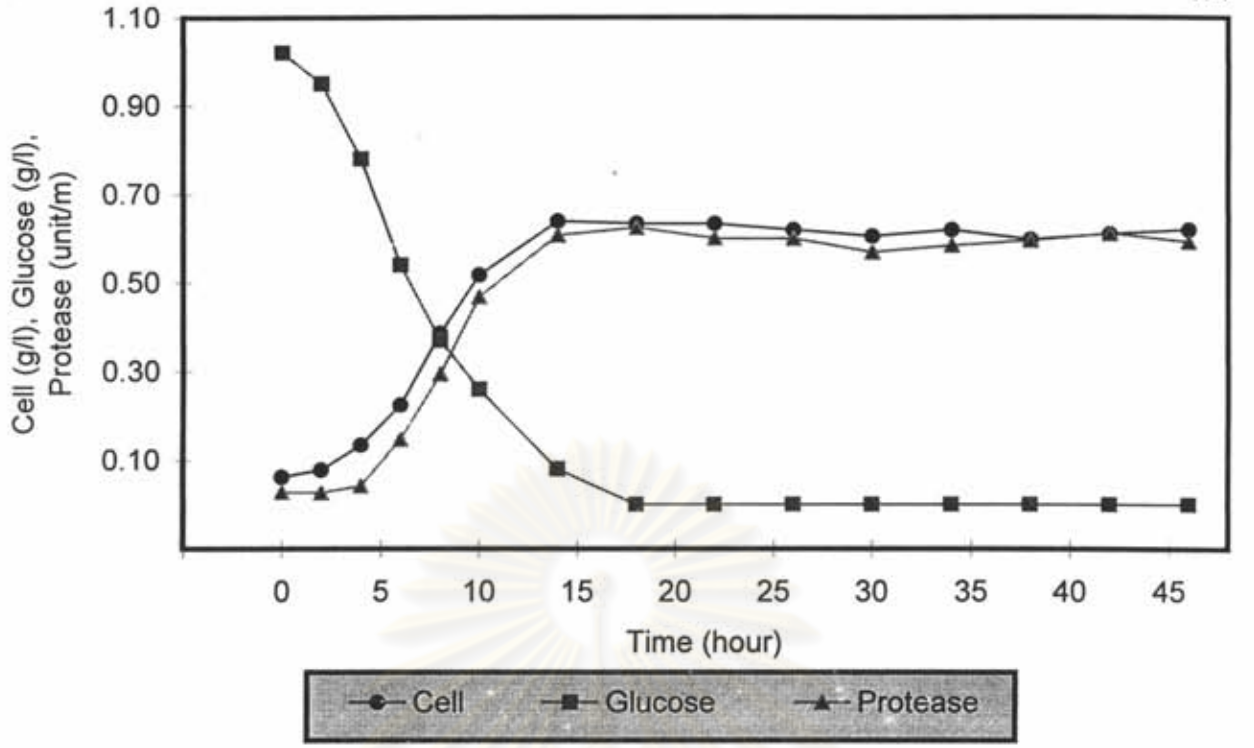

Figure A-4 Batch fermentation of B. subtilis TISTR 25 at 1.0 g/l initial glucose concentration.

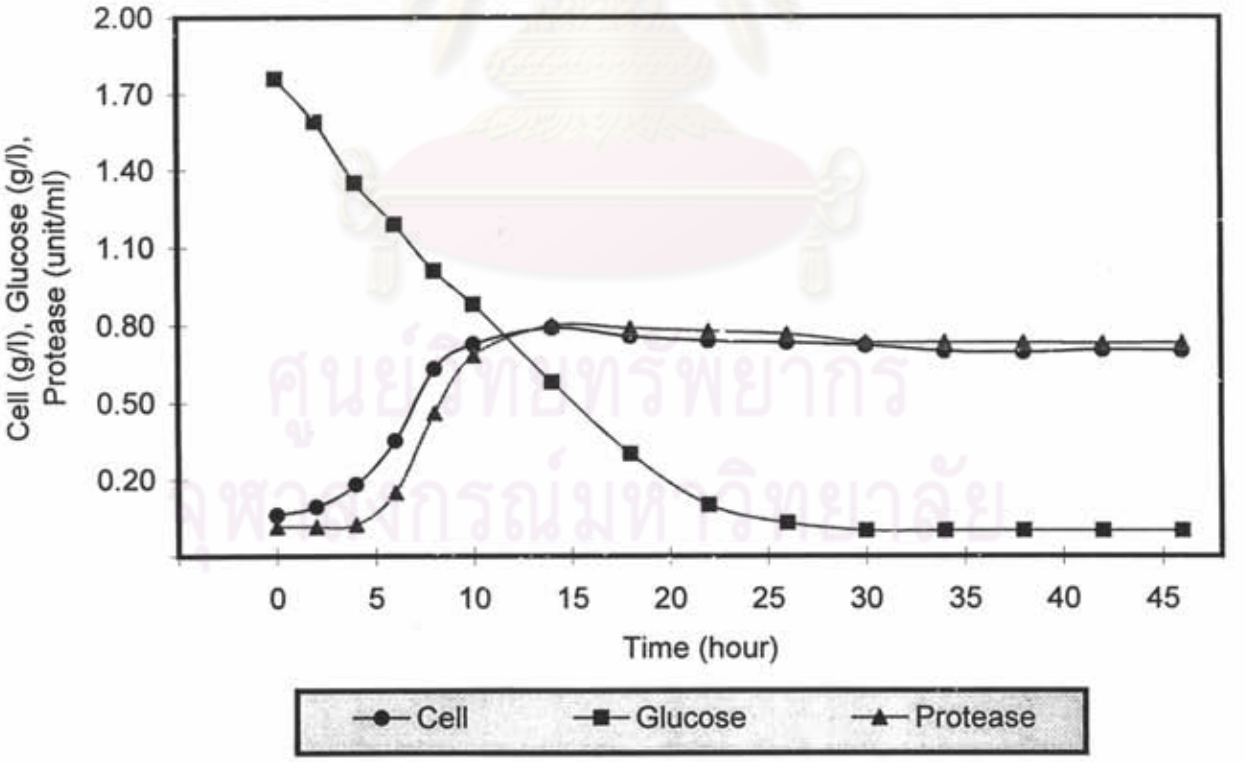

Figure A-5 Batch fermentation of B. subtilis TISTR 25 at 1.8 g/l initial glucose concentration.

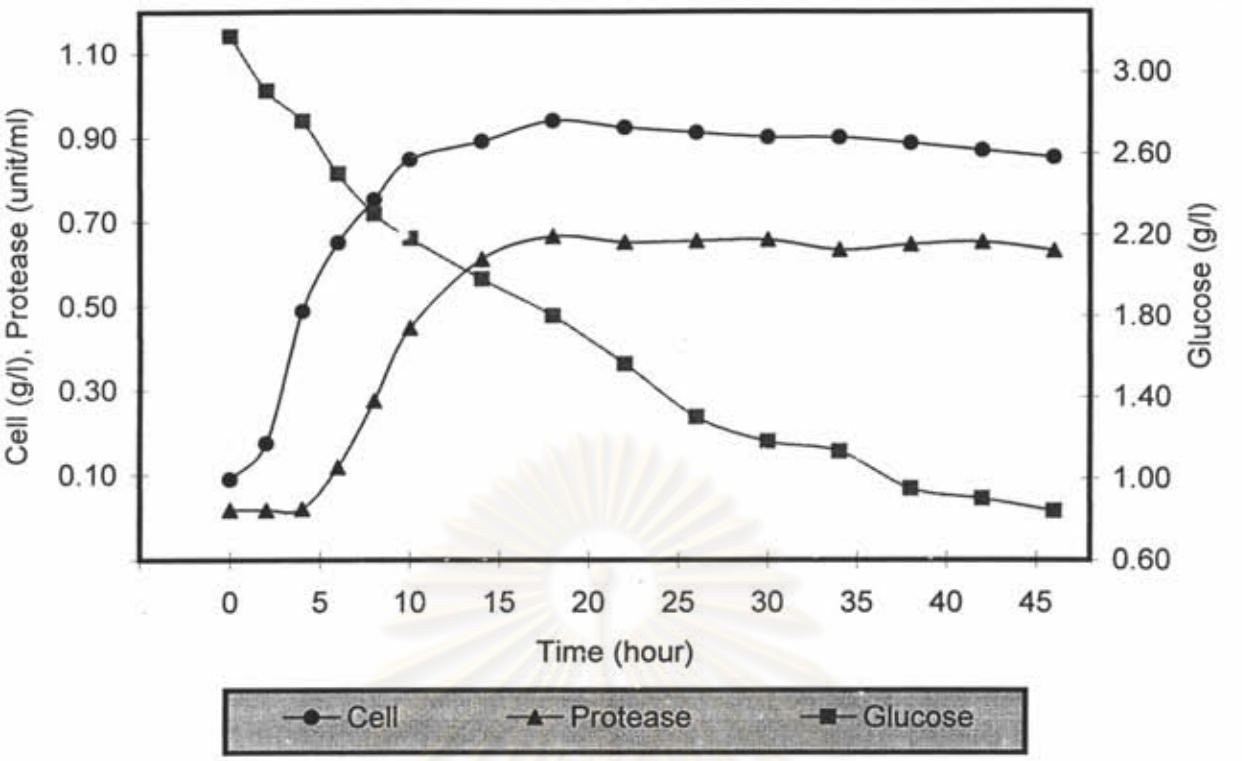

Figure A-6 Batch fermentation of B. subtilis TISTR 25 at 3.2 g/l initial glucose concentration.

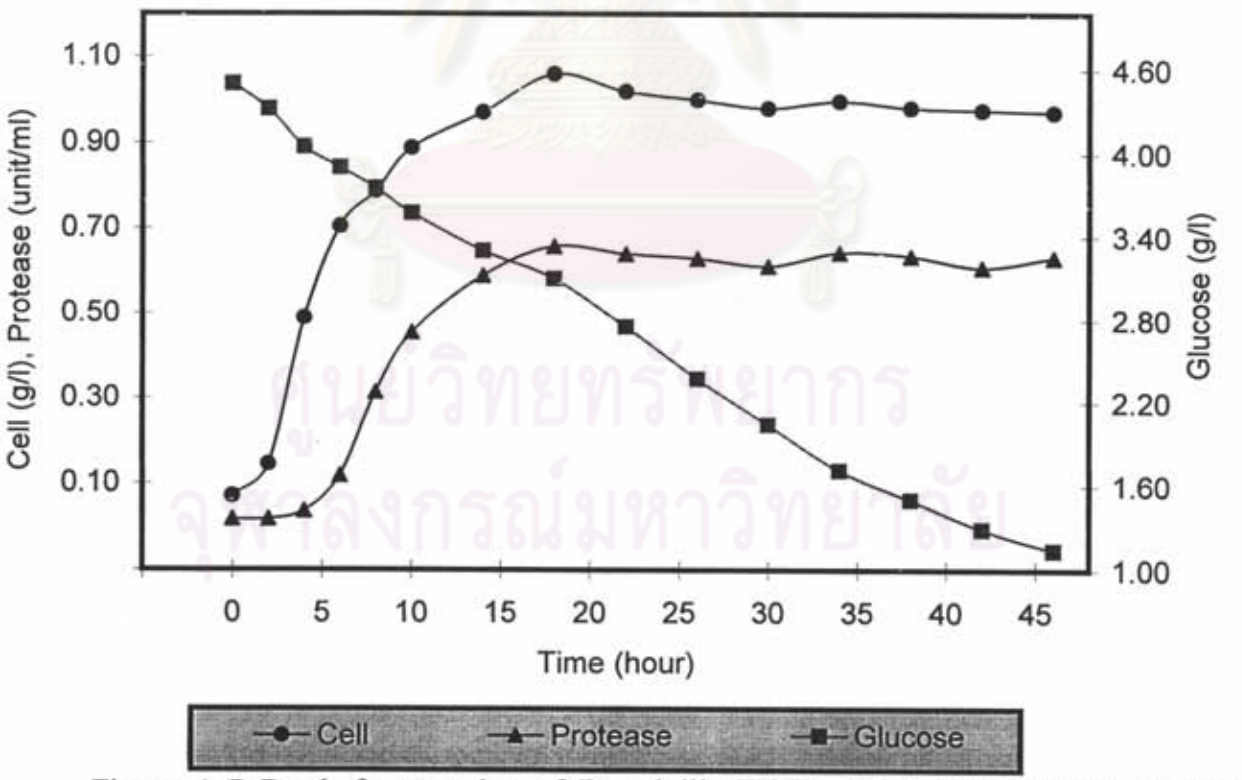

Figure A-7 Batch fermentation of B. subtilis TISTR 25 at 4.5 g/l initial glucose concentration.

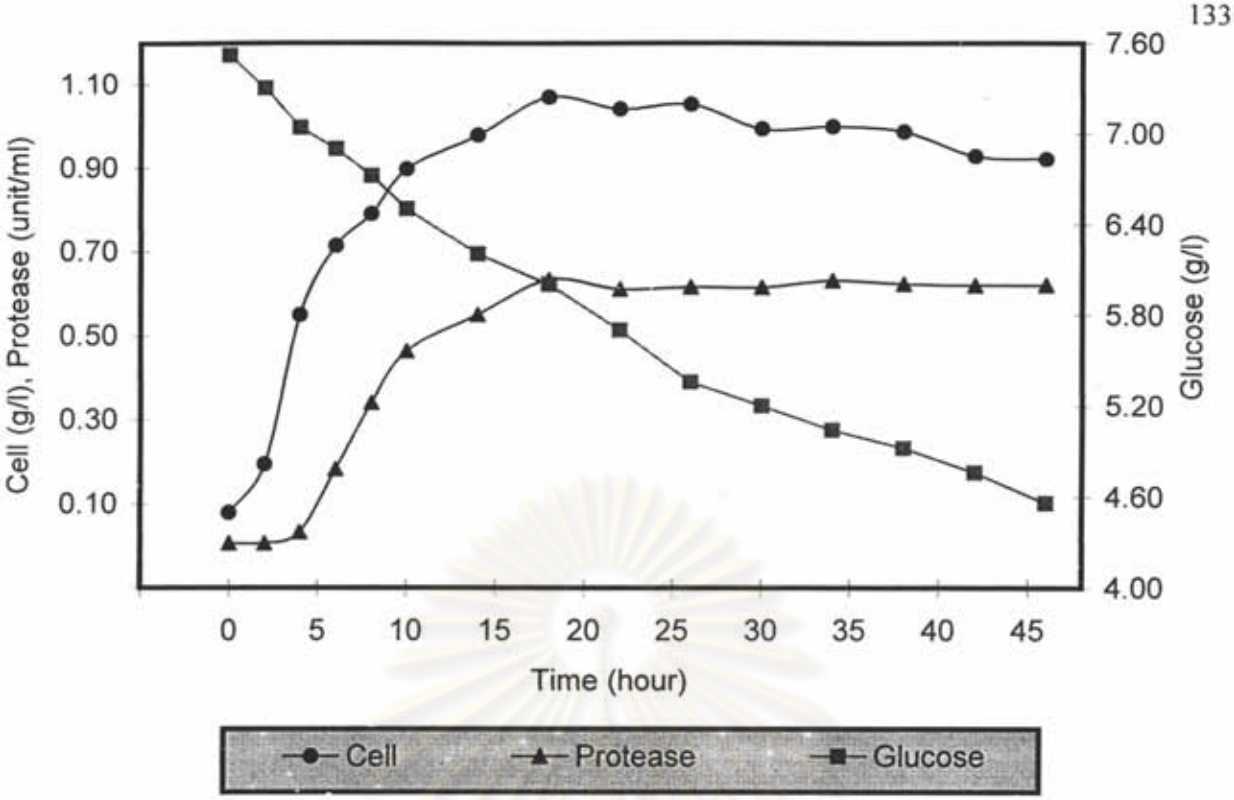

Figure A-8 Batch fermentation of B. subtilis TISTR 25 at 7.5 g/l initial glucose concentration.

Table A-13 Maximum Specific growth rate, Specific consumption rate, and Specific product rate among various initial glucose concentration

Glucose Concentration (g/l)	Specific Growth Rate (hr^{-1})	Specific Consumption Rate (g-glucose/g-cell-hr)	Specific Product Rate (Act/mg-cell-hr)
1.0	0.243	0.802	0.294
1.8	0.322	1.101	0.316
3.2	0.451	0.745	0.112
4.5	0.536	0.757	0.131
7.5	0.498	0.764	0.119

Table A-14 Results of the effects of pressure, recirculation flow rate and cell concentration on permeate flux at operating temperature of 37 °C .

Liquid	Pressure inlet (kg_f/cm^2)	Recirculation flow rate (m^3/hr)	Permeate flow rate (m^3/hr) x 10^{-3}	Permeation flux (m/hr) x 10^{-2}
Distilled Water	0.0+	0.4	12.86	6.3350
	0.0+	0.5	14.64	7.2118
	0.0+	0.6	17.92	8.8276
	0.2+	0.4	31.20	15.3695
	0.4+	0.4	50.98	25.1133
	0.6+	0.4	68.48	33.7340
	0.8+	0.4	86.40	42.5616
Fermentation broth with cell dry weight of 1.81 g/l	0.0+	0.4	2.76	1.3596
	0.0+	0.5	3.60	1.7734
	0.0+	0.6	4.56	2.2463
	0.2+	0.4	3.48	1.7143
	0.4+	0.4	3.96	1.9507
	0.6+	0.4	4.12	2.0296
	0.8+	0.4	4.14	2.0394
Fermentation broth with cell dry weight of 4.24 g/l	0.0+	0.4	2.40	1.1823
	0.0+	0.5	3.08	1.5172
	0.0+	0.6	3.80	1.8719
	0.2+	0.4	3.06	1.5074
	0.4+	0.4	3.36	1.6552
	0.6+	0.4	3.46	1.7044
	0.8+	0.4	3.52	1.7340

Table A-14 (continue) Results of the effects of pressure, recirculation flow rate and cell concentration on permeate flux at operating temperature of 37 °C .

Liquid	Pressure inlet (kg_f/cm^2)	Recirculation flow rate (m^3/hr)	Permeate flow rate (m^3/hr) x 10^{-3}	Permeation flux (m/hr) x 10^{-2}
Fermentation broth	0.0+	0.4	1.92	0.9458
with cell dry weight	0.0+	0.5	2.64	1.3005
of 8.70 g/l	0.0+	0.6	3.24	1.5961
	0.2+	0.4	2.64	1.3005
	0.4+	0.4	3.02	1.4877
	0.6+	0.4	3.06	1.5074
	0.8+	0.4	3.06	1.5074
Fermentation broth	0.0+	0.4	1.56	0.7685
with cell dry weight	0.0+	0.5	1.98	0.9773
of 14.42 g/l	0.0+	0.6	2.33	1.1488
	0.2+	0.4	2.46	1.2118
	0.4+	0.4	2.80	1.3793
	0.6+	0.4	2.94	1.4483
	0.8+	0.4	3.00	1.4778

Table A-15 Experimental data of continuous fermentation coupling with microfiltration.

Cultivation	Time (hour)	Cell (g/l)	Glucose (g/l)	Protease (unit/ml)
Batch 1 L	0	0.268	1.71	0.000
	4	0.400	1.69	0.000
	8	0.498	1.40	0.000
	12	0.589	1.08	0.107
	16	0.739	0.95	0.314
	20	0.880	0.80	0.399
	24	0.977	0.72	0.429
	28	1.100	0.60	0.453
	32	1.200	0.57	0.502
	36	1.086	0.55	0.515
	40	1.118	0.55	0.515
	44	1.056	0.55	0.466
Batch 2.9 L	45	0.431	0.75	0.215
	49	0.567	0.60	0.140
	53	0.676	0.43	0.125
	57	0.653	0.20	0.047
D = 0.11	58	0.688	0.40	0.039
	62	0.836	0.20	0.037
	66	0.929	0.08	0.043
	70	1.162	0.00	0.032
	74	1.235	0.00	0.041
	78	1.256	0.00	0.021
D =0.20	80	1.482	1.00	0.032
	84	1.766	0.50	0.049
	88	2.033	0.30	0.064
	92	2.497	0.04	0.086
	96	2.694	0.04	0.097
	100	3.399	0.05	0.103
	104	3.528	0.04	0.097
	108	4.002	0.04	0.043
	112	4.355	0.04	0.043
	116	4.626	0.05	0.039
	120	4.524	0.06	0.052
	124	4.592	0.04	0.015
	128	4.700	0.04	0.086
	132	4.632	0.04	0.021

Table A-15 (continue) Experimental data of continuous fermentation coupling with microfiltration.

Cultivation	Time (hour)	Cell (g/l)	Glucose (g/l)	Protease (unit/ml)
D =0.30	133	4.585	1.60	0.107
	137	4.866	1.05	0.172
	140	5.849	0.12	0.193
	144	6.188	0.10	0.193
	148	6.527	0.07	0.243
	152	6.933	0.08	0.215
	156	7.204	0.06	0.107
	160	7.408	0.08	0.064
	164	7.882	0.07	0.043
	168	8.289	0.07	0.086
	172	8.330	0.06	0.021
	176	8.696	0.06	0.043
	180	8.899	0.05	0.043
	184	9.129	0.07	0.064
	188	8.933	0.06	0.043
	192	9.068	0.04	0.039
	196	8.628	0.05	0.021
	200	8.763	0.06	0.021

Table A-16 Experimental data of twice cell bleeding continuous fermentation coupling with microfiltration.

Cultivation	Time (hour)	Cell (g/l)	Glucose (g/l)	Protease (unit/ml)
Batch 1 L	0	0.002	1.60	0.000
	4	0.190	1.45	0.000
	8	0.432	1.20	0.079
	12	0.682	1.00	0.116
	16	0.758	0.88	0.279
	20	0.775	0.83	0.322
	24	0.810	0.78	0.387
	28	0.841	0.74	0.408
	32	0.841	0.65	0.429
	36	0.897	0.57	0.472
	40	0.909	0.46	0.494
	44	0.917	0.43	0.466
Batch 2.9L	45	0.426	0.80	0.129
	49	0.513	0.64	0.086
	53	0.524	0.58	0.064
	57	0.533	0.52	0.021
	61	0.597	0.48	0.043
D=0.3	62	0.695	1.47	0.043
	66	0.841	1.46	0.064
	70	0.928	1.41	0.107
	74	1.294	1.28	0.150
	78	2.324	1.30	0.167
	82	2.934	1.28	0.200
	86	3.747	1.32	0.202
	90	4.425	1.20	0.174
	94	4.832	1.23	0.146
	98	5.103	1.15	0.064
	102	5.347	1.04	0.041
	106	5.510	1.09	0.039
	110	5.645	1.02	0.037
	114	6.052	0.98	0.034
	118	6.323	0.90	0.030
	122	6.594	0.91	0.043
	126	6.865	0.85	0.052
	130	7.137	0.82	0.049
	134	7.543	0.80	0.064
	138	8.085	0.82	0.086
	142	8.628	0.76	0.077
	146	9.441	0.73	0.075
	150	9.983	0.71	0.086
	154	9.848	0.70	0.101
	158	9.712	0.71	0.094
	162	9.848	0.70	0.137
	166	9.712	0.72	0.110
	170	9.848	0.69	0.144

Table A-16 (continue) Experimental data of twice cell bleeding continuous fermentation coupling with microfiltration.

Cultivation	Time (hour)	Cell (g/l)	Glucose (g/l)	Protease (unit/ml)
	174	9.848	0.67	0.129
	178	9.916	0.70	0.150
	182	9.848	0.69	0.144
	186	9.712	0.70	0.131
	190	9.712	0.70	0.127
Bleed cell out = 1.0 L	191	9.983	0.98	0.021
	194	6.865	0.85	0.052
	198	8.899	0.67	0.054
	202	10.255	0.54	0.037
	206	11.271	0.49	0.064
	210	11.610	0.45	0.090
	214	11.610	0.40	0.140
	218	12.288	0.33	0.159
	222	12.288	0.30	0.172
	226	12.288	0.29	0.193
	230	12.288	0.28	0.172
	234	12.152	0.31	0.219
	238	12.220	0.30	0.208
	242	12.288	0.28	0.176
	244	12.424	0.29	0.204
	248	12.288	0.30	0.210
Bleed cell out = 2.0 L	249	12.220	0.70	0.052
	253	6.188	0.65	0.062
	257	7.543	0.60	0.052
	261	8.899	0.57	0.064
	265	9.577	0.50	0.101
	269	10.255	0.45	0.146
	273	10.932	0.40	0.170
	277	11.881	0.34	0.183
	280	12.288	0.27	0.215
	284	12.220	0.25	0.258
	288	12.424	0.20	0.290
	292	12.288	0.18	0.268
	296	12.220	0.19	0.273
	300	12.424	0.20	0.225

APPENDIX B

Starch hydrolyzation processes

30 % w/w starch concentration was dissolved in water and then are put into the following steps :

STEP I : Liquefaction

- alpha amylase
- 90°C / pH 6.0 / 180 minutes

STEP II : Saccharification

- glucoamylase
- 60°C / pH 4.2 / 2 days

The glucose concentration was obtain around 314 g/l per 300 g/l starch which was equal to 95 % conversion.

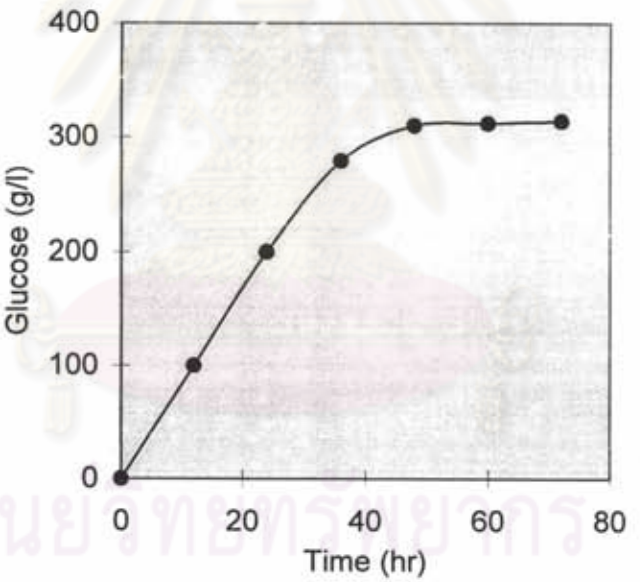

Figure A-9 Two-step enzymatic hydrolysation of 30% w/w starch

Dissolved oxygen in fermentation broth.

The dissolved oxygen of the fermentation broth was off-line measured by D.O. meter. The air flow rate to the fermenter was set at 1 vvm. and the various agitation speed was observed. The result was shown if Figure A-10. The 350 rpm agitation speed was selected to achieved the maximum dissolved oxygen condition.

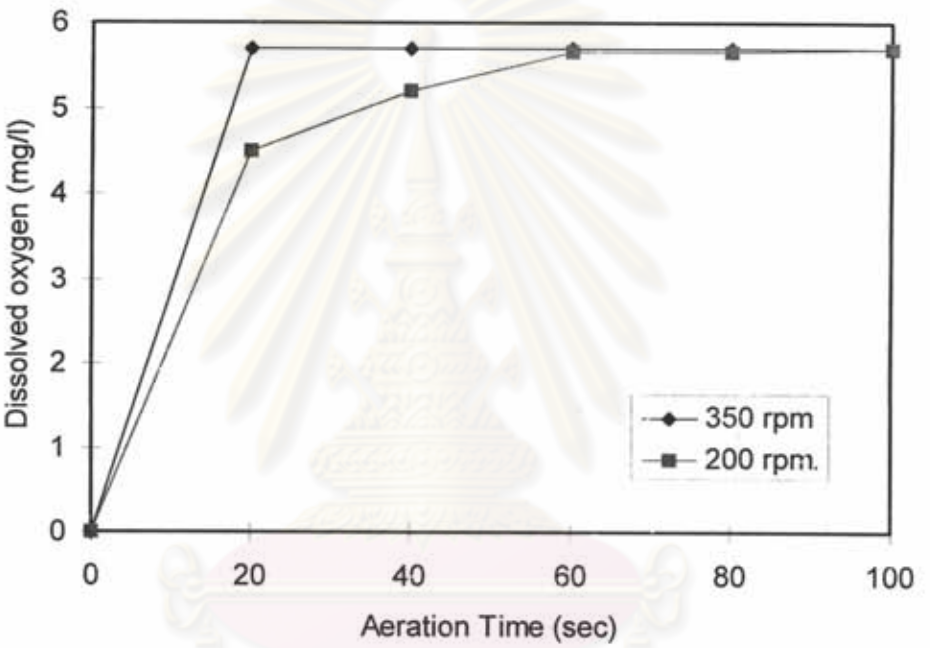

Figure A-10 The effect of agitation speed on dissolved oxygen

BIOGRAPHY

Nuntapon Sukmongkol was born on 2nd December,1971 in Bangkok, Thailand. He finished his secondary school from Santirajvitayalai in the year 1989. After that, he took the entrance examination to study in the faculty of Agro-Industry, Kasetsart University. He proudly chose Biotechnology to be his majorfield of study. When he graduated from Kasetsart University, he began to continue his further study for Master's degree in Chemical Engineering at Chulalongkorn University. He actively participated in the Biochemical Engineering research group. He always dedicated himself to his thesis with all of his effort. After 3 years of his impressively successful work, he eventually achieve his Master's degree in Chemical Engineering (Biochemical Engineering) in April,1997.