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**APPENDIX A**

### A.1 Calibration curve of cell dry weight

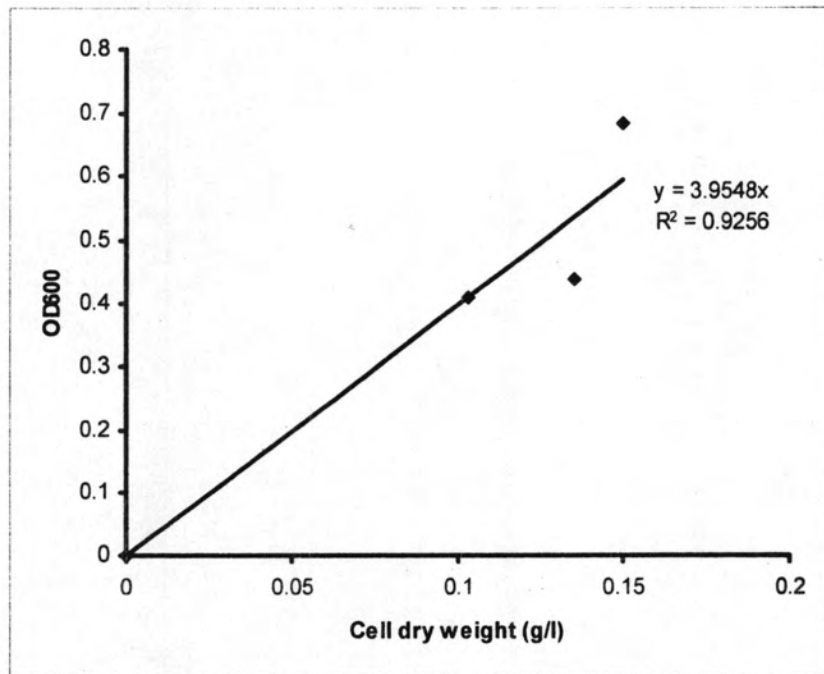


Figure A-1 Calibration curve of cell dry weight

### A.2 Calibration curve of glucose concentration

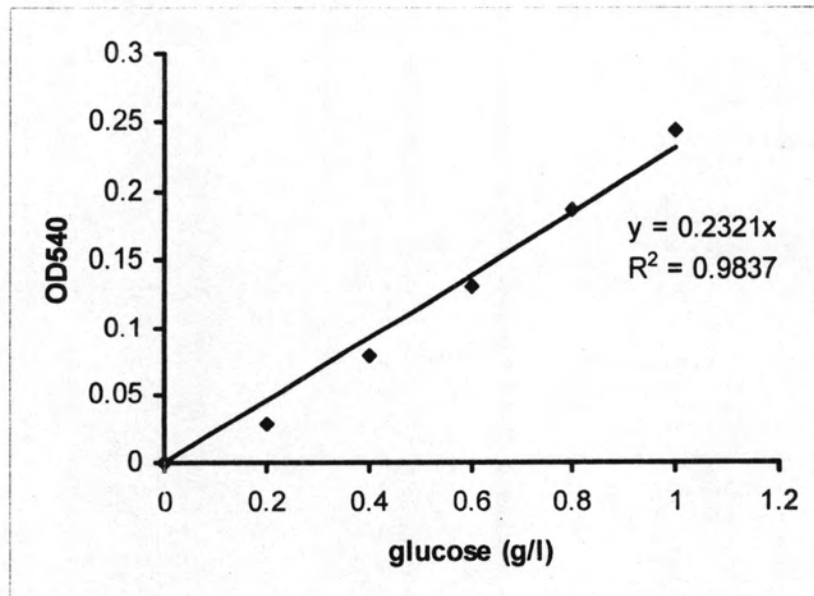
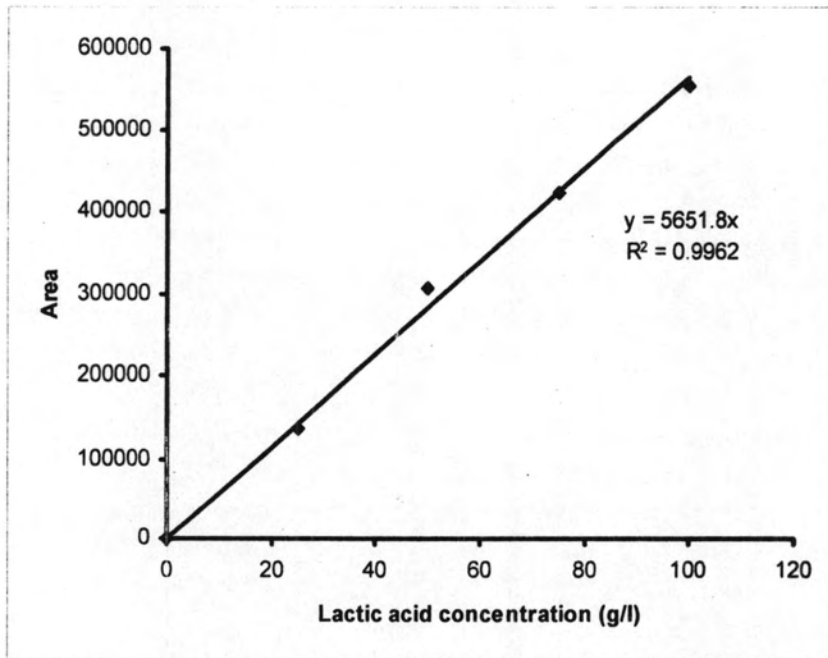


Figure A-2 Calibration curve of glucose concentration

### A.3 Calibration curve of lactic acid concentration



**Figure A-3** Calibration curve of lactic acid concentration



**APPENDIX B**

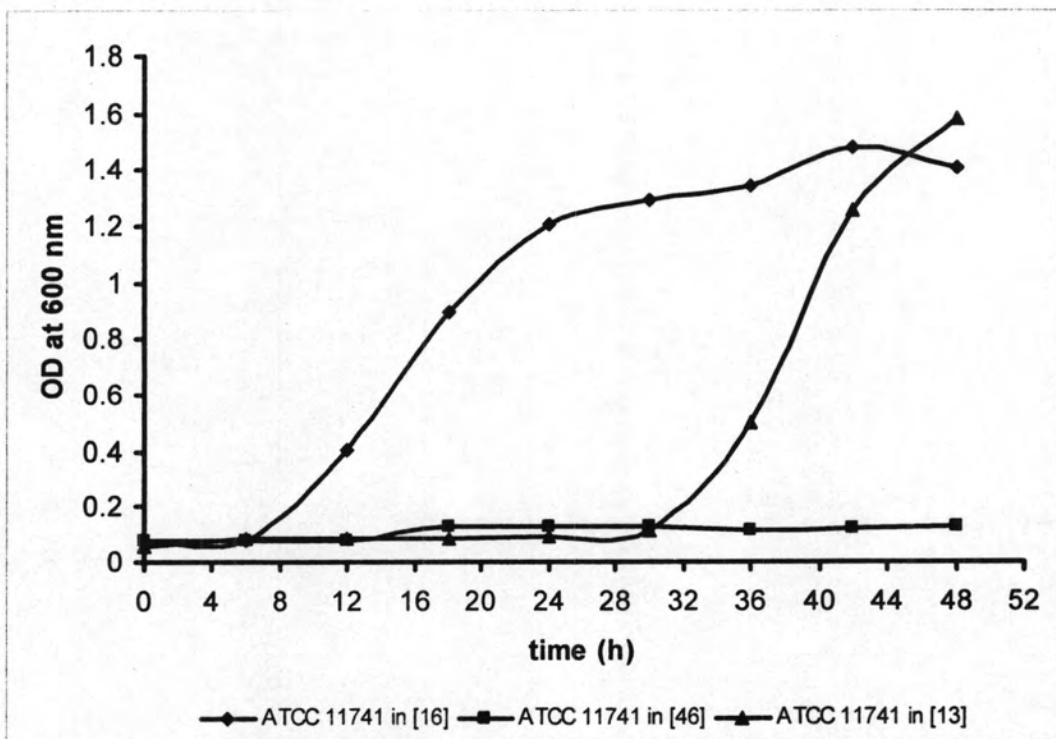
**B.1 Brewer's yeast autolysate utilization for lactic acid production in batch fermentor (varying the component of fermentor)**

**Table B-1** Component of fermentation medium for lactic acid production using *L.salivarius subsp. salivarius* ATCC 11741

<b>Component</b>	<b>[13]</b>	<b>[16]</b>	<b>[46]</b>
	<b>(g/l)</b>	<b>(g/l)</b>	<b>(g/l)</b>
Casein	10	–	–
Meat extract	10	10	–
Yeast Extract	5	5	15
peptone	–	10	–
Glucose	20	20	100
Tween-80	1	1	–
K <sub>2</sub> HPO <sub>4</sub>	2	2	0.5
Na-acetate	3	–	–
(NH <sub>4</sub> ) <sub>2</sub> citrate	2	–	–
NaCl	–	–	0.5
MgSO <sub>4</sub> •7H <sub>2</sub> O	0.2	0.2	2
MnSO <sub>4</sub> •H <sub>2</sub> O	0.05	0.2	–

**Table B-2** Experiment data of optical density at 600 nm in batch fermentation

Time (h)	OD <sub>600</sub>		
	[13]	[16]	[46]
0	0.06	0.08	0.08
6	0.09	0.08	0.08
12	0.09	0.40	0.08
18	0.09	0.89	0.13
24	0.09	0.12	0.13
30	0.12	0.13	0.13
36	0.50	0.13	0.11
42	0.13	0.15	0.12
48	0.16	0.14	0.13

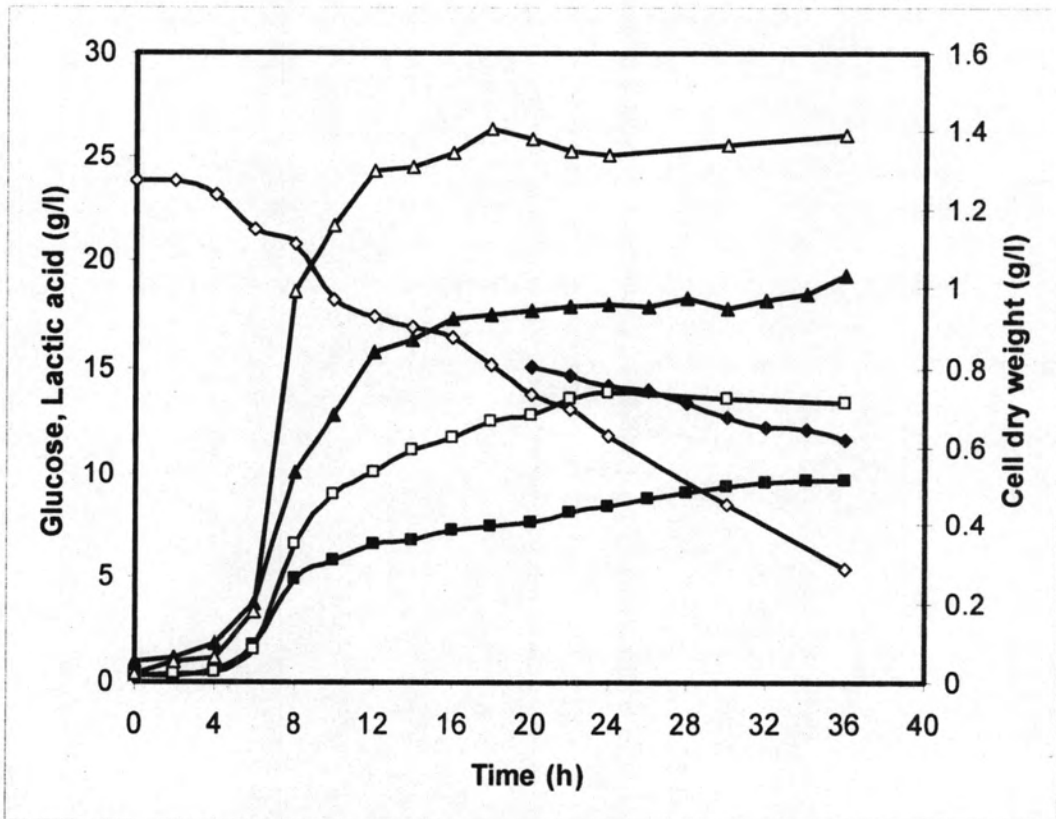
**Figure B-1** The effect of fermentation medium component on cell growth of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741.

**APPENDIX C**

**C.1 Brewer's yeast autolysate utilization for lactic acid production in batch fermentor (varying the size of fermentor)**

**Table C-1** Experiment data of cell dry weight, glucose and lactic acid concentration in batch fermentation

Time (h)	conducted in 250 Erlenmeyer flask			conducted in 1l fermentor		
	cell dry weight (g/l)	glucose (g/l)	lactic acid (g/l)	cell dry weight (g/l)	glucose (g/l)	lactic acid (g/l)
0	0.05	-	0.41	0.02	23.89	0.33
2	0.06	-	0.34	0.05	23.81	0.36
4	0.10	-	0.61	0.06	23.18	0.47
6	0.20	-	1.77	0.18	21.46	1.54
8	0.54	-	4.90	0.99	20.79	6.56
10	0.68	-	5.77	1.16	18.13	8.99
12	0.84	-	6.51	1.30	17.35	10.11
14	0.87	-	6.75	1.31	16.88	11.15
16	0.92	-	7.27	1.34	16.41	11.74
18	0.93	-	7.43	1.41	15.16	12.54
20	0.94	15.04	7.65	1.38	13.75	12.77
22	0.96	14.69	8.07	1.35	13.08	13.60
24	0.96	14.15	8.44	1.34	11.87	13.85
26	0.95	13.96	8.77	-	-	-
28	0.98	13.37	9.08	-	-	-
30	0.95	12.72	9.34	1.36	8.54	13.54
32	0.97	12.17	9.53	-	-	-
34	0.98	12.13	9.65	-	-	-
36	1.03	11.65	9.65	1.39	5.33	13.43
48	-	-	-	1.51	1.84	12.64
65	-	-	-	1.50	1.63	12.40



**Figure C-1** Batch fermentation of *L.salivarius subsp. salivarius* ATCC 11741. (▲) cell dry weight, (◆) glucose, (■) lactic acid conducted in 250 Erlenmeyer flask; (△) cell dry weight, (◇) glucose, (□) lactic acid conducted in 1l fermentor

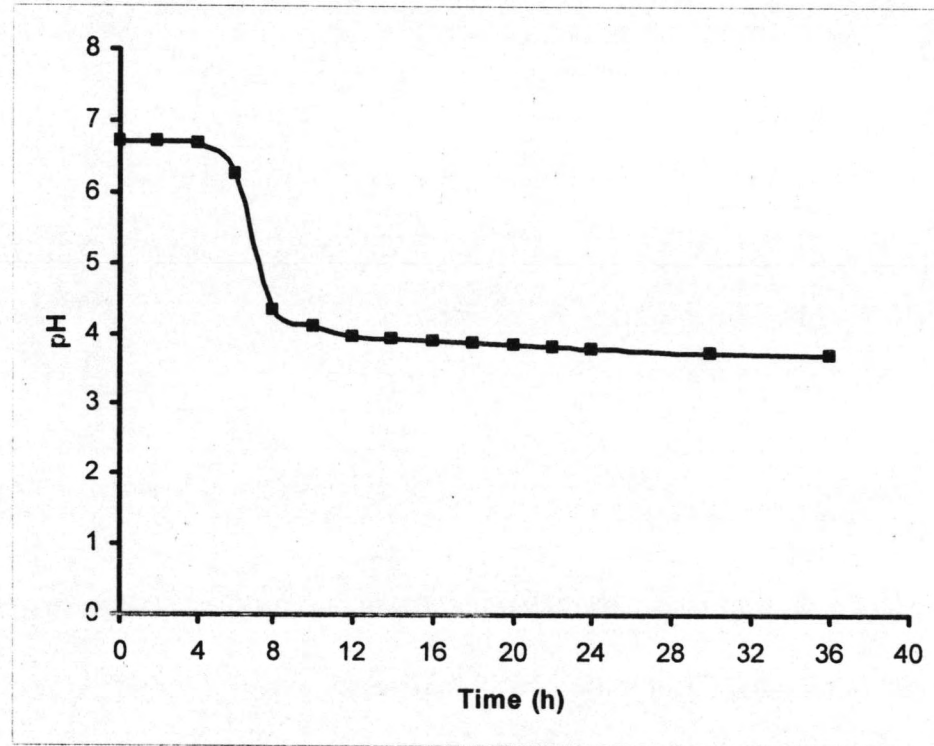
**APPENDIX D**

**D.1 Brewer's yeast autolysate utilization for lactic acid production in batch fermentor (varying pH)**

**Table D-1** The effect of pH on lactic acid production in batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

Time (h)	Cell dry weight (g/l)				Glucose (g/l)				lactic acid (g/l)			
	pH 5.0	pH 5.5	pH 6.0	Uncontrolled pH	pH 5.0	pH 5.5	pH 6.0	Uncontrolled pH	pH 5.0	pH 5.5	pH 6.0	uncontrolled pH
0	0.12	0.12	0.13	0.02	22.26	22.70	22.42	23.89	0.37	0.38	0.39	0.33
2	0.13	0.16	0.17	0.05	19.43	18.39	21.30	23.81	0.63	0.65	0.68	0.36
4	0.20	0.41	0.51	0.06	18.03	18.19	19.11	23.18	1.17	2.04	2.85	0.47
6	0.48	1.25	1.49	0.18	17.67	14.12	7.94	21.46	3.70	10.21	9.36	1.54
8	1.15	1.72	1.70	0.99	14.16	0.68	0.56	20.79	7.94	4.16	4.33	6.56
10	1.84	1.32	1.32	1.16	11.21	0.64	0.32	18.13	8.52	3.46	4.25	8.99
12	2.31	1.06	1.13	1.30	4.87	0.56	0.64	17.35	7.80	4.02	4.36	10.11
14	1.85	0.93	0.97	1.31	0.48	0.52	0.64	16.88	-	-	-	11.15
16	0.84	0.91	0.82	1.34	0.04	0.68	0.64	16.41	-	-	-	11.74
18	0.86	0.91	0.75	1.41	0.08	0.60	0.56	15.16	-	-	-	12.54
20	0.87	0.83	0.65	1.38	0.04	0.60	0.56	13.75	-	-	-	12.77
22	0.85	0.77	0.55	1.35	0.02	0.64	0.60	13.08	-	-	-	13.60
24	0.86	0.72	0.49	1.34	0.12	0.48	0.56	11.87	-	-	-	13.85
30	0.96	0.78	0.53	1.36	0.04	0.64	0.64	8.54	-	-	-	13.54





**Figure D-1** pH in batch fermentation of lactic acid using *L.salivarius subsp. salivarius* ATCC 11741 (YE = 5 g/l, glucose 20 g/l)

## D.2 Brewer's yeast autolysate utilization for lactic acid production in batch fermentor (varying initial glucose concentration)

**Table D-2** The effect of the initial glucose concentration (Commercial grade, CG) on lactic acid production in batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

Initial glucose (g/l)		Fermentation time (h)												
		0	2	4	6	8	10	12	14	16	24	30	36	48
20	Cell dry weight (g/l)	0.12	0.16	0.41	1.25	1.72	1.32	1.06	0.93	0.91	0.72	-	-	-
	Glucose (g/l)	22.28	18.05	17.86	13.86	0.67	0.63	0.55	0.51	0.67	0.47	-	-	-
	Lactic acid (g/l)	0.38	0.65	2.04	10.21	4.16	3.46	4.02				-	-	-
30	Cell dry weight (g/l)	0.08	0.10	0.29	0.93	1.82	2.22	1.92	1.66	1.38	0.99	-	-	-
	Glucose (g/l)	31.19	30.21	25.59	22.51	10.81	1.06	0.62	0.53	0.53	0.44	-	-	-
	Lactic acid (g/l)	0.54	0.73	6.70	10.43	26.41	37.77	37.19	37.78	37.43	39.29	-	-	-
40	Cell dry weight (g/l)	0.07	0.09	0.22	0.87	1.73	2.07	2.19	2.13	1.90	1.12	-	-	-
	Glucose (g/l)	39.07	36.95	33.49	33.23	22.06	11.25	5.05	0.97	0.27	0.27	-	-	-
	Lactic acid (g/l)	0.09	0.31	1.17	9.60	21.48	34.85	42.29	44.78	44.39	44.97	-	-	-
50	Cell dry weight (g/l)	0.09	0.11	0.25	0.87	1.80	2.20	2.36	2.35	2.29	1.45	-	-	-
	Glucose (g/l)	53.16	51.57	49.44	47.05	36.33	24.01	14.53	8.77	4.52	1.33	-	-	-
	Lactic acid (g/l)	0.16	0.29	1.47	10.15	24.96	35.75	40.30	44.78	48.77	51.61	-	-	-
70	Cell dry weight (g/l)	0.04	0.07	0.30	1.35	2.02	2.52	2.70	2.73	2.74	2.93	3.02	2.82	2.14
	Glucose (g/l)	67.89	66.77	64.53	60.43	52.97	32.08	29.84	15.29	11.94	5.22	0.75	0	0
	Lactic acid (g/l)	2.26	3.20	6.33	12.64	28.08	36.54	39.08	42.87	45.12	60.44	60.79	71.41	70.53
80	Cell dry weight (g/l)	0.02	0.05	0.25	1.08	1.56	2.35	2.40	2.44	2.56	2.57	2.61	2.59	2.31
	Glucose (g/l)	83.55	81.69	78.70	74.45	66.53	58.56	55.20	39.16	37.30	22.38	16.41	13.80	3.36
	Lactic acid (g/l)	0.56	2.23	6.93	14.21	28.47	36.27	40.30	42.68	45.21	48.73	56.28	55.34	60.61
100	Cell dry weight (g/l)	0.03	0.06	0.24	0.90	1.60	2.00	2.14	2.26	2.24	2.17	2.12	1.92	1.80
	Glucose (g/l)	103.32	101.08	94.74	94.74	78.33	81.31	61.17	49.61	45.88	28.35	26.86	25.74	17.53
	Lactic acid (g/l)	0.66	2.73	4.24	12.78	24.27	36.38	38.36	38.84	43.17	47.31	52.06	51.63	66.41

**D.3 Brewer's yeast autolysate utilization for lactic acid production in batch fermentor (varying initial glucose concentration (from cassava starch hydrolysate))**

**Table D-3** The effect of glucose concentration (from Cassava starch hydrolysate, CSH) on lactic acid production in batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741

Initial glucose (g/l)	Fermentation time (h)													
	0	2	4	6	8	10	12	14	16	24	30	36	48	
70	Cell dry weight (g/l)	0.02	0.03	0.09	0.30	1.19	2.27	2.89	3.00	3.32	3.76	3.72	3.30	2.32
	Glucose (g/l)	65.78	65.08	62.27	59.99	52.97	41.40	32.10	22.28	17.01	3.68	0	0	0
	Lactic acid (g/l)	1.06	1.92	2.15	3.48	9.73	23.18	31.83	40.99	50.03	59.57	60.80	64.27	65.88
80	Cell dry weight (g/l)	0.01	0.02	0.06	0.29	1.28	2.43	2.53	2.71	2.90	3.14	3.31	3.27	2.54
	Glucose (g/l)	79.81	77.18	76.83	69.99	65.78	47.36	36.48	27.71	21.05	8.07	1.58	0	0
	Lactic acid (g/l)	1.02	1.69	2.00	3.28	11.89	24.71	38.39	45.35	52.85	57.90	58.73	66.47	67.56
100	Cell dry weight (g/l)	0.01	0.02	0.04	0.15	0.66	1.45	2.07	2.53	2.81	3.36	3.71	3.80	3.84
	Glucose (g/l)	99.28	98.93	98.75	95.60	93.84	83.32	70.34	63.15	58.59	37.01	26.84	23.33	5.96
	Lactic acid (g/l)	1.22	1.81	2.04	2.67	6.35	13.92	27.06	34.21	42.59	54.60	62.95	63.72	70.72

**D.4 Brewer's yeast autolysate utilization for lactic acid production in batch fermentor (varying brewer's yeast autolysate concentration)**

**Table D-4** The effect of brewer's yeast autolysate (BYA) on lactic acid production in batch fermentation using *L.salivarius subsp. salivarius*

ATCC 11741

BYA conc. (ml/l)		Fermentation time (h)												
		0	2	4	6	8	10	12	14	16	24	30	36	48
48	Cell dry weight (g/l)	0.05	0.07	0.23	1.41	2.31	2.57	2.60	2.61	2.45	2.31	1.22	1.14	1.07
	Glucose (g/l)	70.23	68.07	67.64	63.77	52.13	25.85	14.22	6.89	3.45	0	0	0	0
	Lactic acid (g/l)	2.40	2.92	3.35	12.45	27.94	39.45	45.72	48.12	48.99	50.69	52.65	52.71	52.53
95	Cell dry weight (g/l)	0.03	0.04	0.11	0.21	1.03	2.18	2.38	2.65	2.69	2.34	1.23	1.10	1.08
	Glucose (g/l)	70.66	69.80	65.49	64.63	59.46	36.62	23.27	9.91	4.31	0	0	0	0
	Lactic acid (g/l)	1.84	2.63	2.83	3.41	8.99	25.26	42.10	47.20	48.81	53.87	52.11	51.83	53.81
190	Cell dry weight (g/l)	0.01	0.01	0.06	0.12	0.34	0.23	0.24	0.29	0.30	0.32	2.75	2.82	2.93
	Glucose (g/l)	59.46	57.30	60.32	61.18	62.47	64.63	61.18	58.60	56.01	53.86	6.46	0	0
	Lactic acid (g/l)	4.21	1.84	2.37	4.13	5.10	4.17	4.27	4.36	4.48	5.04	33.25	46.41	48.33

**D.5 Brewer's yeast autolysate utilization for lactic acid production in batch fermentor (effect of the bitterness of brewer's yeast autolysate)**

**Table D-5** The effect of the bitterness of brewer's yeast autolysate (BYA) on lactic acid production in batch fermentation using *L.salivarius* subsp. *salivarius* ATCC 11741

Time (h)	Cell dry weight (g/l)		Glucose (g/l)		lactic acid (g/l)	
	BYA 48 ml/l	BYA 48 ml/l (Debitterness)	BYA 48 ml/l	BYA 48 ml/l (Debitterness)	BYA 48 ml/l	BYA 48 ml/l (Debitterness)
0	0.01	0.01	69.40	70.72	0.15	0.16
2	0.02	0.05	63.59	64.51	0.60	0.60
4	0.64	0.72	58.29	61.96	6.62	6.47
6	1.63	1.73	49.12	51.36	18.68	17.45
8	1.91	2.17	43.11	46.47	31.95	29.80
10	1.80	2.39	32.00	34.55	39.45	37.75
12	1.84	2.34	30.98	29.55	43.97	42.13
14	1.69	2.31	27.41	29.15	45.77	45.01
16	1.66	2.04	24.66	27.11	48.09	46.31
24	1.43	1.83	18.55	22.22	53.21	51.64
30	1.27	1.89	14.88	18.24	55.12	53.53
36	1.36	1.80	11.41	15.59	57.44	58.62
48	1.47	1.97	7.54	9.07	62.35	61.25

**D.6 The comparison of the commercial grade glucose (CG) and glucose from cassava starch hydrolysate (CSH) on lactic acid production of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741.**

**Table D-6** The effect of the commercial grade glucose (CG) and glucose from cassava starch hydrolysate (CSH) in batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741 (using YE = 5 g/l)

	Commercial grade glucose (CG)	glucose from CSH
lag time (h)	2	4
$X_{\max}$ (g/l)	3.02	3.76
$P_{\max}$ (g/l)	60.79	59.57
$\mu_{\max}$ (h <sup>-1</sup> )	0.39	0.38
$q_s$ (h <sup>-1</sup> )	5.96	5.89
$q_p$ (h <sup>-1</sup> )	8.31	4.20
$Y_{x/s}$	0.17	0.09
$Y_{p/s}$	1.73	1.00

**D.7 The comparison of yeast extract (YE) and brewer's yeast autolysate (BYA) on lactic acid production of batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741.**

**Table D-7** The effect of yeast extract (YE) and brewer's yeast autolysate (BYA) in batch fermentation of *L.salivarius subsp. salivarius* ATCC 11741 using glucose from CSH (70 g/l)

	Yeast Extract (YE)	Brewer's yeast autolysate (BYA)
lag time (h)	4	4
$X_{\max}$ (g/l)	3.76	2.61
$P_{\max}$ (g/l)	59.57	48.99
$\mu_{\max}$ (h <sup>-1</sup> )	0.38	0.42
$q_s$ (h <sup>-1</sup> )	5.89	5.38
$q_p$ (h <sup>-1</sup> )	4.20	5.56
$Y_{x/s}$	0.09	0.21
$Y_{p/s}$	1.00	1.55

**D.8 The effect of the bitterness of brewer's yeast autolysate (BYA) on lactic acid production in batch fermentation using *L.salivarius* subsp. *salivarius* ATCC 11741**

**Table D-8** The effect of the bitterness of brewer's yeast autolysate (BYA) in batch fermentation of *L.salivarius* subsp. *salivarius* ATCC 11741 (using CSH 70 g/l and BYA 48 ml/l)

	Brewer's yeast autolysate (BYA)	BYA (debittering)
lag time (h)	2	2
$X_{\max}$ (g/l)	1.91	2.39
$P_{\max}$ (g/l)	62.35	61.25
$\mu_{\max}$ (h <sup>-1</sup> )	0.48	0.46
$q_s$ (h <sup>-1</sup> )	1.99	1.08
$q_p$ (h <sup>-1</sup> )	2.26	1.91
$Y_{x/s}$	0.08	0.10
$Y_{p/s}$	1.21	1.22



**APPENDIX E**

**E.1 Brewer's yeast autolysate utilization for lactic acid production in batch fermentor (About the Brewer's yeast autolysate (BYA) and the bitterness)**

**Table E-1** The amount of total nitrogen (%) of yeast extract and brewer's yeast autolysate

	<b>amount of sample</b>	<b>% Total Nitrogen</b>
Yeast extract (YE)	0.1 g	10.801
Brewer's yeast autolysate (BYA) (1 ml = 0.148 g)	1 ml	11.350

**Table E-2** The effect of the debittering of Brewer's yeast autolysate on the amount of total nitrogen

	<b>Brewer's yeast autolysate</b>	<b>Brewer's yeast autolysate (debitterness)</b>	<b>% debitterness</b>
BYA dry weight (g/ml)	0.148	0.1478	71.46
Bitterness (mg iso alpha-acids/ g BYA dry weight)	0.4966	0.1417	-
Total Nitrogen (%)	12.23	11.66	-

## VITA

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