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# APPENDIX A

## A.1 Calibration curve of cell dry weight

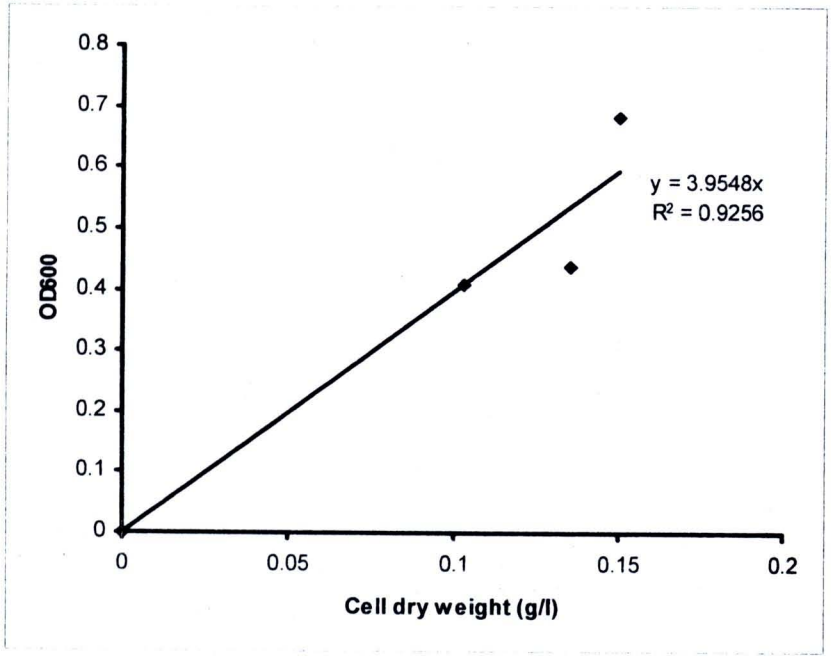


**Figure A-1** Calibration curve of cell dry weight

## A.2 Calibration curve of glucose concentration

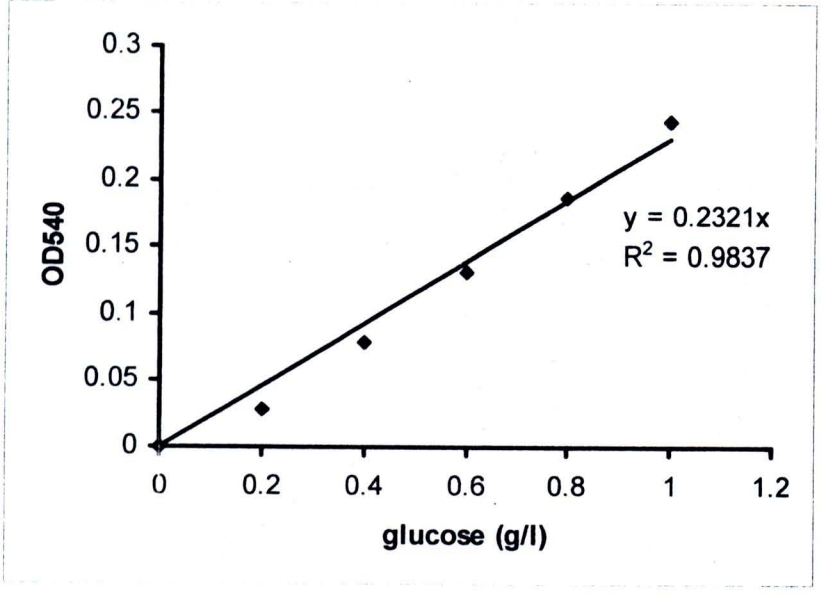


**Figure A-2** Calibration curve of glucose concentration

## A.3 Calibration curve of lactic acid concentration

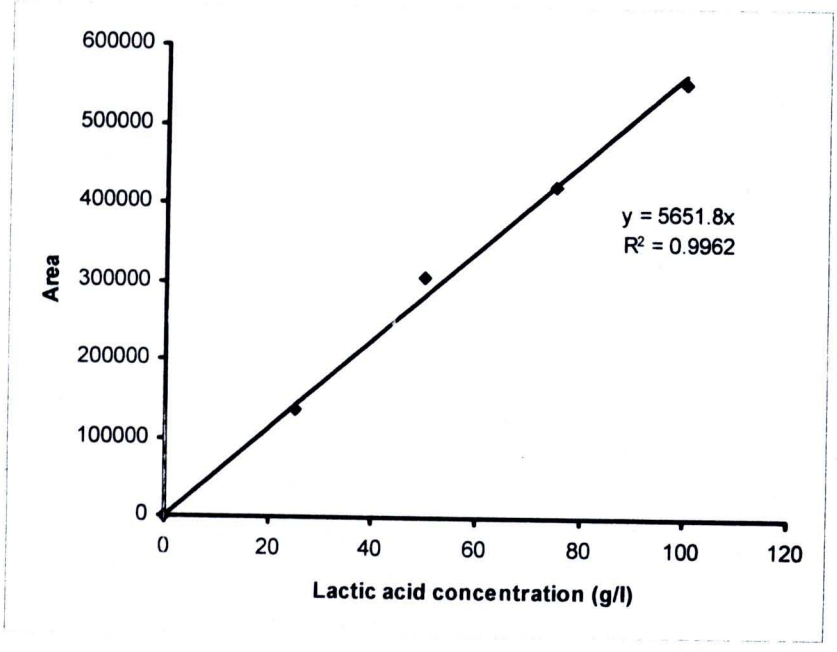


**Figure A-3** Calibration curve of lactic acid concentration

# 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

| Component | [13] (g/l) | [16] (g/l) | [46] (g/l) |
|---|---|---|---|
| Casein | 10 | – | – |
| Meat extract | 10 | 10 | – |
| Yeast Extract | 5 | 5 | 15 |
| peptone | – | 10 | – |
| Glucose | 20 | 20 | 100 |
| Tween-80 | 1 | 1 | – |
| $K_2HPO_4$ | 2 | 2 | 0.5 |
| Na-acetate | 3 | – | – |
| $(NH_4)_2$ citrate | 2 | – | – |
| NaCl | – | – | 0.5 |
| $MgSO_4 \cdot 7H_2O$ | 0.2 | 0.2 | 2 |
| $MnSO_4 \cdot H_2O$ | 0.05 | 0.2 | – |

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

| Time (h) | $OD_{600}$ | | |
|---|---|---|---|
| | [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 |

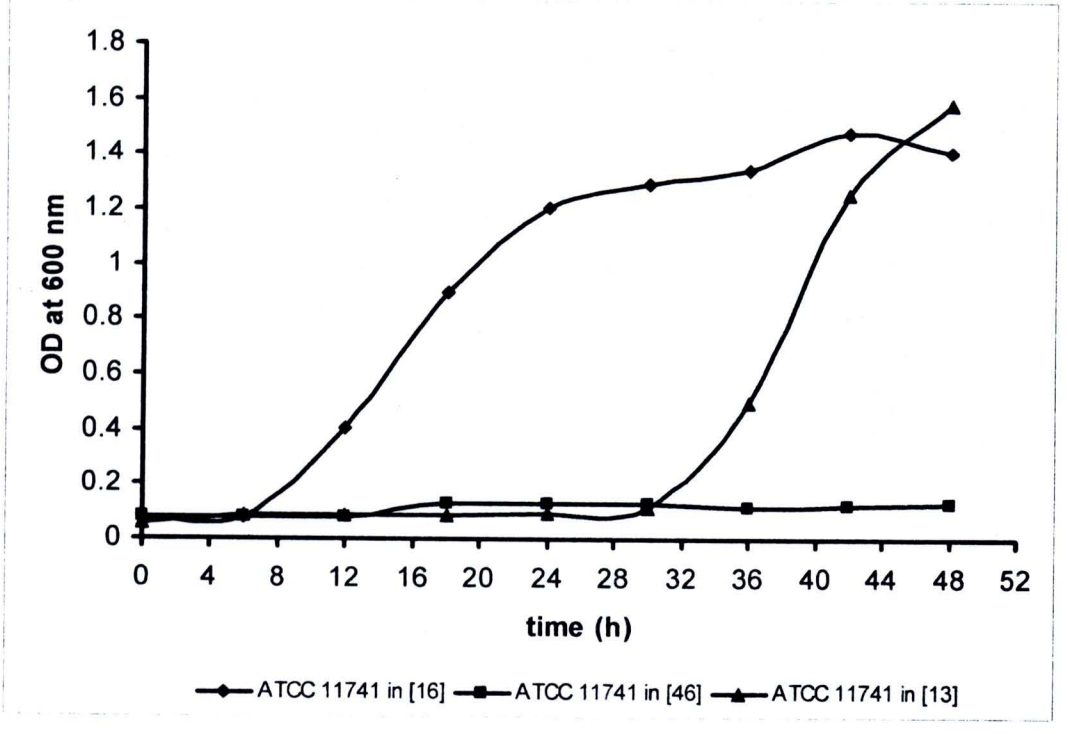


**Figure B-1** The effect of fermentation medium component on cell growth of Batch fermentation using *L.salivarius subsp. salivarius* ATCC 11741.

# 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 |

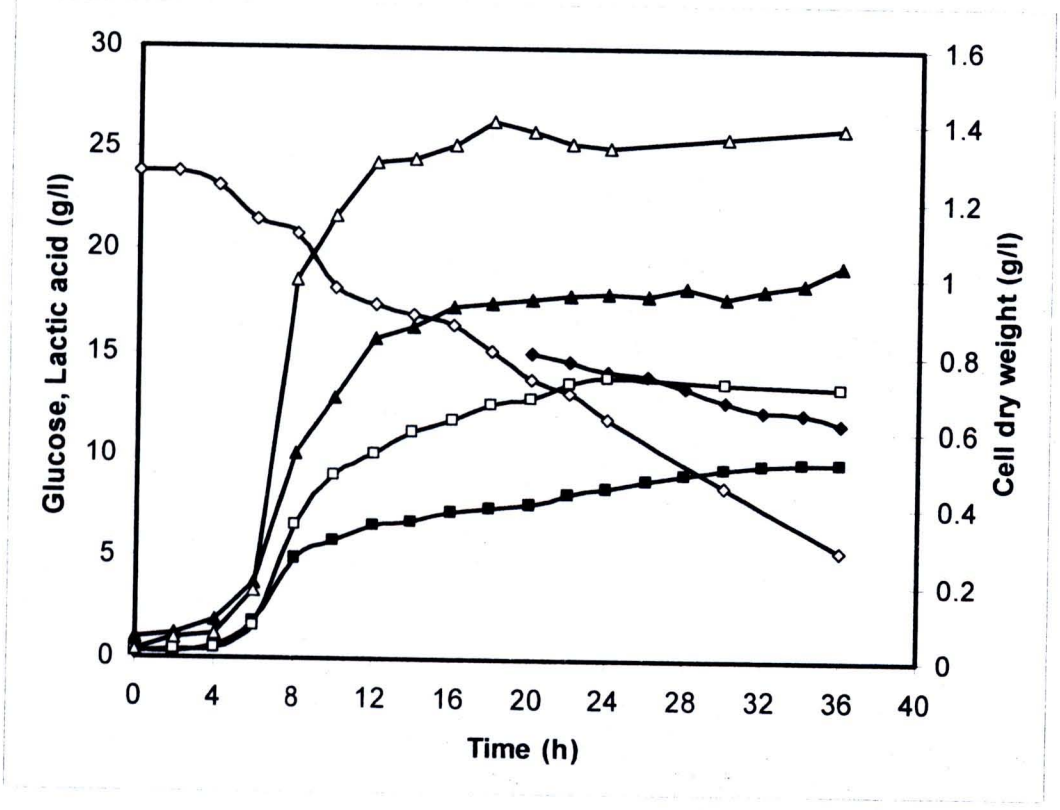


**Figure C-1** Batch fermentation of *L.salivarius subsp. salivarius* ATCC 11741. (▲) cell dry weight, (◆) glucose, ( ■) lactic acid conducted in 250 Erlenmeyer flask; (△) cell dry weight, (◇) glucose, ( □) lactic acid conducted in 1l fermentor

# 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 |

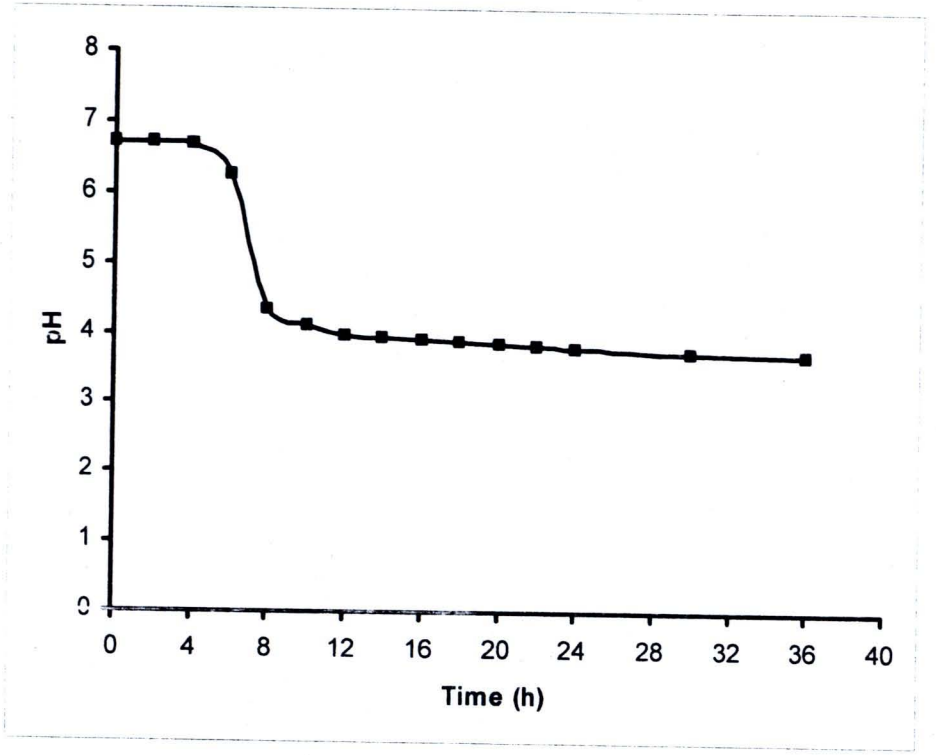


**Figure D-1** pH in batch fermentation of lactic acid using *L.salivarius subsp. salivarius* ATCC 11741 (YE = 5 g/l, glucose 20 g/l)

**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-1) | 0.39 | 0.38 |
| $q_s$ ($h^{-1}$) | 5.96 | 5.89 |
| $q_p$ ($h^{-1}$) | 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-1) | 0.38 | 0.42 |
| $q_s$ ($h^{-1}$) | 5.89 | 5.38 |
| $q_p$ ($h^{-1}$) | 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-1) | 0.48 | 0.46 |
| $q_s$ ($h^{-1}$) | 1.99 | 1.08 |
| $q_p$ ($h^{-1}$) | 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

| | **amount of sample** | **% Total Nitrogen** |
|---|---|---|
| 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

| | **Brewer's yeast autolysate** | **Brewer's yeast autolysate (debitterness)** | **% debitterness** |
|---|---|---|---|
| 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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