APPENDIX

APPENDIX

Appendix I
Calculation of lipase activity

Lipase activity = V ml X 0.05 M X 1/30 minutes
where V = Volume of 0.05 M NaOH required from the activity of lipase

$$= \frac{\text{V ml } 0.05 \text{ M}}{1000} \times {}^{1}/_{30} \text{ minutes}$$

$= 1.67 \text{ V X } 10^{-6}$ mole/min
$= 1.67 \text{ V X } 10^{-6} \text{ X } 10^{6}$ µmole/min
$= 1.67 \text{ X V}$ µmole/min

Appendix II
OD readings for Bovine serum albumin

Distilled water (ml)	BSA stock solutions (mg/ml)	Volume of BSA added (ml)	Weight of the BSA (mg)	OD_{750}
1.0	0.0	0.0	0.0	-
0.9	0.05	0.1	0.05	-
0.8	0.05	0.2	0.001	-
0.7	0.5	0.1	0.05	0.097
0.8	0.5	0.2	0.1	0.137
0.7	0.5	0.5	0.15	0.183
0.6	0.5	0.4	0.2	0.227
0.5	0.5	0.5	0.25	0.277
0.4	0.5	0.6	0.30	0.340
0.3	0.5	0.7	0.35	0.352
0.2	0.5	0.8	0.40	0.383

Appendix III
Calculation of specific activity of citrate synthase

Example: 40 µl (From 10 times dilution) of crude enzyme extract of yeast *Yarrowia lipolytica* which contained 0.084 mg of protein was added into assay system and reaction was carried for 3 minutes.

Absorbance obtained (A/t) = $OD_{\text{at 3 minutes}}$ - $OD_{\text{at 0 time}}$ /t = 0.435/3 min = 0.145 min^{-1}

$\Delta E_{412} = K = 13{,}000\ mol^{-1}\ L\ cm^{-1}$
l = lenght of light path in solution = 1 cm
V = Activity of the enzyme
C = Concentration of absorbing species in µmoles/ml
t = Times

From Beer-Lambart,

$A/t = Kcl/t \qquad V = c/t$
$A/t = c/t \times Kl$
$V = 1/Kl \times A/t$

$K = 13{,}000\ mol^{-1}\ L\ cm^{-1}$
$= 1.3 \times 10^4\ mol^{-1}\ L\ cm^{-1}$
$= 1.3 \times 10^4 \times 1/10^6 \times 1/cm \times 1000\ ml$
$= 13\ \mu mol^{-1}\ cm^{-1}\ ml$
$= 13\ ml / \mu mol\ cm$
$\therefore = 1/K = \mu mol \times cm / 13\ ml$
$\therefore = 1/l = 1 / cm$

Enzyme activity V'= µmol X cm /13 ml X 1/cm X A/t
V'= µmol/13 X 0.145 min^{-1}

The assay volume contained 1 ml volume,
∴ Activity of enzyme in 1 ml solution = 1 ml X µmol /13 ml X 0.145 min^{-1}
= 0.011 µmol / min

Specific activity = Total amount of enzyme activity / Total amount of protein activity

= 0.011 µmol / min / 0.084 mg protein
= 0.131 µmol / mg/ protein / min X 10 (From 10 times dilution)
= 1.31 µmol / mg/ protein / min

Appendix IV
Calculation of specific activity of isocitrate lyase

Example: 50 µl (From 5 times of dilution) of crude enzyme extract of yeast *Y. lipolytica* which contained 0.0228 mg of protein was added into assay system and reaction was carried for 4 minutes.

Absorbance obtained (A/t) = $OD_{\text{at 4 minutes}}$ - $OD_{\text{at 0 time}}$ /t = 0.652-0.264/4 min
= 0.097 min^{-1}

$\Delta E_{324} = K = 1.7 \times 10^4 mol^{-1} L cm^{-1}$
l = lenght of light path in solution = 1 cm
V = Activity of the enzyme
C = Concentration of absorbing species in µmoles/ml
t = Times

From Beer-Lambart,

$A/t = Kcl/t$ $\quad V = c/t$
$A/t = c/t \times Kl$
$V = 1/Kl \times A/t$

$K = 1.7 \times 10^4 mol^{-1} Lcm^{-1}$
$= 1.7 \times 10^4 \times 1/10^6 \times 1/cm \times 1000 ml$
$= 17 \mu mol^{-1} cm^{-1} ml$
$= 17 ml / \mu mol\ cm$
$\therefore = 1/K = \mu mol \times cm / 17 ml$
$\therefore = 1/l = 1 / cm$

Enzyme activity V' = µmol X cm /17 ml X 1/cm X A/t
V' = µmol/17 ml X 0.097 min^{-1}

The assay volume contained 1 ml volume,
∴ Activity of enzyme in 1 ml solution = 1 ml X µmol /17 ml X 0.097 min^{-1}
= 0.0057 µmol / min

Specific activity = Total amount of enzyme activity / Total amount of protein activity

= 0.0057 µmol / min / 0.0228 mg protein
= 0.25 µmol / mg/ protein / min X 5 (from 5 times of dilution)
= 1.25 µmol / mg/ protein / min

Appendix V
Calculation of specific activity of malate synthase

Example: 50 µl of crude enzyme extract of yeast *Y. lipolytica* which contained 0.0513 mg mg of protein was added into assay system and reaction was carried for 1 minute.

Absorbance obtained (A/t) = $OD_{at\ 1\ minute}$ - $OD_{at\ 0\ time}$ /t = 0.513-0.236/1 min
= 0.277 min^{-1}

$\Delta E_{324} = K = 4.5 \times 10^3\ mol^{-1}\ L\ cm^{-1}$
l = lenght of light path in solution = 1 cm
V= Activity of the enzyme
C = Concentration of absorbing species in µmoles/ml
t = Times

From Beer-Lambart,

$A/t = Kcl/t$ $\quad V = c/t$
$A/t = c/t \times Kl$
$V = 1/Kl \times A/t$

$K = 4.5 \times 10^3\ mol^{-1}\ Lcm^{-1}$
$= 4.5 \times 10^3 \times 1/10^6 \times 1/cm \times 1000\ ml$
$= 4.5\ \mu mol^{-1}\ cm^{-1}\ ml$
$= 4.5$ ml / µmol cm
$\therefore = 1/K = \mu mol \times cm / 4.5$ ml
$\therefore = 1/l = 1$ / cm

Enzyme activity V' = µmol X cm /4.5 ml X 1/cm X A/t
V' = µmol/4.5 ml X 0.277 min^{-1}

The assay volume contained 0.5 ml volume,
∴ Activity of enzyme in 1 ml solution = 0.5 ml X µmol /4.5 ml X 0.277 min^{-1}

= 0.061 µmol / min

Specific activity = Total amount of enzyme activity / Total amount of protein activity

= 0.0308 µmol / min / 0.0513 mg protein

= 0.60 µmol / mg protein / min

Appendix VI
Calculation of specific activity of isocitrate dehydrogenase

Example: 50 µl of crude enzyme extract of yeast *Y. lipolytica* which contained 0.0858 mg mg of protein was added into assay system and reaction was carried for 3 minute.

Absorbance obtained (A/t) = $OD_{\text{at 3 minute}} - OD_{\text{at 0 time}} / t$ = 0.307-0.262/3 min
= 0.0045 min^{-1}

$\Delta E_{340} = K = 6.22 \times cm^2\ mol^{-1}$
l = lenght of light path in solution = 1 cm
V= Activity of the enzyme
C = Concentration of absorbing species in µmoles/ml
t = Times

From Beer-Lambart,

$A/t = Kcl/t \qquad V = c/t$
$A/t = c/t \times Kl$
$V = 1/Kl \times A/t$

$K = 6.22 \times 10^6\ cm^2\ mol^{-1}$
$= 6.22 \times 10^6 \times 1/10^6\ \mu mol \times cm^2$
$= 6.22\ cm^2\ \mu mol^{-1}$
$\therefore = 1/K = \mu mol / 6.22\ cm^2$
$\therefore = 1/l = 1 / cm$

Enzyme activity V' = µmol / 6.22 cm^2 X 1/cm X A/t
V' = µmol/ 6.22 cm^3 X 0.045 min^{-1}
= µmol/ 6.22 ml X 0.045 min^{-1}
The assay volume contained 1 ml volume,
∴ Activity of enzyme in 1 ml solution = 1 ml X µmol /6.22 ml X 0.045 min^{-1}
= 0.0024 µmol / min

Specific activity = Total amount of enzyme activity / Total amount of protein activity

= 0.0024 µmol / min / 0.0858 mg protein

= 0.028 µmol / mg/ protein / min

Appendix VII
Calculation of specific activity of aconitate hydratase

Example: 100 µl of crude enzyme extract of yeast *Y. lipolytica* which contained 0.0684 mg mg of protein was added into assay system and reaction was carried for 1 minute.

Absorbance obtained (A/t) = $OD_{\text{at 1 minute}}$ - $OD_{\text{at 0 time}}$ /t = 0.012-0.035/1 min
= -0.023 min^{-1}

Each 0.001 change in optical density is equivalent to 2.84 X 10^{-4} µmol of cis-aconitate per ml

0.01..... 2.84 X 10^{-4} µmol/ml
0.023.....0.023 min^{-1} / 0.001 X 2.84 X 10^{-4} µmol/ml

$\therefore$ 6.53 X 10^{-3} µmol/ml/min

The assay volume contained 1 ml volume,
$\therefore$ Activity of enzyme in 1 ml solution = 1 ml X 6.53 X 10^{3} µmol/ml/min
= 6.53 X 10^{-3} µmol / min

Specific activity = Total amount of enzyme activity / Total amount of protein activity

= 0.0065 µmol / min / 0.0684 mg protein

= 0.095 µmol / mg / protein / min

Appendix VIII
Calculation colony forming unit per ml

CFU/ml = Average colony count per plates X $^{1}/_{\text{volume of cell suspension}}$ X Dilution factor of the cell suspension

Appendix IX

Growth and citric acid production of different strains *Yarrowia lipolytica* utilising 2 % glucose										
	ATCC 8661 (Laboratory strain no. M240)					Strain F21A				
Fermentation time (Hours)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)
0	0.26	6.9	1.82	0.01	0.11	0.24	6.9	0.24	0.00	0.14
24	0.86	3.8	4.5	0.1	0.2	1.23	4.8	7.40	0.02	0.06
32	1.04	3.6	4.6	0.78	1.46	1.57	3.6	7.70	2.01	0.75
48	1.28	3.5	5.8	1.23	1.41	1.62	3.5	8.00	2.41	0.80
72	1.40	3.5	6.7	1.56	1.35	1.68	3.5	8.4	2.45	0.79
96	1.48	3.6	7.2	1.87	0.65	1.64	3.6	7.31	2.50	0.75
120	1.47	3.7	7.0	1.49	0.58	1.58	3.7	7.15	1.28	0.63
144	1.40	3.8	5.8	0.37	0.06	1.59	3.8	6.4	1.26	0.5

Growth and citric acid production of different strains *Yarrowia lipolytica* utilising 2 % glucose										
	M243 (IFO no. 1545/ S-22)									
Fermentation time (Hours)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)
0	0.19	6.8	1.98	0	0					
24	1.31	5.8	7.4	0.06	0.03					
32	1.58	5.7	7.6	1.96	0.09					
48	1.65	5.4	8.0	2.27	0.26					
72	1.67	5.4	8.4	2.51	0.89					
96	1.56	5.5	7.6	2.64	0.96					
120	1.51	5.4	7.3	1.26	0.08					
144	1.48	5.3	7.2	1.18	0.06					

Appendix X

Growth and citric acid production of different strains *Yarrowia lipolytica* utilising 2 % NBD palm olein (Ajam *et al.*,1991)

	ATCC 8661 (Laboratory strain no. M240)					M243 (IFO no. 1545/ S-22)				
Fermentation time (Hours)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)
0	0.23	6.9	0.6	0	0	0.22	6.9	0.42	0	0
4	0.54	6.8	1.2	ND	ND	0.61	6.7	1.2	ND	ND
8	0.64	6.7	1.8	ND	ND	0.73	6.8	2.5	ND	ND
20	0.79	3.4	4.6	1.5	1.0	0.85	6.1	5.0	0.5	0.6
40	0.90	2.8	8.0	3.6	2.2	1.05	3.4	8.4	6.0	0.5
80	0.96	2.8	8.2	5.8	4.0	1.15	3.2	10.5	9.0	1.0
120	0.99	2.8	5.8	8.0	6.2	1.20	3.4	7.2	11.5	1.2
160	0.95	2.6	4.3	7.3	6.0	1.10	3.2	6.1	7.1	0.8

Growth and citric acid production of different strains *Yarrowia lipolytica* utilising 2 % NBD palm olein

	Strain F21					Strain F21A				
Fermentation time (Hours)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)
0	0.021	6.9	0.64	0.021	0.018	0.018	6.8	0.37	0.00	0.00
24	1.33	4.0	6.05	2.24	1.25	1.48	4.4	8.1	2.02	1.58
48	1.43	3.3	8.1	3.15	2.26	1.56	4.3	7.9	5.10	2.50
72	1.45	3.0	7.8	6.35	3.51	1.58	3.1	8.2	10.79	3.62
96	1.39	3.3	6.5	1.95	1.65	1.56	2.9	7.9	4.62	6.75
120	1.39	3.1	6.35	1.55	1.34	1.56	3.1	6.5	4.58	7.82
144	1.28	3.3	6.28	1.43	1.43	1.51	3.2	6.25	3.38	5.25
168	1.36	3.0	6.0	1.50	1.72	1.48	3.3	5.98	2.78	4.76

Appendix XI

Growth and citric acid production of different strains *Yarrowia lipolytica* utilising 4 % NBD palm olein										
	ATCC 8661 (Laboratory strain no. M240)					M243 (IFO no. 1545/ S-22)				
Fermentation time (Hours)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)
0	0.20	6.8	1.82	0.00	0.00	0.19	6.0	1.06	0.00	0.00
24	1.27	3.5	7.0	1.74	2.02	1.05	4.9	7.8	1.65	0.75
48	1.36	3.0	7.2	2.52	3.38	1.42	3.0	8.2	4.40	4.80
72	1.40	2.9	6.9	4.0	5.22	1.40	3.0	7.4	3.50	5.20
96	1.39	2.9	6.5	3.92	4.42	1.38	3.0	6.4	3.35	3.75
120	1.29	3.0	6.0	3.25	3.51	1.36	3.0	6.2	3.20	2.80
144	1.30	2.9	5.2	2.80	3.00	1.32	3.2	5.8	2.80	2.02
168	1.36	3.0	4.9	2.60	3.00	1.28	3.4	4.8	2.40	1.80

Growth and citric acid production of different strains *Yarrowia lipolytica* utilising 4 % NBD palm olein										
	Strain F21A									
Fermentation time (Hours)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)	OD_{560}	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)
0	0.21	6.8	1.02	0.00	0.00					
24	154	5.3	8.40	2.41	1.02					
48	1.60	3.3	8.61	2.80	5.03					
72	1.68	3.1	7.93	3.85	6.52					
96	1.66	3.0	7.42	5.6	7.04					
120	1.65	3.2	6.06	7.8	10.52					
144	1.62	3.0	5.91	7.2	7.82					
168	1.60	3.1	5.23	5.2	7.51					

Appendix XII

Growth and extracellular lipase activity of different strains *Yarrowia lipolytica* utilising 2 % NBD palm olein as a carbon source						
	ATCC 8661 (Laboratory strain no. M240		Strain F21A		M243 (IFO no. 1545/ S-22)	
Fermentation time (Hours)	Lipase activity (µmol/min)	OD_{560}	Lipase activity (µmol/min)	OD_{560}	Lipase activity (µmol/min)	OD_{560}
0	0.17	0.19	0.19	0.12	0.18	0.15
4	1.59	0.21	1.67	0.38	0.33	0.20
8	5.70	0.48	3.05	0.52	0.68	0.38
12	13.00	0.61	8.52	0.63	8.18	0.52
16	6.30	0.76	13.42	0.73	13.2	0.86
20	1.50	1.21	4.53	0.84	18.87	1.14
24	0.90	1.32	2.15	1.08	11.37	1.38
48	0.67	1.49	0.87	1.40	5.01	1.59
52	-	-	-	-	0.84	1.62

Appendix XIII

Growth and extracellular lipase activity of different strains *Yarrowia lipolytica* utilising 4% NBD palm olein as a carbon source						
ATCC 8661(Laboratory strain no. M240)			Strain F21A		M243 (IFO no. 1545/S-22)	
Fermentation time (Hours)	Lipase activity (µmol/min)	OD_{560}	Lipase activity (µmol/min)	OD_{560}	Lipase activity (µmol/min)	OD_{560}
0	0.17	0.16	0.0	0.12	0.18	0.12
4	0.67	0.30	1.0	0.58	0.25	0.16
8	10.60	0.58	2.09	1.18	1.92	0.42
12	12.02	0.80	2.59	1.42	12.86	0.82
16	5.34	1.28	17.37	1.56	7.10	0.96
20	3.76	1.19	14.28	1.68	4.84	1.08
24	0.92	1.17	1.17	1.78	1.75	1.10
48	0.67	1.18	0.84	1.82	0.33	1.34
52	ND	ND	ND	ND	ND	ND

ND not determined

Appendix XIV

TCA and glyoxylate enzymes activity of different strains *Yarrowia lipolytica* utilising 2 % NBD palm olein as the carbon source										
	ATCC 8661(Laboratory strain no. M240)					M243 (IFO no. 1545/ S-22)				
	Enzymes activity (μmol/mg protein/min)					Enzymes activity (μmol/mg protein/min)				
Fermentation time (Day)	CS	ICL	MS	AH	ICDH	CS	ICL	MS	AH	ICDH
20 hours	0.92	0.36	0.52	0.092	0.071	1.28	0.80	0.14	0.012	0.016
1	1.76	0.82	1.48	0.17	0.12	2.56	0.86	2.57	0.102	0.052
2	1.80	0.90	1.20	0.190	0.146	3.49	1.38	1.01	0.098	0.080
3	1.46	0.75	0.80	0.14	0.10	2.62	0.95	0.98	0.074	0.071
4	1.20	0.65	0.56	0.06	0.031	1.85	0.84	0.72	0.070	0.060
5	1.10	0.38	0.50	0.04	0.020	1.51	0.27	0.57	0.062	0.054

CS	Citrate synthase
ICL	Isocitrate lyase
MS	Malate synthase
AH	Aconitate hydratase
ICDH	Isocitrate dehydrogenase

Appendix XV

TCA and glyoxylate enzymes activity of different strains *Yarrowia lipolytica* utilising 2 % NBD palm olein as the carbon source										
	Strain F21A									
	Enzymes activity (µmol/mg protein/min)					Enzymes activity (µmol/mg protein/min)				
Fermentation time (Day)	CS	ICL	MS	AH	ICDH	CS	ICL	MS	AH	ICDH
20 hours	1.02	0.50	0.60	0.085	0.065					
1	1.70	0.88	1.42	0.24	0.19					
2	1.95	0.90	1.88	0.20	0.122					
3	1.88	0.98	1.25	0.015	0.092					
4	1.68	0.80	0.88	0.092	0.085					
5	1.45	0.45	0.60	0.081	0.051					

CS	Citrate synthase
ICL	Isocitrate lyase
MS	Malate synthase
AH	Aconitate hydratase
ICDH	Isocitrate dehydrogenase

Appendix XVI

TCA enzymes activity of different strains *Yarrowia lipolytica* utilising 4 % NBD palm olein as the carbon source

	ATCC 8661 (Laboratory strain no. M240)					M243 (IFO no. 1545/ S-22)				
	Enzymes activity (μmol/mg protein/min)					Enzymes activity (μmol/mg protein/min)				
Fermentation time (Day)	CS	ICL	MS	AH	ICDH	CS	ICL	MS	AH	ICDH
20 hours	0.50	0.25	0.45	0.095	0.036	0.70	0.84	0.23	0.040	0.010
1	1.88	1.25	1.50	0.23	0.19	1.43	0.95	0.80	0.092	0.036
2	1.65	1.00	1.20	0.145	0.126	1.81	1.07	1.27	0.052	0.042
3	1.10	0.58	0.70	0.20	0.16	1.55	1.17	1.63	0.048	0.038
4	0.94	0.42	0.58	0.09	0.06	1.27	0.95	0.74	0.026	0.024
5	0.80	0.28	0.52	0.015	0.042	0.85	0.55	0.60	0.020	0.015

CS Citrate synthase
ICL Isocitrate lyase
MS Malate synthase
AH Aconitate hydratase
ICDH Isocitrate dehydrogenase

Appendix XVII

TCA enzymes activity of different strains *Yarrowia lipolytica* utilising 4 % NBD palm olein as the carbon source										
	Strain F21A									
	Enzymes activity (µmol/mg protein/min)					Enzymes activity (µmol/mg protein/min)				
Fermentation time (Day)	CS	ICL	MS	AH	ICDH	CS	ICL	MS	AH	ICDH
20 hours	0.88	0.25	0.60	0.065	0.035					
1	2.05	1.21	2.38	0.095	0.085					
2	2.80	1.50	2.18	014	0.10					
3	2.38	1.18	1.85	0.19	0.05					
4	1.75	0.75	1.15	0.185	0.041					
5	1.30	0.50	0.68	0.165	0.036					

CS Citrate synthase
ICL Isocitrate lyase
MS Malate synthase
AH Aconitate hydratase
ICDH Isocitrate dehydrogenase

Appendix XVIII

Growth production of citric acid by *Yarrowia lipolytica* on 2% NBD palm olein at fermenter level.

Fermentation time (hour)	OD measured at 560 nm	pH	Dry weight (g/l)	Citric acid (g/l)	Isocitric acid (g/l)
0	0.31	6.9	2.1	-	ND
24	1.89	6.8	10.5	-	ND
36	ND	6.7	13.6	-	ND
48	2.08	6.7	13.8	-	ND
72	ND	6.8	12.6	-	ND
96	2.18	6.7	11.5	-	ND
120	ND	6.5	10.7	-	ND
144	1.83	6.4	8.8	-	ND
168	ND	6.5	9.2	-	ND
192	1.90	6.5	9.3	-	ND

- Not detected
ND Not determined