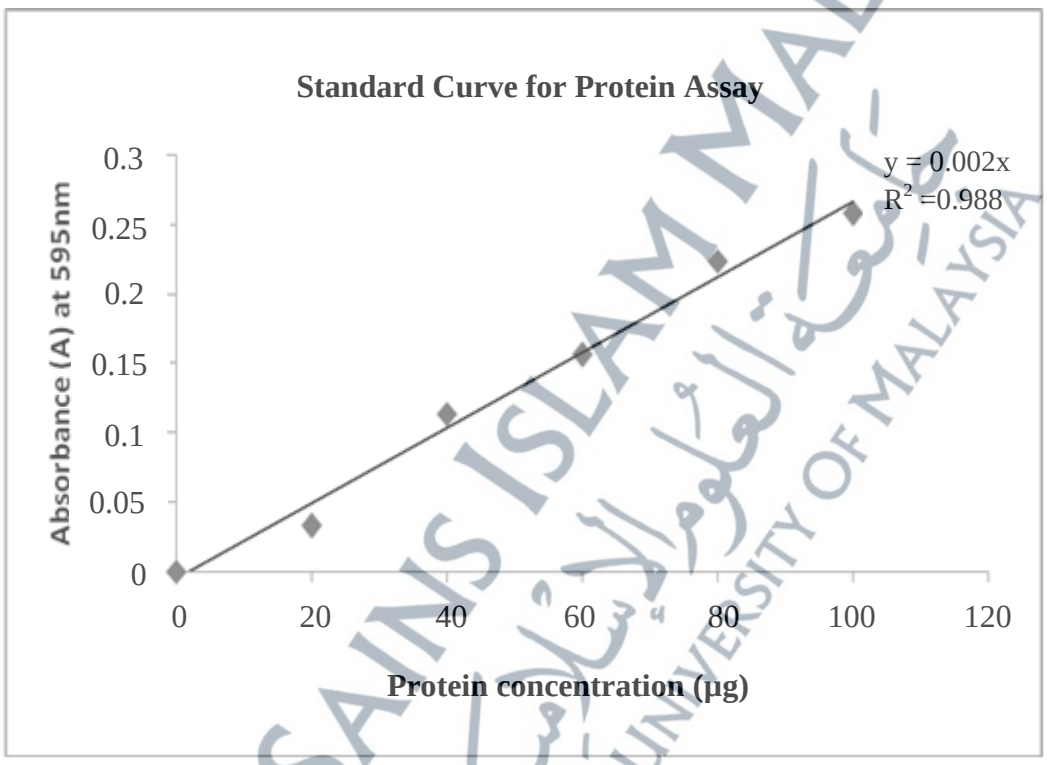
APPENDIX A

THE STANDARD CURVE OF ABSORBANCE VERSUS CONCENTRATION OF BOVINE SERUM ALBUMIN (BSA)



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APPENDIX B

PREPARATION OF BRADFORD REAGENT, BOVINE SERUM ALBUMIN (BSA) STANDARD SOLUTION AND DISTILLED WATER IN PROTEIN ASSAY PROCEDURE

Tube	BSA[a] (µL)	Distilled water (µL)	Bradford Reagent (mL)
1	0	100.0	5.0
2	20.0	80.0	5.0
3	40.0	60.0	5.0
4	60.0	40.0	5.0
5	80.0	20.0	5.0
6	100.0	0	5.0
	Sample[b] (µL)		
Free lipase	50.0	50.0	5.0
Immobilized lipase (before and after immobilization	50.0	50.0	5.0

Sample[a] = 1mg/mL of BSA concentration was prepared and used as a standard protein

Sample[b] = 50 µL was used in the determination of protein content

APPENDIX C

CALCULATION OF SPECIFIC ACTIVITY

$$\text{Specific Activity (mmol.min/mg)} = \frac{[\,(V_{control} - V_{sample})\,M]\,/t}{W}$$

Whereby,

$V_{control}$ = titration volume of NaOH for control (mL)

V_{sample} = titration volume of NaOH for sample (mL)

M = molarity of NaOH

t = reaction time

W = amount of protein (mg)

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APPENDIX D

CALCULATION OF RELATIVE ACTIVITY

The relative activity was determined as percentage of yield of certain activities compared to the activity of reaction at maximum yield by using the following equation:

$$\text{Relative activity (\%)} = \frac{\text{Specific activity in treatment}}{\text{Maximum yield specific activity}} \times 100$$

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APPENDIX E

CALCULATION THE PERCENTAGE CONVERSION OF ESTER

The percentage conversion of ester was determined by using the following equation:

$$\% \text{ conversion} = [(V_{control} - V_{sample}) / V_{control}] \times 100$$

Whereby,

$V_{control}$ = titration volume of NaOH for control (mL)

V_{sample} = titration volume of NaOH for sample (mL)

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APPENDIX F

CALCULATION THE PERCENTAGE OF IMMOBILIZATION AND PROTEIN LOADING

The immobilized enzyme was calculated in term of percentage of immobilization (%) by using the equation as stated below:

$$\% \text{ immobilization} = \frac{X_b - X_a}{X_b} \times 100$$

Whereby,

X_a = Amount of lipase after immobilization (*from standard curve*)

X_b = Amount of lipase before immobilization (*from standard curve*)

$$\text{Protein loading } (\mu g/g) = \frac{X_b - X_a}{A}$$

A= initial mass of support used

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APPENDIX G

CALCULATION OF THE REACTION RATE, V (mmol/L/min/mg)

$$V = \frac{Vmax\,[But][Ol]}{K_{m(But)}[Ol](1 + [Ol]/K_{i(Ol)}) + K_{m(Ol)}[But] + [But][Ol]}$$

$$V = \frac{Vmax\,[But][Ol]}{K_{m(Ol)}[But](1 + [But]/K_{i(But)}) + K_{m(Ol)}[Ol] + [But][Ol]}$$

$$V = \frac{Vmax\,[But][Ol]}{K_{m(But)}[Ol](1 + [Ol]/K_{i(Ol)}) + K_{m(Ol)}[But](1 + [But]/K_{i(But)} + [But][Ol]}$$

Whereby,

V = initial reaction rate

[But] = concentration of butanol

[Ol] = concentration of oleic acid

Vmax = maximum velocity

$K_{m(But)}$, $K_{m(Ol)}$ = Michaelis constant of butanol, oleic acid

$K_{i(But)}$, $K_{i(Ol)}$ = inhibition constant of butanol, oleic acid

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