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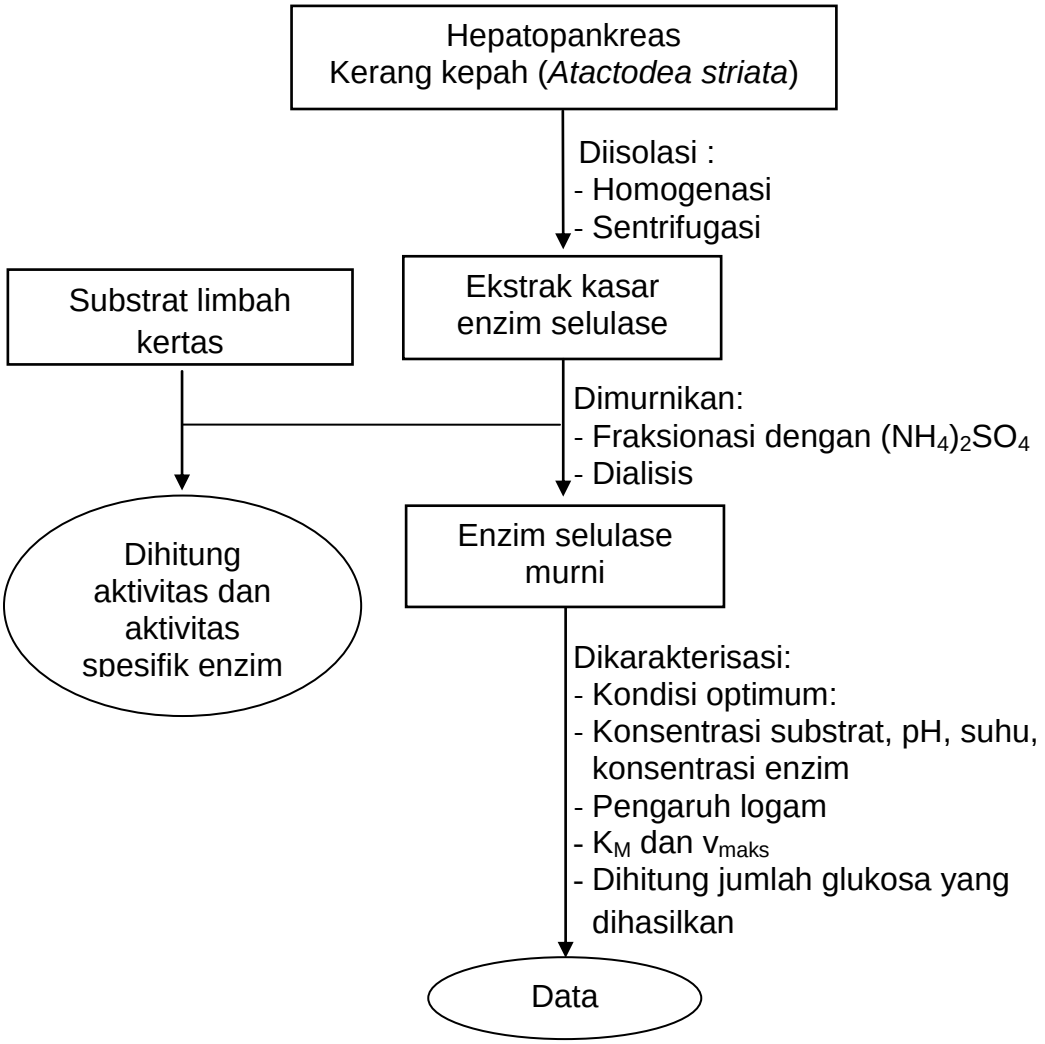
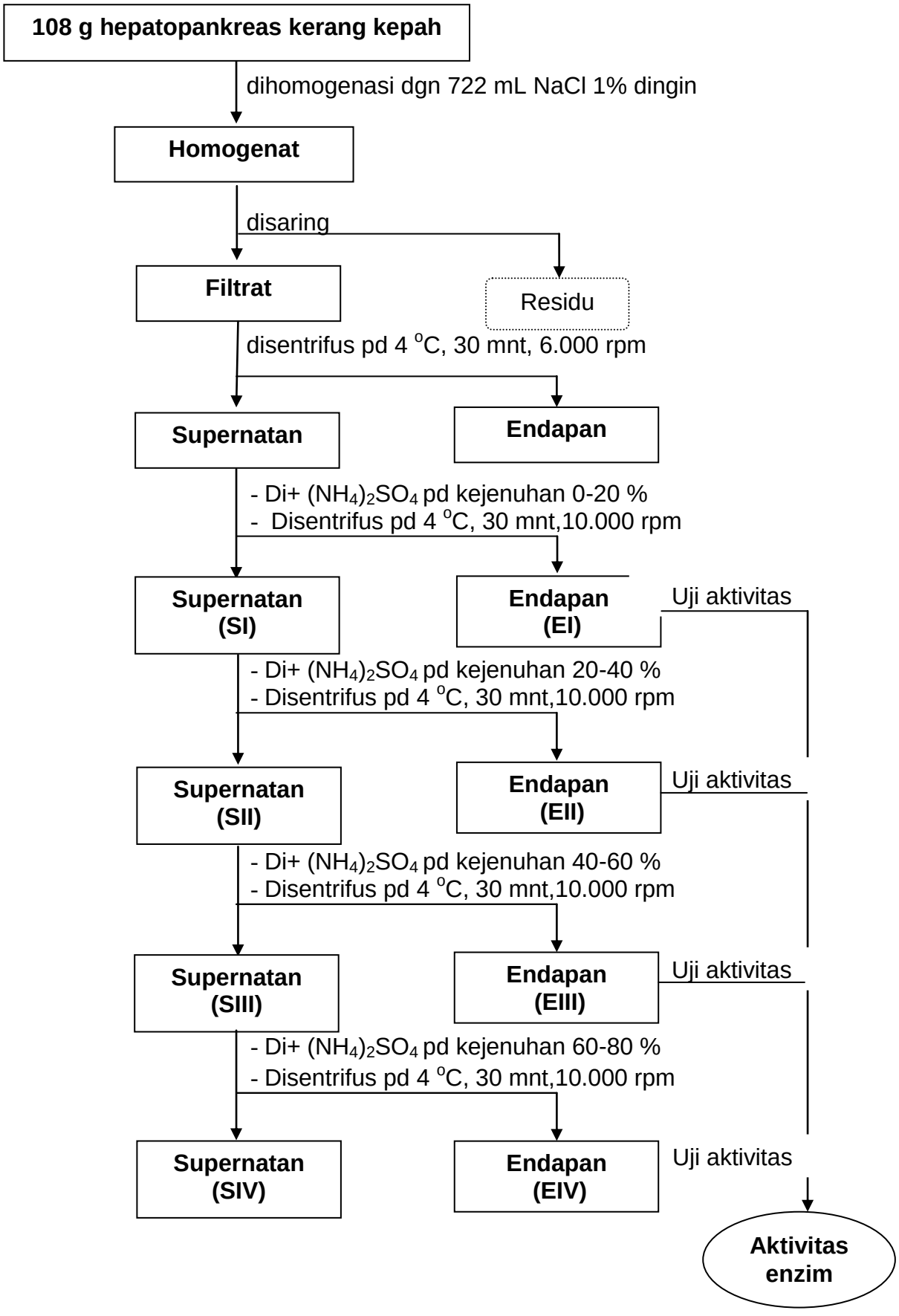
LAMPIRAN

Lampiran 1.

Gambaran Umum Prosedur Penelitian

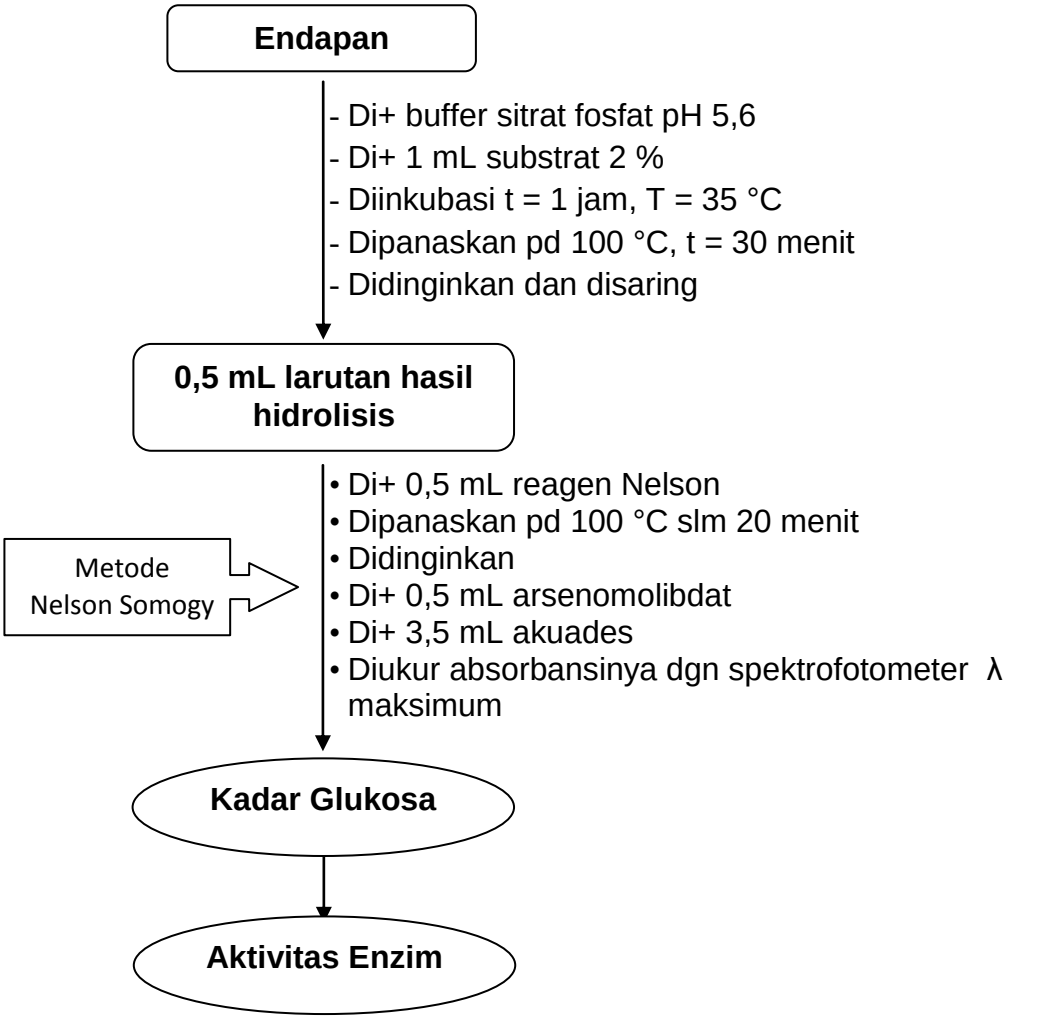


Lampiran 2. Isolasi enzim selulase dari kerang kepah (*Atactodea striata*)

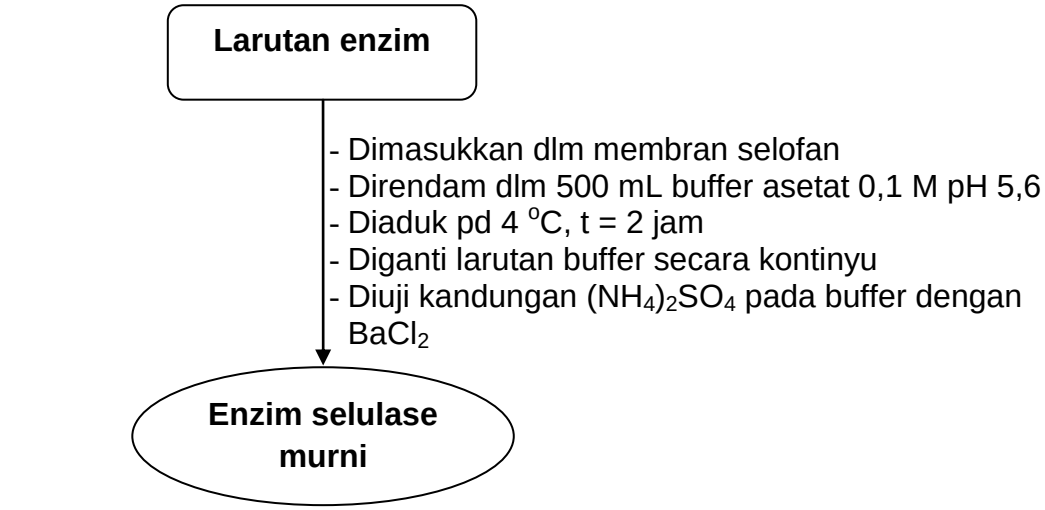


Lampiran 3. Uji Aktivitas Enzim, Dialisis, dan Penentuan Kadar Protein

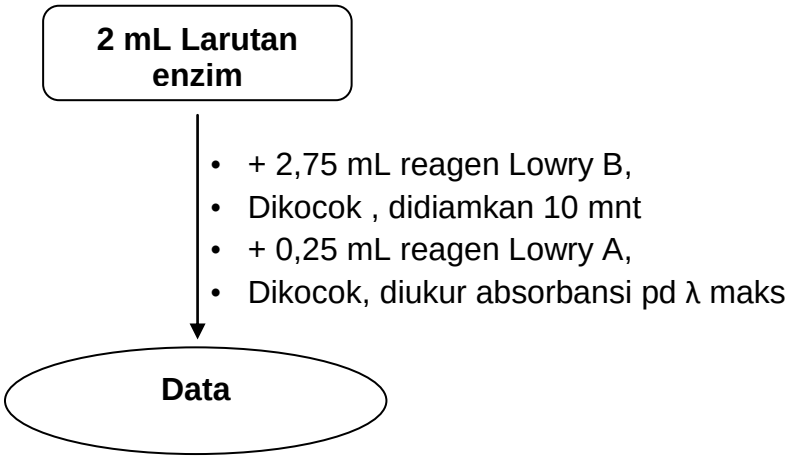
I. Uji Aktivitas



II. Dialisis

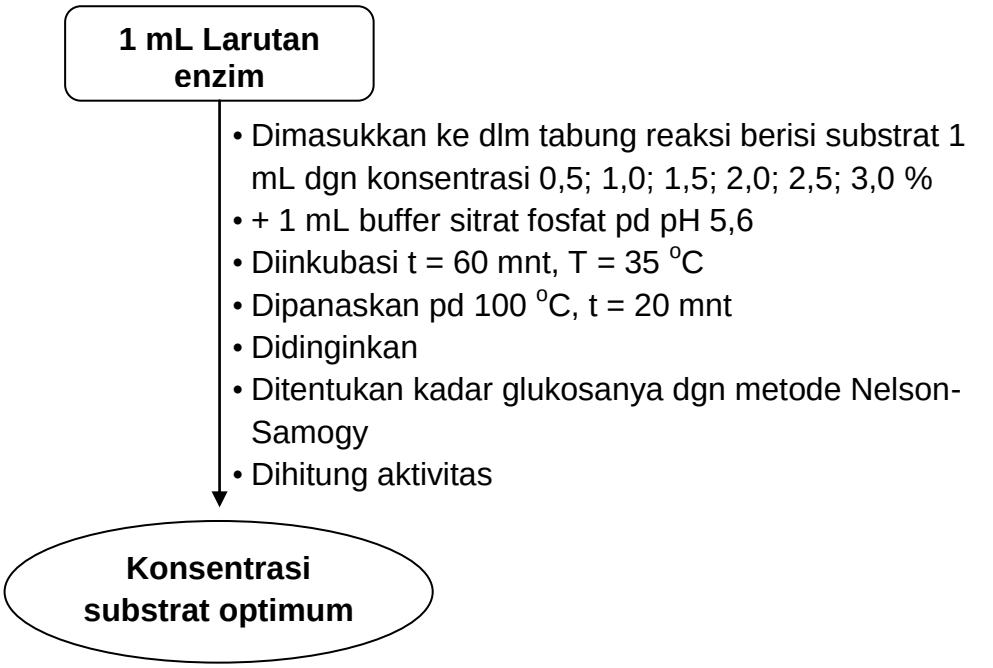


III. Penentuan Kadar Protein

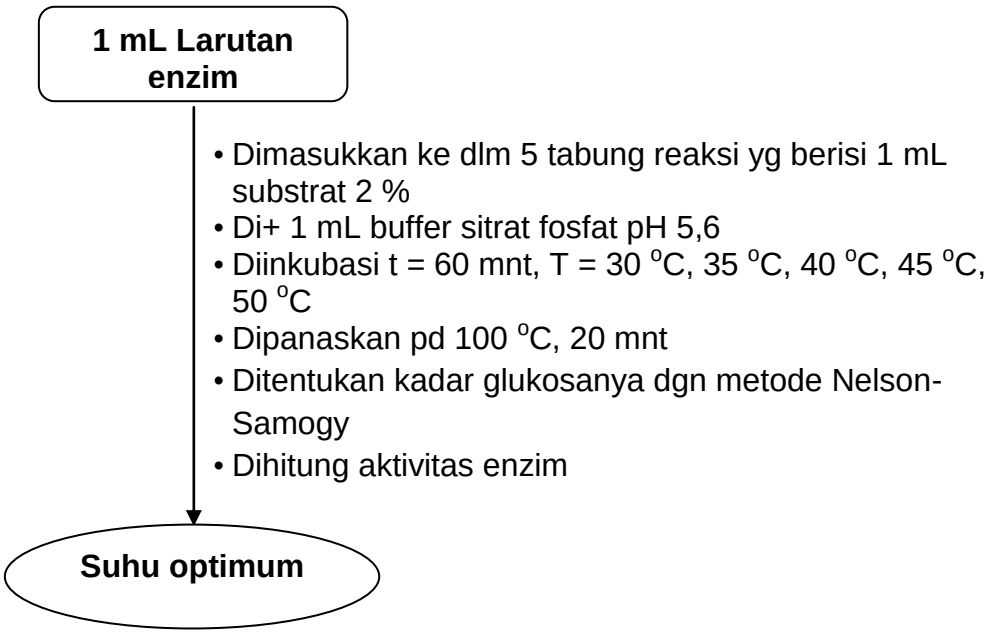


Lampiran 4. Karakterisasi Enzim Selulase

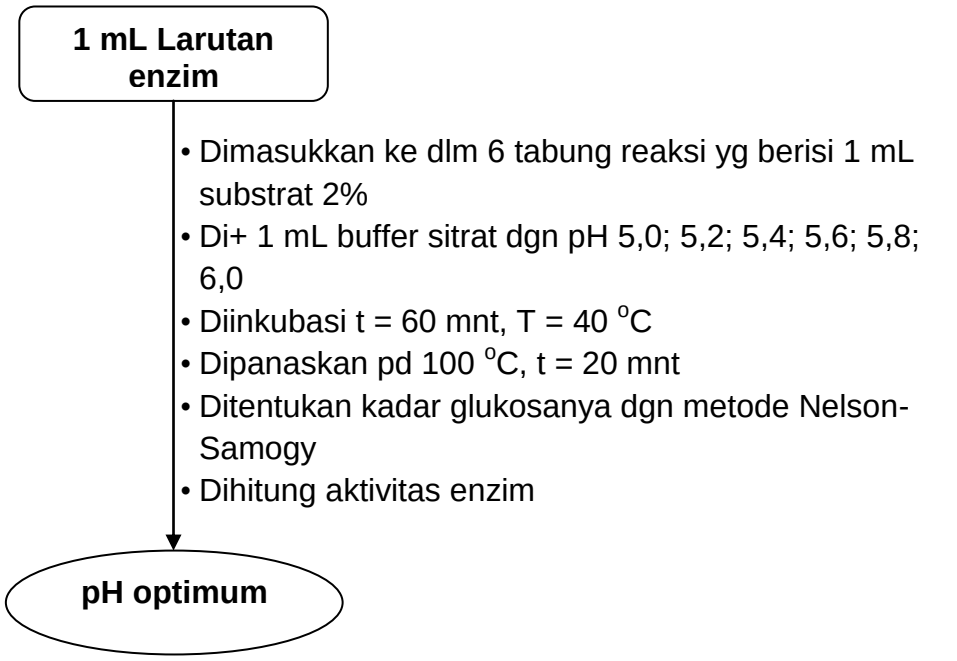
a. Penentuan konsentrasi substrat optimum



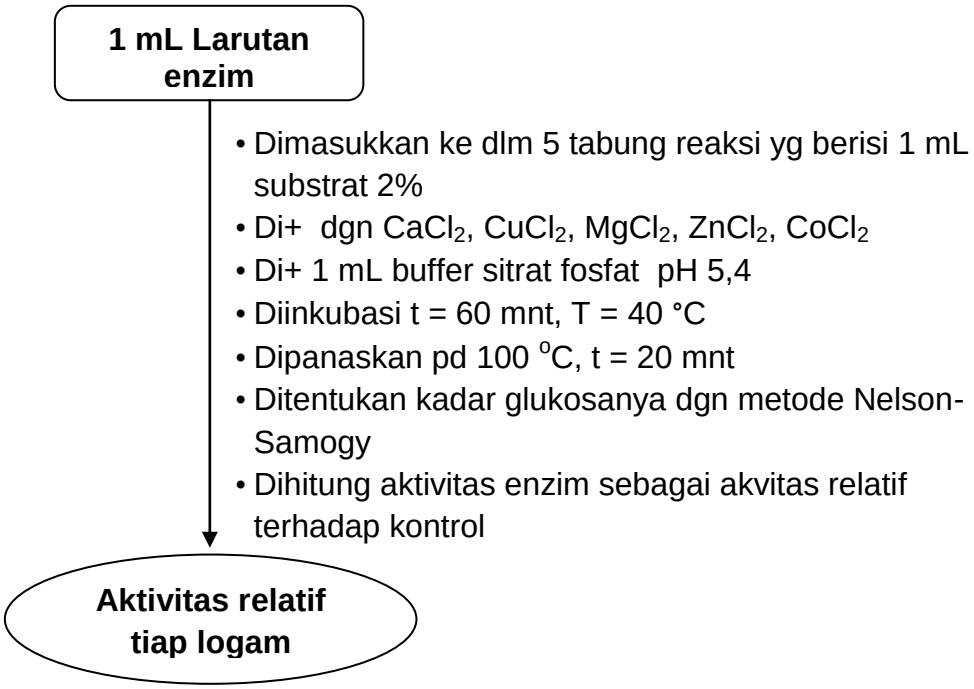
b. Penentuan suhu optimum inkubasi



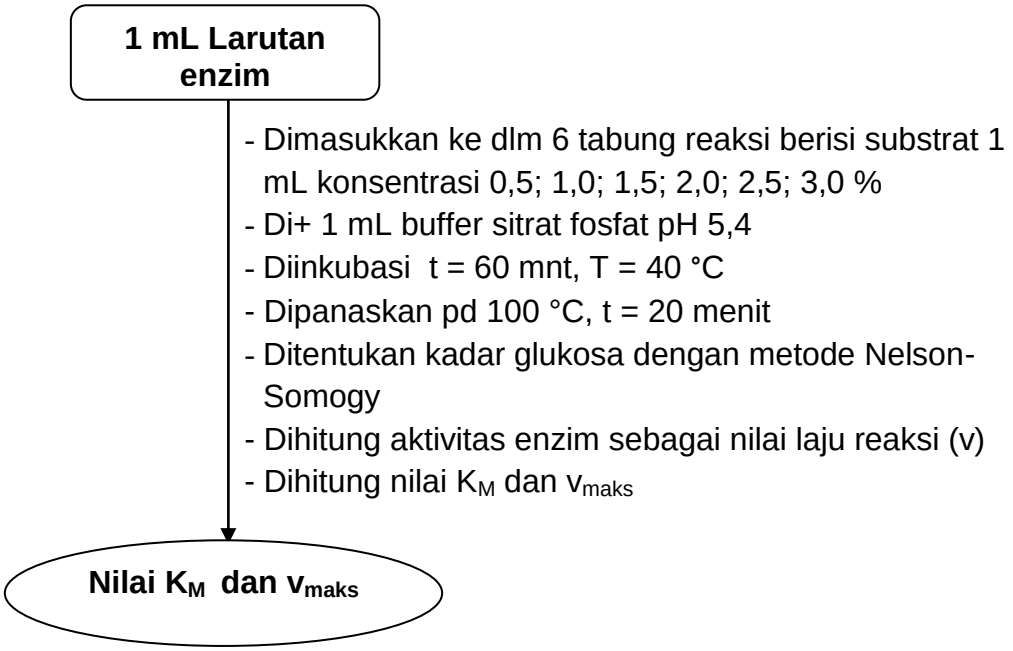
c. Penentuan pH optimum



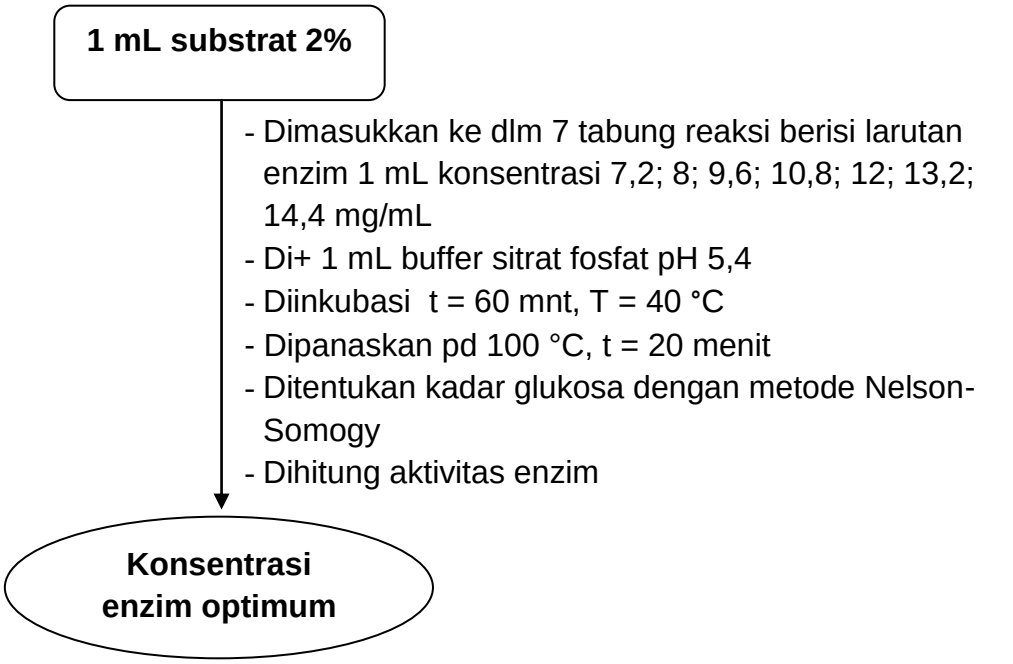
d. Penentuan Pengaruh Aktivator dan Inhibitor



e. Penentuan parameter kinetik nilai K_M dan v_{maks}

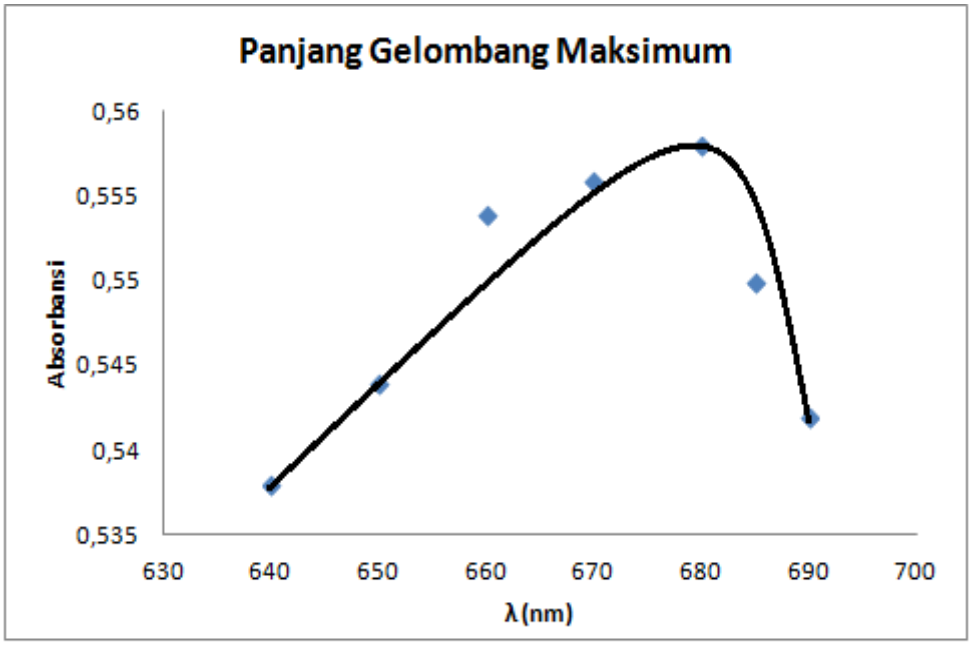


f. Penentuan konsentrasi enzim optimum



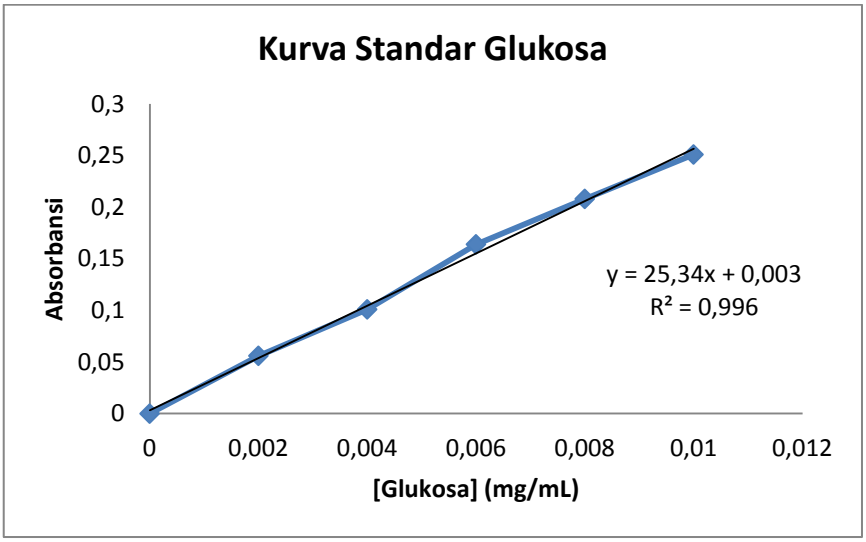
Lampiran 5. Hasil pengukuran panjang gelombang maksimum untuk penentuan kurva standar dan kadar glukosa

λ (nm)	Absorbansi
640	0,538
650	0,544
660	0,554
670	0,556
680	0,558
685	0,550
690	0,542



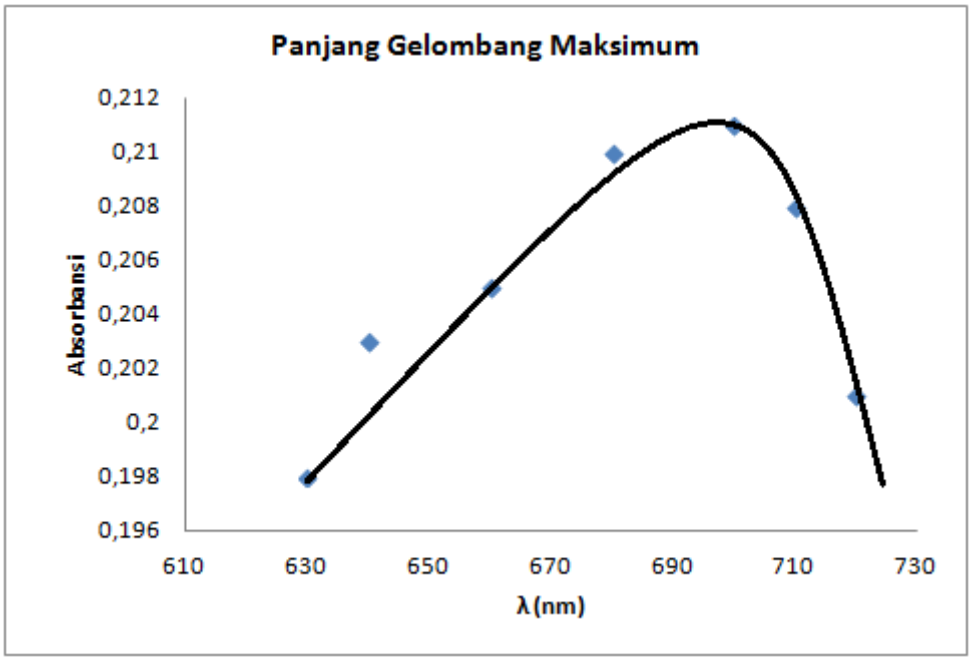
Lampiran 6. Hasil pengukuran kurva standar glukosa

[Glukosa] (mg/mL)	Absorbansi
0	0
0,002	0,056
0,004	0,101
0,006	0,164
0,008	0,208
0,010	0,251



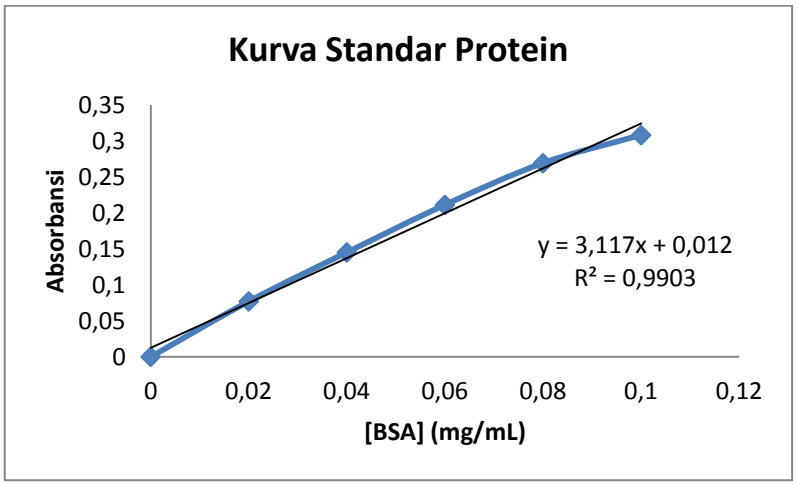
Lampiran 7. Hasil pengukuran panjang gelombang maksimum untuk penentuan kurva standar dan kadar protein

λ (nm)	Absorbansi
630	0,198
640	0,203
660	0,205
680	0,210
700	0,211
710	0,208
720	0,201



Lampiran 8. Hasil pengukuran kurva standar protein

[BSA] (mg/mL)	Absorbansi
0	0
0,02	0,077
0,04	0,145
0,06	0,211
0,08	0,269
0,10	0,308



Lampiran 9. Pembuatan larutan buffer sitrat fosfat

20 mL campuran dari X mL Na_2HPO_4 0,2 M dan Y mL asam sitrat 0,1 M

X (mL) Na_2HPO_4	Y (mL) Asam sitrat	pH
7,71	12,29	4,0
8,28	11,72	4,2
8,82	11,18	4,4
9,35	10,65	4,6
9,86	10,14	4,8
10,30	9,70	5,0
10,72	9,28	5,2
11,15	8,85	5,4
11,60	8,40	5,6
12,09	7,91	5,8
12,63	7,37	6,0
13,22	6,78	6,2
13,85	6,15	6,4

Lampiran 10. Contoh perhitungan penentuan aktivitas enzim

Fraksi 70% ⟶ Absorbansi = 0,303

Persamaan garis lurus: y= 25,34x + 0,003

$$\text{Kadar glukosa} = \frac{0{,}303 - 0{,}003}{25{,}34}$$

$$= 0{,}0118$$

$$\text{Aktivitas enzim} = \frac{\text{Kadar glukosa x } 1000}{\text{BM x t}}$$

$$= \frac{0{,}0118 \text{ x } 1000}{180 \text{ x } 60}$$

$$= 1{,}09 \text{ x } 10^{-3} \text{ U/mL}$$

Lampiran 11. Contoh perhitungan penentuan kadar protein dan aktivitas spesifik

a. Penentuan kadar protein

Fraksi 70% ⟶ Absorbansi = 0,736 dengan fp = 100

Persamaan garis lurus: y= 3,117x + 0,012

$$\text{Kadar Protein} = \frac{0{,}736 - 0{,}012}{3{,}117}$$

$$= 0{,}23227$$

$$\text{Kadar Protein} = 0{,}23227 \times \text{fp}$$

$$= 0{,}23227 \times 100$$

$$= 23{,}227 \text{ mg/mL}$$

b. Penentuan aktivitas spesifik

$$\text{Aktivitas spesifik} = \frac{\text{Aktivitas enzim (U/mL)}}{\text{Kadar protein (mg/mL)}}$$

$$= \frac{0{,}00109 \text{ U/mL}}{23{,}227 \text{ mg/mL}}$$

$$= 4{,}719 \times 10^{-5} \text{ U/mg}$$

Lampiran 12. Data aktivitas enzim setiap fraksi

Fraksi	Absorbansi [glukosa]	[Glukosa] (mg/mL)	Aktifitas Enzim (U/mL)
0-20 %	0,120	$4{,}60 \times 10^{-3}$	$4{,}30 \times 10^{-4}$
0-30 %	0,115	$4{,}40 \times 10^{-3}$	$4{,}10 \times 10^{-4}$
20-40 %	0,170	$6{,}60 \times 10^{-3}$	$6{,}10 \times 10^{-4}$
30-50 %	0,210	$8{,}20 \times 10^{-3}$	$7{,}60 \times 10^{-4}$
40-60 %	0,266	$1{,}04 \times 10^{-2}$	$9{,}60 \times 10^{-4}$
50-70 %	0,303	$1{,}18 \times 10^{-2}$	$1{,}10 \times 10^{-3}$
60-80 %	0,104	$3{,}90 \times 10^{-3}$	$3{,}70 \times 10^{-4}$

Lampiran 13. Data Penentuan Konsentrasi Substrat Optimum

[substrat] (%)	Absorbansi	Kadar Glukosa (mg/mL)	Aktivitas Enzim (U/mL)
0,5	0,091	$3,47 \times 10^{-3}$	$3,22 \times 10^{-4}$
1	0,159	$6,16 \times 10^{-3}$	$5,70 \times 10^{-4}$
1,5	0,173	$6,71 \times 10^{-3}$	$6,21 \times 10^{-4}$
2	0,205	$7,97 \times 10^{-3}$	$7,38 \times 10^{-4}$
2,5	0,203	$7,89 \times 10^{-3}$	$7,31 \times 10^{-4}$
3	0,200	$7,77 \times 10^{-4}$	$7,20 \times 10^{-4}$

Lampiran 14. Data Penentuan pH Optimum

pH	Absorbansi	Kadar Glukosa (mg/mL)	Aktivitas Enzim (U/mL)
5	0,032	$1,14 \times 10^{-3}$	$1,06 \times 10^{-4}$
5,2	0,102	$3,91 \times 10^{-3}$	$3,62 \times 10^{-4}$
5,4	0,118	$4,54 \times 10^{-3}$	$4,20 \times 10^{-4}$
5,6	0,076	$2,88 \times 10^{-3}$	$2,67 \times 10^{-4}$
5,8	0,063	$2,37 \times 10^{-3}$	$2,19 \times 10^{-4}$
6	0,034	$1,22 \times 10^{-3}$	$1,13 \times 10^{-4}$

Lampiran 15. Data Penentuan Suhu Optimum

Suhu (°C)	Absorbansi	Kadar Glukosa (mg/mL)	Aktivitas Enzim (U/mL)
30	0,039	$1,42 \times 10^{-3}$	$1,32 \times 10^{-4}$
35	0,105	$4,03 \times 10^{-3}$	$3,73 \times 10^{-4}$
40	0,110	$4,22 \times 10^{-3}$	$3,91 \times 10^{-4}$
45	0,071	$2,68 \times 10^{-3}$	$2,48 \times 10^{-4}$
50	0,046	$1,69 \times 10^{-3}$	$1,57 \times 10^{-4}$

Lampiran 16. Data penentuan pengaruh konsentrasi enzim terhadap aktivitas enzim

[Enzim] (mg/mL)	Absorbansi	Kadar Glukosa (mg/mL)	Aktivitas enzim (U/mL)
7,2	0,089	$3,39 \times 10^{-3}$	$3,14 \times 10^{-4}$
8	0,196	$7,62 \times 10^{-3}$	$7,05 \times 10^{-4}$
9,6	0,309	$1,21 \times 10^{-2}$	$1,12 \times 10^{-3}$
10,8	0,418	$1,64 \times 10^{-2}$	$1,52 \times 10^{-3}$
12	0,52	$2,04 \times 10^{-2}$	$1,89 \times 10^{-3}$
13,2	0,515	$2,02 \times 10^{-2}$	$1,88 \times 10^{-3}$
14,4	0,5	$1,96 \times 10^{-2}$	$1,82 \times 10^{-3}$

Lampiran 17. Data penentuan pengaruh logam terhadap aktivitas enzim

Logam	Absorbansi	Kadar Glukosa (mg/mL)	Aktivitas Enzim (U/mL)	Aktivitas Relatif (%)
Kontrol	0,183	$7{,}10 \times 10^{-3}$	$6{,}58 \times 10^{-4}$	100
Mg	0,155	$5{,}99 \times 10^{-3}$	$5{,}55 \times 10^{-4}$	84,44
Co	0,198	$7{,}69 \times 10^{-3}$	$7{,}13 \times 10^{-4}$	108,33
Ca	0,138	$5{,}33 \times 10^{-3}$	$4{,}93 \times 10^{-4}$	75
Cu	0,086	$3{,}28 \times 10^{-3}$	$3{,}03 \times 10^{-4}$	46,11
Zn	0,092	$3{,}51 \times 10^{-3}$	$3{,}25 \times 10^{-4}$	49,44

Lampiran 18. Data Aktivitas Spesifik Enzim Selulase pada Kondisi Optimum

Kondisi Optimum:[substrat]=2 %; suhu=40 °C; pH=5,4; [enzim]= 12 mg/mL

[Glukosa] (mg/mL)	Aktifitas Enzim (U/mL)	Kadar Protein (mg/mL)	Faktor Pengenceran (fp)	Kadar Protein x fp	Aktivitas Spesifik
0,0204	$1,89 \times 10^{-3}$	0,23	50	11,65	$1,62 \times 10^{-4}$

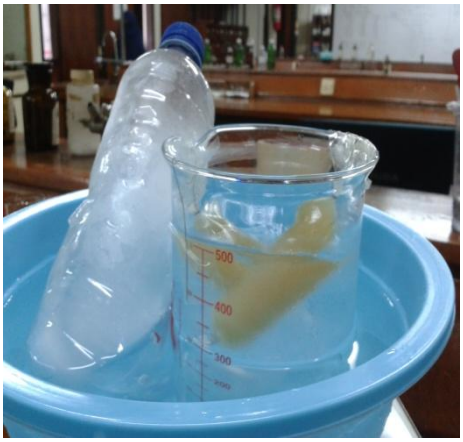
Lampiran 20. Foto Penelitian

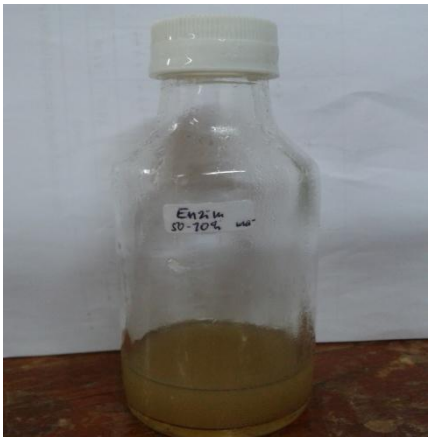
Preparasi kertas koran sebagai substrat

Proses isolasi enzim selulase dari kerang kepah

Fraksinasi dengan amonium sulfat

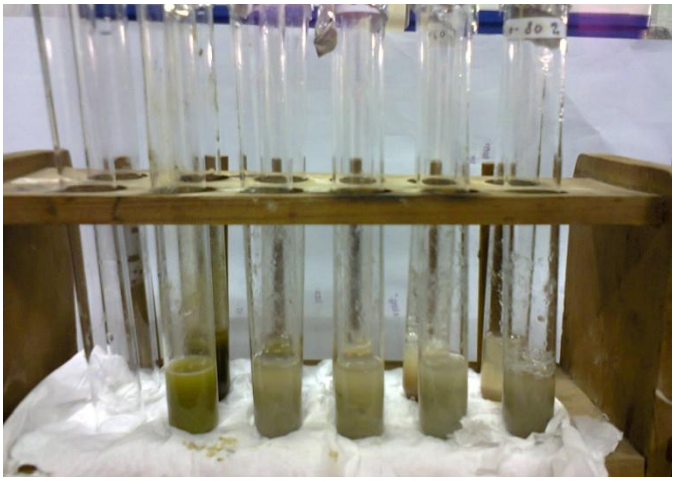
Proses Dialisis





Penentuan Aktivitas Enzim

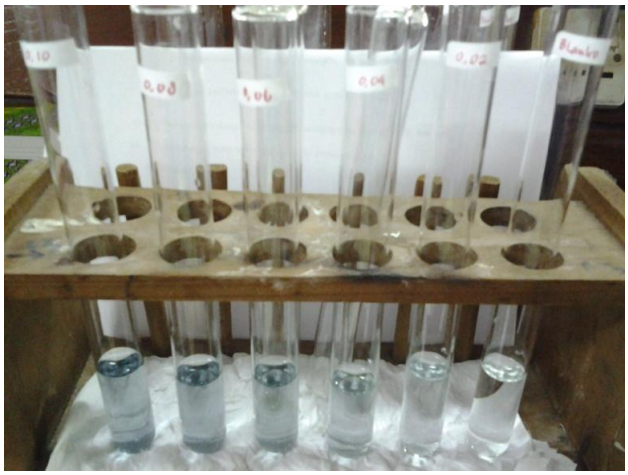
Larutan standar



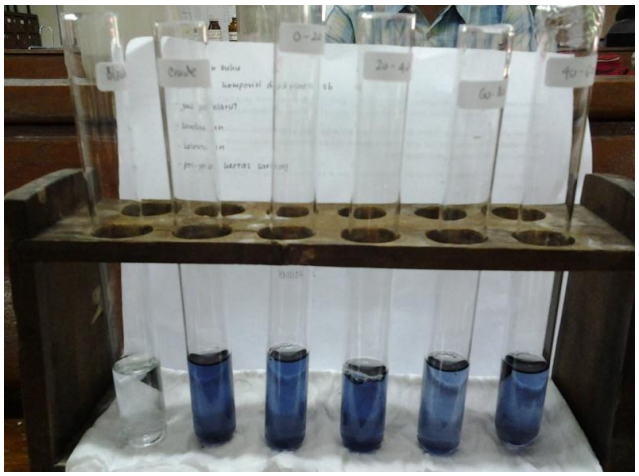
Proses hidrolisis selulosa (enzim selulase + substrat kertas + buffer)

Penentuan kadar glukosa (hasil hidrolisis + reagen Nelson + arsenomolibdar + H_2O)

Penentuan kadar protein



Larutan standar



Sampel

Pengukuran absorbansi dengan spektronik 20 D^+