

HYDROLASES (pancreatic amylase, pancreatic lipase)

Experiment 1

The aim of the task is to determine α -amylase activity in the pancreatic extract.

Protocol

Add 5 cm³ of 0.5% starch solution and 1 cm³ of pancreatic extract to the test tube using a pipette (to obtain a working solution). Put the probe in water bath at 37°C.

After the time periods specified in the table follow analysis and note results:

- a. Fehling's test for reducing sugars. For this purpose mix 0.5 cm³ Fehling I and Fehling II solution and 0.5 cm³ of working solution. Carefully heat working solution over the burner for a few minutes.
- b. 0.001 mol/dm³ J₂ in potassium iodide (KJ) to detect the presence of a starch. For this purpose pour 10 drops of J₂ in KJ into the 0.5 cm³ of working solution.

Number of probe	Incubation period [min]	Test for reducing sugars	Iodine test
1	0		
2	5		
3	10		
4	20		
5	30		



Experiment 2

The aim of the task is to determine lipase activity in the pancreatic extract.

Protocol

Add 1 cm³ of 0.1 mol/dm³ Na₂CO₃ to the 15 cm³ of oil and shake it vigorously to obtain oil-in-water emulsion. Place the appropriate amounts of pancreatic extract, water, oil-in-water extract and bile to the 3 test tubes, according to the table.

Number of probe	Pancreatic extract [cm ³]	Water [cm ³]	Oil-in water emulsion [cm ³]	Bile [cm ³]	0.05 mol/dm ³ NaOH [cm ³]
1	2	1	3	0	
2	2	0.75	3	0.25	
3	-	2.75	3	0.25	

Probe 1 - probe „0“, probe 3 - control probe relative to probe 2.

Incubate all test tubes for 60 min (37°C). During incubation shake the tubes every 5 minutes. After finishing the incubation pour solutions from the tubes into the small Erlenmeyer flask. Add 3 drops of phenolphthalein to each flask and titrate with 0,05 mol/dm³ NaOH until the permanent pink color (fuchsia).

Enter the results in the table. Explain.

