Isolation of invertase enzyme from Saccharomyces cerevisiae and calculation of enzyme activity

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Background

- Invertase is an important enzyme that has many applications in the food industry, including the production of high-fructose corn syrup and other sweeteners.
- The isolation and purification of invertase from Saccharomyces cerevisiae is an important step in the production of this enzyme at an industrial scale.
- The activity of invertase can be measured by its ability to break down sucrose into glucose and fructose, and this activity can be quantified using a spectrophotometer and a colorimetric assay.
- The standard curve generated in this experiment can be used to determine the concentration of glucose in the samples, which can then be used to calculate the activity of the invertase enzyme.

Procedure: Isolation of Invertase

The yeast extract solution was centrifuged at 7500rpm for 2 minutes at 4°C.

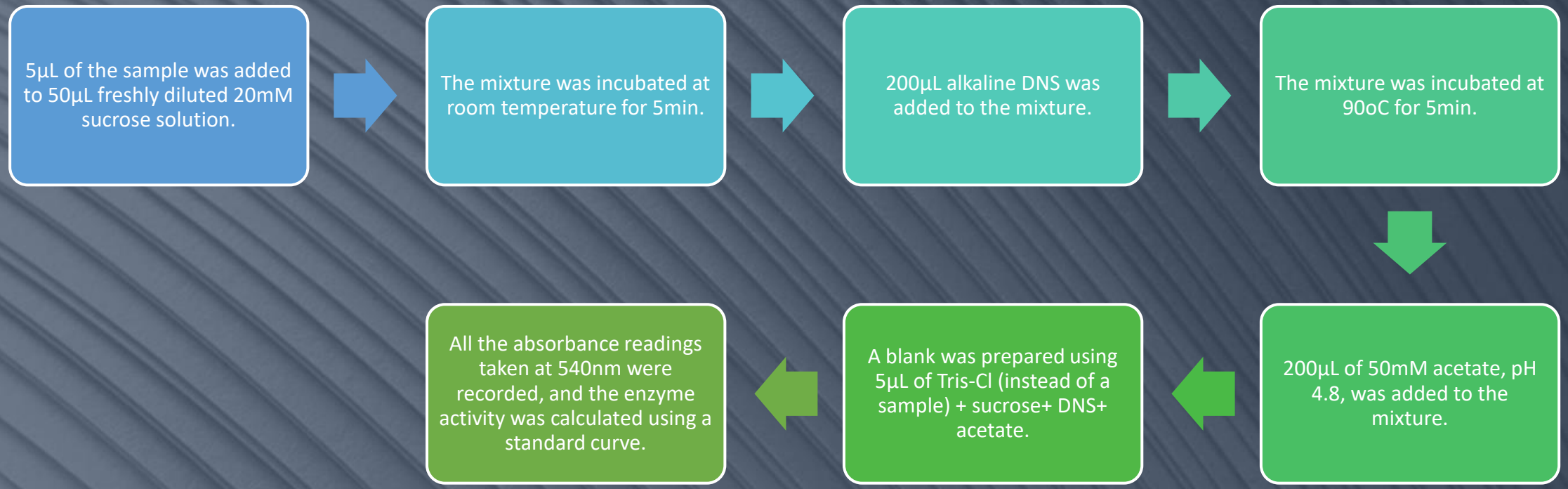
Ice-cold ethanol was added to the supernatant to obtain a final concentration of 29%, followed by inversion, and centrifugation at 10000rpm for 10 minutes at 4°C.

The supernatant was transferred to another tube, and the ethanol was completely removed. The pellet was resuspended in 600µL of 5mM Tris-Cl, pH 7.4.

Ice-cold ethanol was added to the supernatant to obtain a final concentration of 40%. The mixture was inverted, placed on ice for 2 minutes, and centrifuged at 10000rpm for 10 minutes at 4°C.

The activity of the five suspensions obtained after centrifugation was tested.

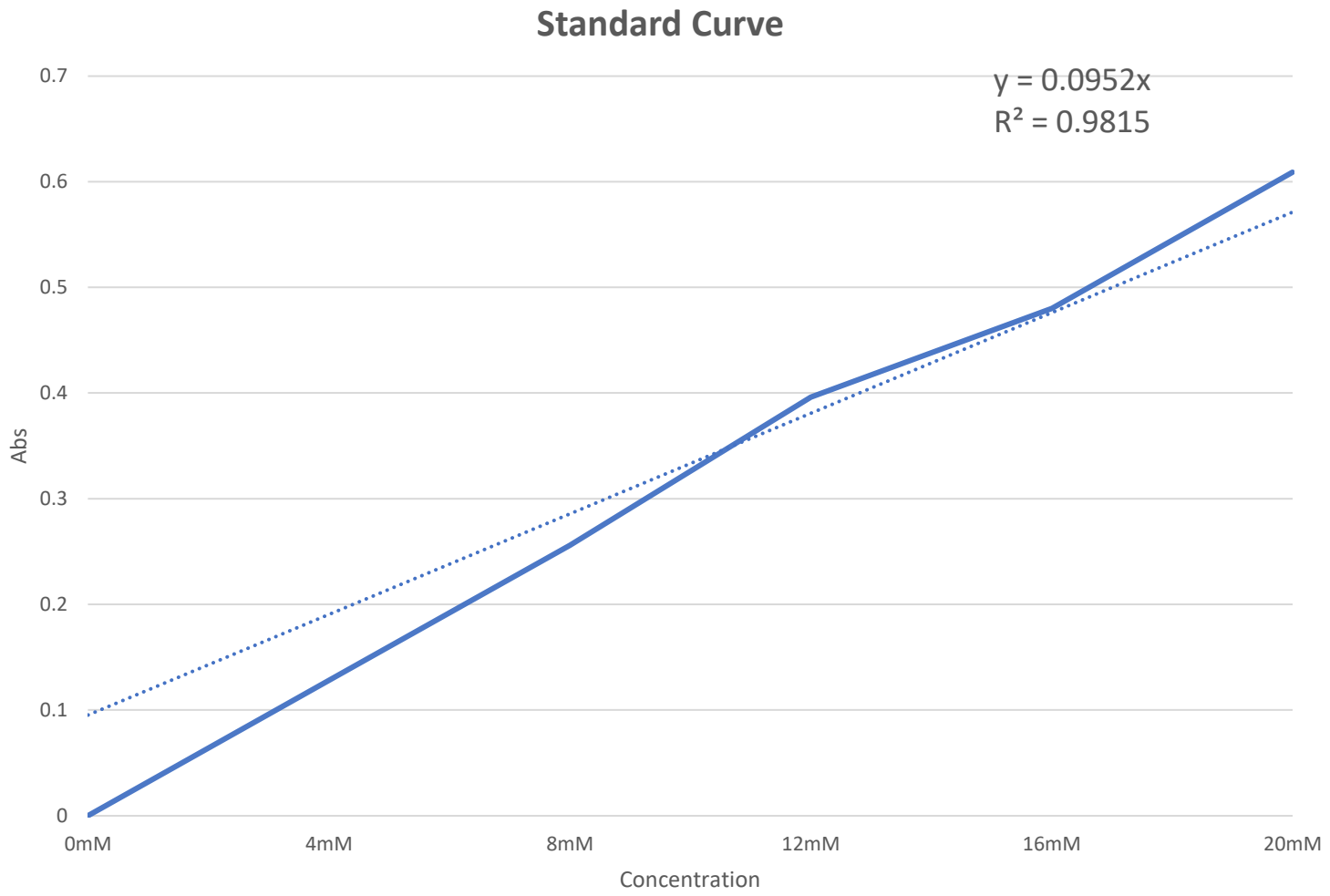
Procedure: Activity testing



Observation

Std curve readings					
Blank	**4mM**	**8mM**	**12mM**	**16mM**	**20mM**
0	0.128	0.256	0.396	0.48	0.609
Sample readings					
S0	**S1**	**S2**	**S3**	**S4**	**S5**
0.000	0.042	0.231	0.085	0.266	0.272

Standard Curve



Calculating glucose concentrations

Sample	Abs	Concentration
S0	0	0.000
S1	0.042	4.412
S2	0.231	24.265
S3	0.085	8.929
S4	0.266	27.941
S5	0.272	28.571

- Concentration = (Abs*Dilution factor)/0.0952
- Since y = 0.0952 and Dilution factor = 10

Enzyme activity calculation

Sample	Abs	Conc	ΔC = (20 – Conc)	Activity
S0	0	NA		
S1	0.042	4.412	15.588	3.118
S2	0.231	24.265	-4.265	-0.853
S3	0.085	8.929	11.071	2.214
S4	0.266	27.941	-7.941	-1.588
S5	0.272	28.571	-8.571	-1.714
			AVG =	0.235

Result

Enzyme activity of Invertase

= 0.235*1000

= 235 U/mL

Thank you