Questions Lab 4: Enzymes and Fermentation

Utsav Acharya

Dr. Christina Minassian, BIO 1120L, Section 03

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Table 4.1: Fermentation of compounds by yeast cultures

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Test Tube | | Gas column Height  (measured at 10-minute intervals) | | | | | | |
| Number | Contents | 0 | 10 | 20 | 30 | 40 | 50 | 60 |
| 1 | Boiled yeast, glucose | 0 | 1.4 | 1.4 | 1.5 | 1.5 | 1.5 | 1.5 |
| 2 | Active yeast,  Glucose | 0 | 3.2 | 3.5 | 4.2 | 4.8 | 5 | 5.5 |
| 3 | Active Yeast,  galactose | 0 | 2.9 | 3.2 | 3.2 | 3.4 | 3.5 | 3.7 |
| 4 | Active yeast, sucrose | 0 | 1.5 | 2.2 | 3.1 | 3.5 | 3.9 | 4.5 |
| 5 | Active yeast, Swerve | 0 | 2.7 | 3 | 3.5 | 4 | 4.5 | 5 |

Key Findings:

* Glucose (Test Tube 2) showed the highest rate of fermentation, producing 5.5 mm of gas by the end of 60 minutes. This indicates that glucose is efficiently metabolized by yeast.
* Galactose (Test Tube 3) produced slightly less gas (3.7 mm), suggesting that yeast ferments galactose but at a slower rate than glucose.
* Sucrose (Test Tube 4) had a moderate fermentation rate (4.5 mm), likely because yeast first hydrolyzes sucrose into glucose and fructose before fermentation.
* Swerve (Test Tube 5), a sugar substitute, exhibited significant fermentation (5.0 mm), suggesting it contains fermentable components.
* Boiled yeast (Test Tube 1) had minimal fermentation (1.5 mm), confirming that heat denatures yeast enzymes, inhibiting fermentation.

Table 4.2: Result of Yeast catalase experiment

|  |  |  |  |
| --- | --- | --- | --- |
| Tube | pH | Temperature  (degree Celsius) | Height of Foam Produced(cm) |
| 1 | 3 | 5 | 2.2 |
| 2 | 3 | 22 | 1.4 |
| 3 | 3 | 85 | 1.4 |
| 4 | 7 | 5 | 2.0 |
| 5 | 7 | 22 | 2.7 |
| 6 | 7 | 85 | 3.5 |
| 7 | 10 | 5 | 1.9 |
| 8 | 10 | 22 | 2.1 |
| 9 | 10 | 85 | 1.3 |

Key Findings:

* The highest catalase activity was observed at pH 7 and 85°C (3.5 cm foam, Tube 6), indicating that neutral pH and higher temperatures enhance enzyme efficiency.
* At low pH (3), enzyme activity was reduced, with the lowest foam height (1.4 cm in Tubes 2 and 3), suggesting that acidic conditions partially denature catalase.
* At high pH (10), enzyme activity was moderate, with a foam height of 2.1 cm at 22°C (Tube 8) but lower activity at extreme temperatures (1.3 cm at 85°C in Tube 9).
* Temperature also played a significant role—enzyme activity increased with temperature up to 85°C at neutral pH but decreased under acidic or highly basic conditions.

1. What creates the foaming in the catalase reaction?

The foaming in the catalase reaction is caused by the breakdown of hydrogen peroxide (H₂O₂) by the enzyme catalase, which produces oxygen gas (O₂) and water (H₂O). The oxygen gas forms bubbles, which get trapped in the liquid, creating foam.

Reaction:

2H2​O2​→2H2​O+O2​(gas)

The breakdown of hydrogen peroxide into oxygen and water creates foam. The oxygen gas forms bubbles, causing the foaming effect.

2. What is the optimal pH for catalase activity? What result do you predict if the enzyme was tested at pH 6 or 8?

Based on Table 4.2, the highest foam production occurred at pH 7 and 85°C (3.5 cm foam height), suggesting that catalase works best at a neutral pH.

Prediction:

pH 6 or 8: The enzyme would still function but with slightly reduced activity, producing less foam than at pH 7. Catalase tends to work well in a neutral to slightly acidic/basic range.

3. What is the optimal temperature for catalase activity, and how does it compare with the temperature at which the enzyme normally acts?

From Table 4.2, catalase activity was highest at 85°C (3.5 cm foam). However, this result is unusual, as catalase generally functions best at body temperature (~37°C).

* At low temperatures (5°C): Catalase activity was lower (2.2 cm foam at pH 3), indicating that the reaction slowed down.
* At high temperatures (85°C): Catalase may start to denature, but in this experiment, it still produced foam.
* Comparison: Normally, catalase functions best at body temperature (~37°C). Higher temperatures (above 60°C) typically denature enzymes, reducing activity.

4. What happens to catalase at low and high temperatures? How does that affect reaction speed?

* Low temperatures (5°C): The enzyme activity slows down because molecules have less kinetic energy, leading to fewer enzyme-substrate interactions.
* High temperatures (85°C): Enzymes typically start denaturing (losing shape), reducing activity. However, in this experiment, catalase still produced foam, indicating some stability at high heat.

Reaction Speed Effects:

At low temperatures → Slower reaction, less foam

At optimal temperature (~37°C) → Fastest reaction

At high temperatures → Denaturation reduces activity over time

5. What variables other than temperature and pH might alter the activity of catalase?

* Substrate concentration (H₂O₂ levels): More hydrogen peroxide = more reaction, up to saturation.
* Enzyme concentration: More catalase = faster reaction, up to a limit.
* Presence of inhibitors: Certain chemicals can block catalase function.
* Salt concentration: Too much or too little salt can affect enzyme shape and function.

6. How different were the hydrogen ion concentrations of the buffers you used?

Hydrogen ion concentration varies with pH, following the equation:

[H⁺]=10-pH

* **pH 3:** 10-3 M
* **pH 7:** 10-7 M
* **pH 10:** 10-10M

This means that pH 3 has 10,000 times more H⁺ ions than pH 7, and pH 10 has 1,000 times fewer H⁺ ions than pH 7, explaining why extreme pH levels affect catalase activity.

7. Which of the four compounds tested gave living yeast cells the most energy during fermentation, and why?

Glucose, as it had the highest gas production, indicates rapid yeast metabolism.

From Table 4.1, the highest gas column height at 60 minutes was:

* Active yeast + glucose → 5.5 cm gas (highest)
* Active yeast + Swerve™ → 5.0 cm gas
* Active yeast + sucrose → 4.5 cm gas
* Active yeast + galactose → 3.7 cm gas
* Boiled yeast + glucose → 1.5 cm gas (inactive yeast)

Conclusion:

* Glucose produced the most gas, meaning it provided the most energy.
* Swerve™ was slightly lower, likely because its sugar alcohols are partially fermentable.
* Sucrose (a disaccharide) had to be broken down first, slightly reducing efficiency.
* Galactose resulted in the least fermentation, as yeast prefers glucose.

8. How confident are you in the results of your experiments, and what factors may have led to errors?

Confidence is moderate, but some possible errors include:

* Measurement errors: Foam height might not have been recorded precisely.
* Temperature fluctuations: If incubation was inconsistent, results could vary.
* pH inconsistencies: Buffers might not have been perfectly mixed.
* Human error: Pipetting mistakes or incorrect timing could alter data.
* Enzyme denaturation: If catalase was left too long at 85°C, results may be inaccurate.

The results align with expected enzymatic behavior, but minor errors could arise from measurement inconsistencies or contamination.