

Lab Report: Lac Operon Virtual Lab

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Materials & Methods (refer to the Student's Manual)

Qualitative Procedure (refer to the Student's Manual)

Quantitative Procedure (refer to the Student's Manual)

Results & Discussion

10 minutes after the reaction began, all three culture tubes were consistently clear with no visible change. 20 minutes after the reaction began, the glucose tube was clear, the lactose tube was yellowish, and the tube containing both glucose and lactose was very faintly yellow. Up until 60 minutes after the reaction began, the lactose tube was the only one that turned distinctly yellow, while the glucose tube remained clear and without color, and the tube with both lactose and glucose turned faintly yellow.

It is clear that the *E. coli* in the lactose tube produced the most β -galactosidase because its color is the deepest shade of yellow out of the three tubes, and more β -galactosidase activity means more clear ONPG is cleaved and turned into yellow ONP.

In comparison to the other two tubes, the tube with both lactose and glucose contained more β -galactosidase than the glucose tube, but less β -galactosidase than the lactose tube.

$$1 \text{ Miller unit} = 1000 \times \frac{(A_{420} - (1.75 \times A_{550}))}{t \times v \times A_{600}}$$

A_{420} = the absorbance of the yellow ONP.

A_{550} = a correction factor to take into account that there are bacterial cells in the assay that will scatter light.

t = the reaction time (from the start of the assay until the measurements are made).

v = volume (in mL) of the culture used in the assay. (In this case, v equals 2.4 mL.)

A_{600} = the optical density (OD) of the original culture.

The equation above was used to calculate the level of β -galactosidase enzyme activity present in each of the three cultures in Miller units. The results show that the lactose tube contained the most activity after 60 minutes, with ~112.8 units of activity, while the mixed tube contained ~36.5 units and the glucose tube contained ~1.7 units (Table 1). The results can be seen in Table 1 and Figure 1.

The A_{420} absorbance values of ONP, or the degree of yellow the tubes turned, were also measured. The results also showed that the lactose tube had the overall highest level of A_{420} absorbance, with an optical density of 7.5 after 60 minutes, while the mixed tube measured 4.2 and the glucose tube measured 2.7 (Table 2). These results mean that the lactose tube was the most yellow, the lactose/glucose tube was the

second-most yellow, and the glucose tube was the least yellow. The results can be seen in Table 2 and Figure 2.

Conclusion

As the measurements indicate, the lactose bottle had the most β -galactosidase activity and turned the most yellow due to the change from ONPG to ONP after the β -galactosidase cleaving. Because the other two bottles had glucose, they had significantly less β -galactosidase activity. Therefore, the *lac* operon only produces β -galactosidase when there is only lactose and no glucose present as a food source for the *E. coli* cell, a prime example of how gene regulation and thus energy conservation through enzyme necessity is used in *E. coli*. When glucose is present in the cell, enzymes that break down lactose are not needed and therefore are not produced to save energy; this is a strategy crucial to minimizing the amount of energy an organism expends every day (and therefore the amount of food it needs to consume).

| Time from Start of Reaction | Glucose | Glucose and Lactose | Lactose |
|-----------------------------|---------|---------------------|--------------|
| 10 minutes | Clear | Clear | Clear |
| 20 minutes | Clear | Very faint yellow | Faint yellow |
| 30 minutes | Clear | Very faint yellow | Yellow |
| 40 minutes | Clear | Very faint yellow | Yellow |
| 50 minutes | Clear | Very faint yellow | Yellow |
| 60 minutes | Clear | Very faint yellow | Yellow |

Table 1: Table of Qualitative Results of *E. Coli* Culture Color.

| Time from Start of Reaction | Glucose (in Miller Units) | Glucose and Lactose (in Miller units) | Lactose (in Miller units) |
|-----------------------------|---------------------------|---------------------------------------|---------------------------|
| 10 minutes | 2.8 | 41.7 | 111.1 |
| 20 minutes | 1.7 | 43.4 | 119.8 |
| 30 minutes | 1.2 | 35.9 | 123.8 |
| 40 minutes | 0.9 | 39.1 | 125.9 |
| 50 minutes | 2.8 | 38.9 | 116.7 |
| 60 minutes | 1.7 | 36.5 | 112.8 |

Table 2: Table of Quantitative Results of *E. Coli* Culture Color.

| Time from Start of Reaction | Glucose $A_{600} = 0.3$ | Glucose and Lactose $A_{600} = 0.3$ | Lactose $A_{600} = 0.3$ |
|-----------------------------|------------------------------------|--|------------------------------------|
| 10 minutes | $A_{420} = 0.7$ $A_{550} = 0.4$ | $A_{420} = 1.0$ $A_{550} = 0.4$ | $A_{420} = 1.5$ $A_{550} = 0.4$ |
| 20 minutes | $A_{420} = 0.9$ $A_{550} = 0.5$ | $A_{420} = 1.5$ $A_{550} = 0.5$ | $A_{420} = 2.6$ $A_{550} = 0.5$ |
| 30 minutes | $A_{420} = 1.3$ $A_{550} = 0.7$ | $A_{420} = 2.0$ $A_{550} = 0.7$ | $A_{420} = 3.9$ $A_{550} = 0.7$ |
| 40 minutes | $A_{420} = 1.6$ $A_{550} = 0.9$ | $A_{420} = 2.7$ $A_{550} = 0.9$ | $A_{420} = 5.2$ $A_{550} = 0.9$ |
| 50 minutes | $A_{420} = 2.2$ $A_{550} = 1.2$ | $A_{420} = 3.5$ $A_{550} = 1.2$ | $A_{420} = 6.3$ $A_{550} = 1.2$ |
| 60 minutes | $A_{420} = 2.7$ $A_{550} = 1.5$ | $A_{420} = 4.2$ $A_{550} = 1.5$ | $A_{420} = 7.5$ $A_{550} = 1.5$ |

Table 3: A_{420} and A_{550} Values of *E. Coli* Culture In Glucose, Lactose, and Both Glucose and Lactose.

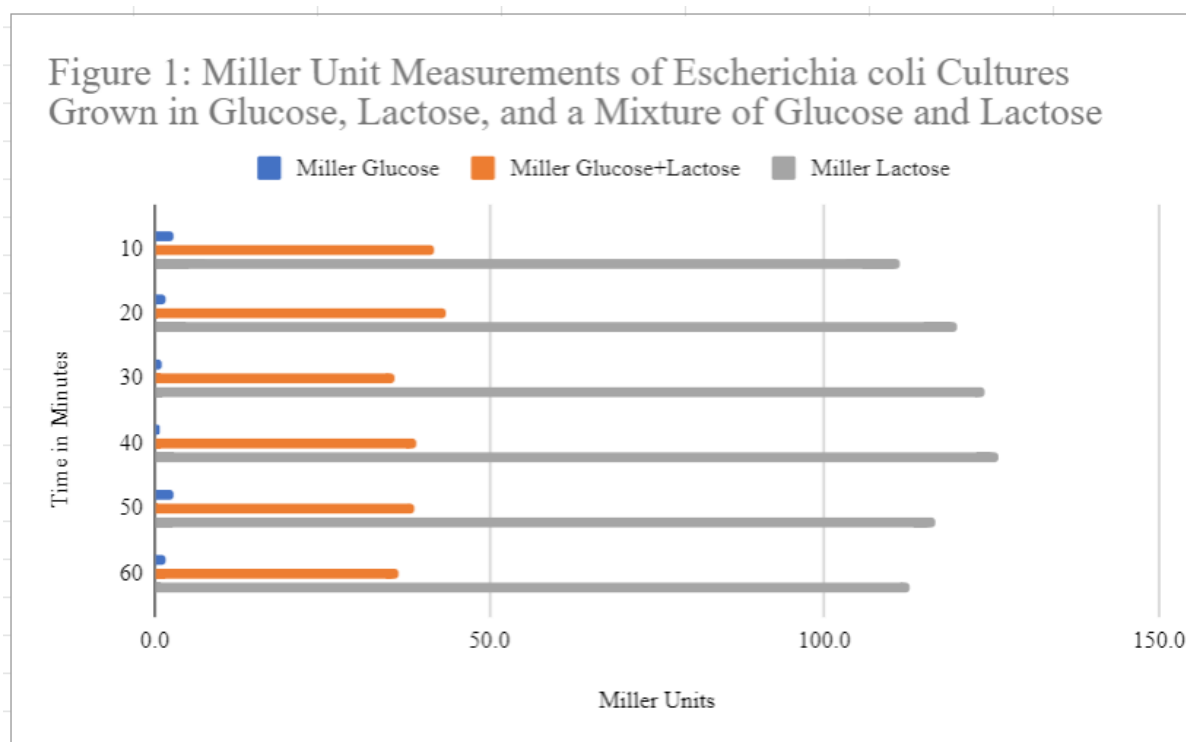


Figure 1. Miller Unit Measurements of Escherichia coli Cultures Grown in Glucose, Lactose, and a Mixture of Glucose and Lactose.

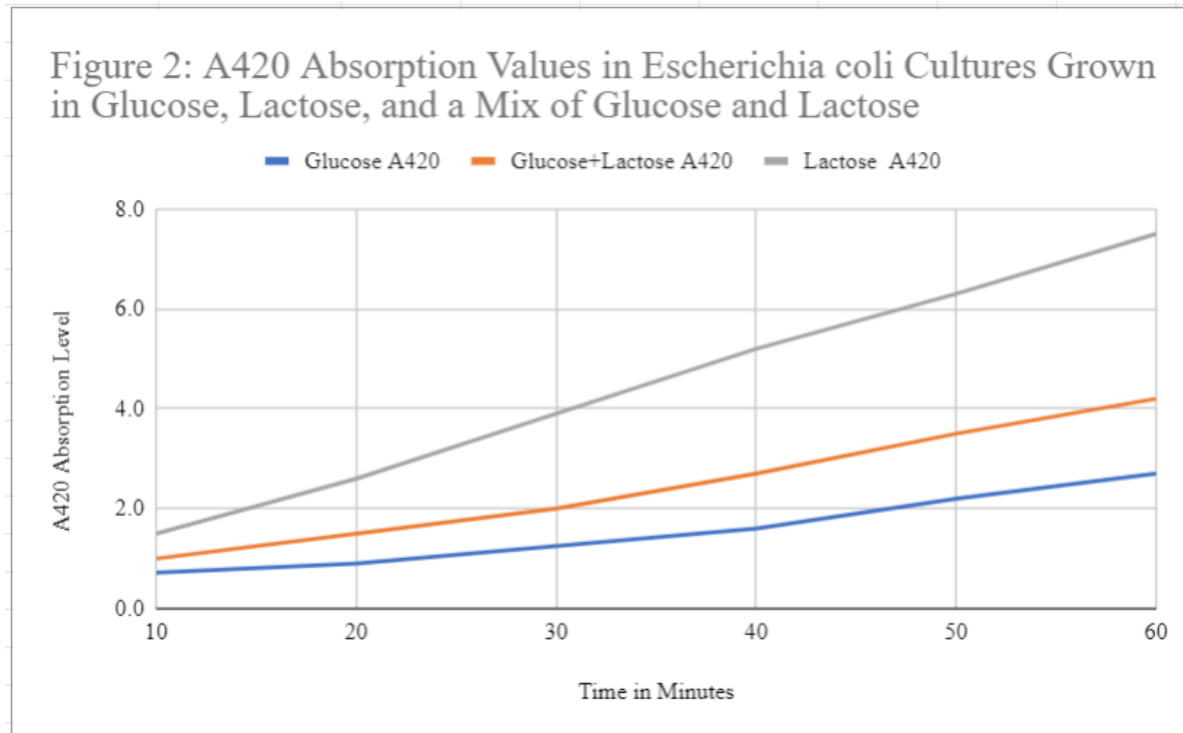


Figure 2. A420 Absorption Values in Escherichia coli Cultures Grown in Glucose, Lactose, and a Mix of Glucose and Lactose.