Lac Operon Lab Report

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Materials & Methods (refers to the Student's Manual)
Quantitative Procedure (refers to the Student's Manual)
Qualitative Procedure (refers to the Student's Manual)

Results & Discussion

The qualitative process assessed the color change of the 3 cultures as ***β***-galactosidase activity increased. All 3 cultures were clear after 10 minutes. During the entire reaction the glucose culture did not change color and was clear. The culture with glucose and lactose turned a very faint yellow color after 20 minutes. After 20 minutes the culture with only lactose was a faint yellow. After 30-60 minutes, the culture with only lactose became a brighter yellow. The specific results at each 10-minute interval are displayed in Table 1.

Table 1. ***Color Change of Cultures Grown with Glucose, Lactose, and both Glucose and Lactose***

Time from Start of Reaction	Glucose	Glucose + 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 shows the observed colors of cultures grown in glucose, lactose, and both glucose and lactose at each 10-minute time interval for 60 minutes.

β-galactosidase separates ONPG, which is clear, from its galactose component to create yellow ONP color. A darker yellow color in the cultures indicates higher production of ONP, and thus higher ***β***-galactosidase activity. So, the difference in colors in Table 1 is because of the different ***β***-galactosidase activity. The qualitative results indicated that the culture with only lactose had the brightest yellow color and so the highest ***β***-galactosidase activity. The culture with glucose and lactose had less ***β***-galactosidase activity, and the culture with only glucose had no activity.

The quantitative process assessed the absorption of light in each culture, representing the colors observed in Table 1. Equation 1 was used to calculate the miller units, which quantified the β-galactosidase activity, of each culture.

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

A_{420} is the absorbance of the yellow ONP. A_{550} is a correction factor that takes into account that there are bacterial cells in the assay that will scatter light. t is the reaction time (from the start of the assay until the measurements are made). v is 2.4, the volume in mL of each culture used in the assay. A_{600} is the optical density (OD) of the original culture.

Table 2 shows the $A_{420,}$, A_{550} , and miller unit measurements for the three cultures used in the experiment.

Table 2. ***Miller Units,*** A_{600}, A_{420}, A_{550} ***Calculations for the 3 Cultures***

Time from Start of Reaction	**Glucose** $\mathbf{A_{600} = 0.3}$	**Glucose + Lactose** $\mathbf{A_{600} = 0.3}$	**Lactose** $\mathbf{A_{600} = 0.3}$
10 minutes	$A_{420} = 0.7$ $A_{550} = 0.4$ Miller Unit = 0.0	$A_{420} = 1.0$ $A_{550} = 0.4$ Miller Unit = 41.7	$A_{420} = 1.5$ $A_{550} = 0.4$ Miller Unit = 111.1
20 minutes	$A_{420} = 0.9$ $A_{550} = 0.5$ Miller Unit = 1.7	$A_{420} = 1.5$ $A_{550} = 0.5$ Miller Unit = 43.4	$A_{420} = 2.6$ $A_{550} = 0.5$ Miller Unit = 119.8
30 minutes	$A_{420} = 1.3$ $A_{550} = 0.7$ Miller Unit = 3.5	$A_{420} = 2$ $A_{550} = 0.7$ Miller Unit = 35.9	$A_{420} = 3.9$ $A_{550} = 0.7$ Miller Unit = 123.8
40 minutes	$A_{420} = 1.6$ $A_{550} = 0.9$ Miller Unit = 0.9	$A_{420} = 2.7$ $A_{550} = 0.9$ Miller Unit = 39.1	$A_{420} = 5.2$ $A_{550} = 0.9$ Miller Unit = 125.9
50 minutes	$A_{420} = 2.2$ $A_{550} = 1.2$ Miller Unit = 2.8	$A_{420} = 3.5$ $A_{550} = 1.2$ Miller Unit = 38.9	$A_{420} = 6.3$ $A_{550} = 1.2$ Miller Unit = 116.7
60 minutes	$A_{420} = 2.7$ $A_{550} = 1.5$ Miller Unit = 1.7	$A_{420} = 4.2$ $A_{550} = 1.5$ Miller Unit = 36.5	$A_{420} = 7.5$ $A_{550} = 1.5$ Miller Unit = 112.8

Table 2 shows the 4 measurements that were determined for each culture at each time interval. The A_{600}, A_{550}, *and* A_{420} *values were used to calculate the Miller Units using Equation (1).*

The results indicated that the culture with only lactose had the highest miller unit value, the culture with glucose and lactose had the second highest miller unit, and the culture with glucose had the least miller unit for each time interval (Table 2/Figure 1).

Figure 1. Miller Units for 3 Cultures over 60 Minutes

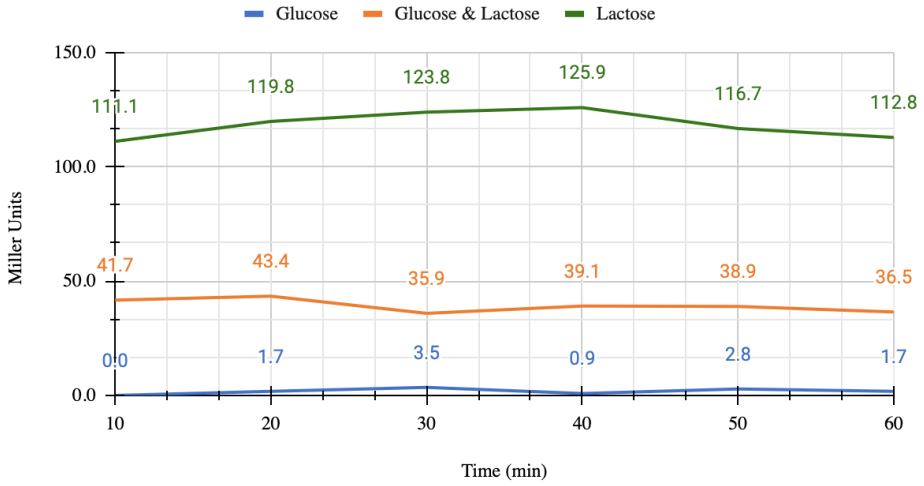

Figure 1 shows the miller units for the cultures grown in glucose, lactose, and both glucose and lactose at each time interval. The data points are the same as in the miller units written in Table 2.

A higher miller unit measurement indicates higher β-galactosidase activity. This means that the order stated above also is true for the β-galactosidase activity. As explained previously, A_{420} is the absorbance of the yellow ONP, and represents how "yellow" the cultures looked. The culture with only lactose also had the highest A_{420} value for each time interval (Table 2/Figure 2). This means that culture was also the most yellow, as confirmed in Table 1 from the qualitative results.

Figure 2. A420 Values for 3 Cultures over 60 Minutes

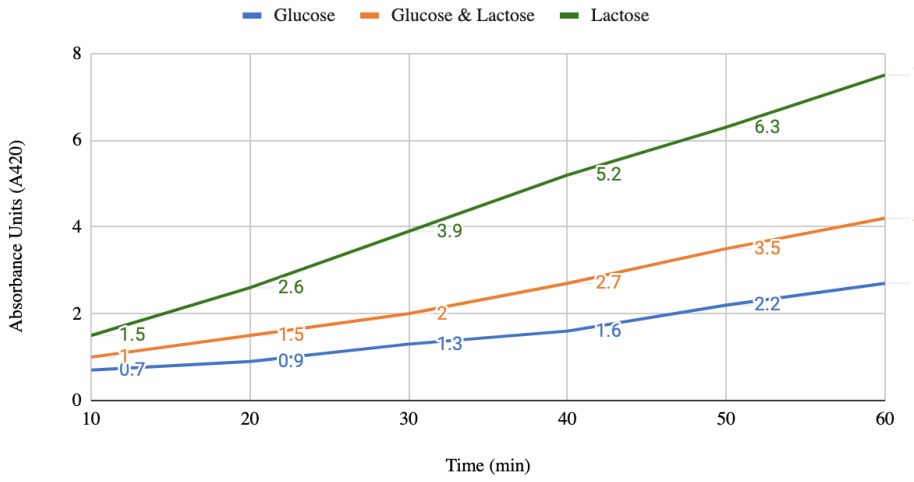

Figure 2 shows the A_{420} values for the cultures grown in glucose, lactose, and both glucose and lactose at each time interval. The data points are the same as in the A_{420} values written in Table 2.

The difference in β-galactosidase activity in the cultures as observed in the qualitative and quantitative results (Table 1, Table 2/Figure 1) can be explained by the conditional gene expression of the *lac* operon. The purpose of the *lac* operon is to metabolize lactose. LacZ is a gene in the *lac* operon, and when expressed produces the β-galactosidase enzyme. So, when lactose is not present, β-galactosidase is not produced or only produced in very low amounts. This was observed as in the culture grown with only glucose, the miller unit values were the lowest of all three cultures (Table 2/Figure 1). The bacterial cell also prefers to use glucose because it is a simpler molecule. This means that when both glucose and lactose are present in the environment, glucose is consumed first and there is less β-galactosidase activity. This was confirmed as the culture with both glucose and lactose had low miller unit values (Table 2/Figure 1). When only lactose is present, then the bacterial cell is fully dependent on lactose; so, the *lac* operon is expressed at a higher level, increasing β-galactosidase activity. This was observed in the culture with only lactose, in which the miller unit values were the highest (Table 2/Figure 1).

Conclusion

From the results of this experiment, we can conclude that the β-galactosidase enzyme is present in high amounts when there is lactose in the sample and no glucose, present in low amounts when there is both glucose and lactose present in the same, and is absent or present in very low amounts when there is no lactose and only glucose.