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Escherichia Coli cell yield in media containing different carbohydrates

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Abstract
The objective of this study was to determine and compare the cell yield of *Escherichia coli* grown in minimal medium containing different concentrations of glucose (0.025%, 0.050% and 0.075%) and lactose (0.025%, 0.050% and 0.075%). Determination of the cell yield was done by oven drying the harvested cells to obtain the dry weight and analysis of the residual sugar in the liquid medium by phenol-sulfuric acid method.
Highest initial concentration of glucose (596.667 ppm) and lactose (563.333 ppm) was recorded in minimal medium supplemented with 0.075% of the respective sugar. *E. coli* grown in 0.050% glucose (354.167 ppm) and 0.025% lactose (266.667 ppm) had the highest substrate consumed. Highest cell dry weight (µg/mL) and cell yield (µg/mL) was obtained in 0.075% glucose and 0.075% lactose. Comparing the two sugars, still, glucose had the highest cell yield (glucose 0.050% = 217.368 µg/mL and lactose 0.050% = 195.146 µg/mL; glucose 0.075% = 572.923 µg/ml and lactose 0.075% = 295.146 µg/ml). Highest $Y^{substrate}$ was also recorded in highest sugar concentration (glucose 0.075% = 2.331; lactose 0.075% = 1.638). No statistical significance was observed when $Y^{substrate}$ of different sugar concentrations were compared.

Keywords: *Escherichia coli*, sugar, yield, Ysubstrate, phenol-sulfuric acid method

Introduction
The growth rate of cells varies depending on growth conditions. Growth rate is influence by a number of factors that include substrate concentration, growth medium, temperature, pH and the supply of oxygen. As a rule of the thumb, fast growing cells contain more DNA, RNA, ribosomes, proteins, phospholipids and cell wall material, and tend to increase in size [1].
There is more than one expression being used for bacterial growth yield. Two of the most commonly used expressions are biomass yield (Y) and Y^{ATP}. Biomass yield (Y) is the biomass produced per unit of substrate consumed. This can be determined by measuring dry weight of cells or estimating their total protein content in a known volume. The amount of substrate consumed is the difference between the initial concentration and the concentration of substrate left after the growth period. Meanwhile, Y^{ATP} is defined as the biomass produced per mole of ATP. According to Bauchop and Elsden [2], the ATP production from the substrate can be calculated if the substrate and its catabolic pathway are known. Gravimetry is being utilized to estimate the amount of biomass produced. With these estimates, one can calculate the amount of biomass that is produced per mole of ATP. Yield can be expressed as mg of biomass per mg or mole of substrate consumed or mg of biomass formed per mole of ATP generated.
The objective of this study was to determine and compare the cell yield of *Escherichia coli* grown in minimal medium containing different concentrations of carbohydrates: 0.025%, 0.050% and 0.075% glucose, and 0.025%, 0.050% and 0.075% lactose.

2. Materials and Method
E. coli was grown overnight in 100 mL minimal medium containing 0.2% glucose. Ten millilitre of the culture was transferred in falcon tube for centrifugation at 10,000 rpm. The resulting pellet was washed three times in distilled water. After washing, the pellet was resuspended in distilled water. Five millilitre of the resuspended pellet was transferred in growth flasks with 100 mL minimal medium that contains varying concentrations of glucose and lactose (0.025%, 0.050% and 0.075%). The flasks were incubated on a shaker under room condition. An initial optical density (OD) reading was taken and the next reading was done after two hours. The subsequent OD readings were taken in an hourly basis.
When maximum OD reading has been attained, the cells were harvested by centrifugation at 10,000 rpm for 10 minutes. The cells were washed three times with distilled water. Three millilitre of the resuspended pellet was placed in pre-weighed small plate with filter paper. Cell wet weight was computed by deducting the weight of the plate with filter paper to the weight of the plate with filter paper and cell. The plate with cell was oven-dried at 80 °C to constant weight. The final dry weight corresponds to the cell yield.
Phenol-sulfuric acid method was employed for the determination of sugar concentration. A glucose stock solution of 1 mg/mL was made and this was used in the preparation of different concentrations of sugar solution: 2 ppm, 6 ppm, 10 ppm and 14 ppm. One millilitre of diluted sample or standard sugar solution

was pipetted into a test tube and 1 mL of 5% phenol was added. Five millilitre of concentrated H_2SO_4 was directly added in the solution. The test tubes were cooled for 10 minutes before incubating in a water bath at 30 to 37 °C for 20 minutes. The absorbance of the sample and standard sugar solution was read at 480 nm using spectrophotometer. A standard curve was prepared by plotting the absorbance against the sugar concentration of the standard solution. The sugar concentration of the sample was determined based on the equation of the line of the standard curve. $Y^{substrate}$ was computed using the formula: biomass produced over substrate consumed.

Significant difference of *E. coli* $Y^{substarte}$ in glucose and lactose across concentration was determined using One Way Analysis of Variance. Meanwhile, paired T-test was used to compare *E. coli* $Y^{substrate}$ between glucose and lactose in every concentration.

3. Results and Discussion

The maximum optical density was first achieved in minimal medium supplemented with 0.025% glucose (after 600 minutes) and 0.025% lactose (after 780 minutes). Meanwhile, 0.075% glucose attained its maximum OD reading (0.130) after 900 minutes while 0.075% lactose had its maximum absorbance (0.113) after 1,140 minutes. The highest OD reading was recorded in 0.050% lactose. Hypothetically, we can expect that higher sugar concentration will result to higher OD reading because the bacterium can have more carbon source to utilize, but, this is not the scenario that happened in the present study. Variation in the inoculum size of *E. coli* in each growth flask might be one possible reason. Another possible source of error is uneven number of bacterium introduced in each flask. Comparing the two sugar sources, *E. coli* grew more abundantly in lactose of lower concentration based on attained maximum OD reading. It is learned from the very start that glucose is the carbon source supporting the fastest growth on *E. coli* [3]. Also, glucose is the preferred sugar if bacteria are exposed to a mixture of carbon sources.

The wet weight of *E. coli* cell placed in plate with filter paper varied from 3.8840 to 2.7982 g. Carbohydrate was analyzed using the phenol-sulfuric acid method. The initial and final concentration of sugar was determined by using the equation of the line derived by plotting the absorbance against the sugar concentration of standard solution. The derived equation of the line ($y = 0.0096x - 0.0018$; $r^2 = 0.987$) was presented in Figure 1.

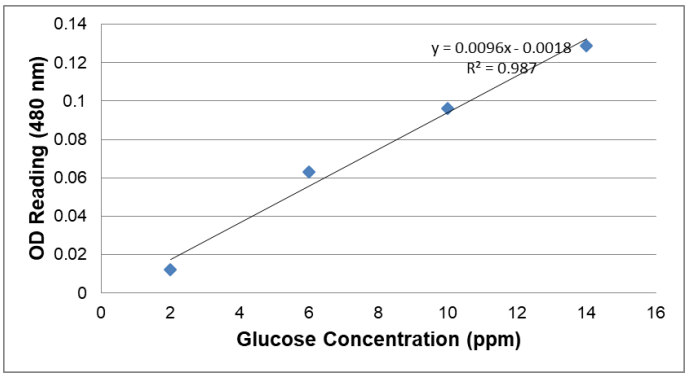

Fig 1: The prepared standard curve by plotting the absorbance against the sugar concentration of the standard solution

As expected, highest initial glucose and initial lactose concentration was recorded in 0.075% and lowest in 0.025%. Substrate consumed was computed by deducting the final sugar concentration to the initial sugar concentration. It appeared that *E. coli* grown in 0.050% glucose (354.167 ppm) and 0.025% lactose (266.667 ppm) had the highest substrate consumed (Table 1). Highest cell dry weight (µg/mL) was obtained in 0.075% glucose (577.778 µg/mL), followed by 0.075% lactose (300.000 µg/mL) (Table 1). The result in lactose was contradicting since maximum OD reading was recorded in 0.050% lactose and not in 0.075% lactose. Result of this experiment showed that, the amount of substrate consumed is not a guarantee to the final dry weight of the cell. For instance, 0.075% glucose and 0.075% lactose had the highest final cell dry weight even though they placed only second with respect on the amount of substrate consumed. Moreover, highest cell yield (µg/mL) was recorded in 0.075% glucose and 0.075% lactose. Comparing the two sugars, still glucose had the highest cell yield (0.050% glucose = 217.368 µg/mL and 0.050% lactose = 195.146 µg/mL; 0.075% glucose = 572.923 µg/mL and 0.075% lactose = 295.146 µg/mL). Highest $Y^{substrate}$ was also recorded in highest sugar concentration (0.075% glucose = 2.331, 0.050% glucose = 0.614, 0.025% glucose = 0.449; 0.075% lactose = 1.638, 0.050% lactose = 1.398, 0.025% lactose = 0.398).

Table 1: Cell yield and $Y^{substrate}$ of *E. coli* grown in minimal media containing different concentrations of carbohydrates.

Sugar Concentration (%)	Substrate Consumed (ppm)	Initial Cell Dry Weight (µg/mL)	Final Cell Dry Weight (µg/mL)	Cell Yield (µg/mL)	$Y^{substrate}$
Glucose					
0.025	236.458	4.854	111.111	106.257	0.449
0.050	354.167	4.854	222.222	217.368	0.614
0.075	245.833	4.854	577.778	572.923	2.331
Lactose					
0.025	266.667	4.854	111.111	106.257	0.398
0.050	139.583	4.854	200.000	195.146	1.398
0.075	180.208	4.854	300.000	295.146	1.638

No statistical significance was observed when *E. coli* $Y^{substrate}$ in glucose or lactose was compared across various concentrations (Table 2). Non-significant result was also obtained when *E. coli* $Y^{substrate}$ in glucose and lactose at each concentration was compared (Table 3). The non-significant result might be due to the small number of replicates used in this study.

Table 2: $Y^{substrate}$ comparison of *E. coli* across sugar concentration.

Sugar	Concentration (%)	Y substrate	Pvalue
Glucose	0.025	0.449±0.163[a]	0.073
	0.050	0.614±0.288[a]	
	0.075	2.331±1.494[a]	
Lactose	0.025	0.398±0.315[a]	0.163
	0.050	1.398±0.861[a]	
	0.075	1.638±0.848[a]	

Table 3: $Y^{substrate}$ comparison between *E. coli* grown in glucose and lactose in every sugar concentration.

Concentration (%)	Glucose	Lactose	Pvalue
0.025	0.449±0.163[a]	0.398±0.315[a]	0.820
0.050	0.614±0.288[a]	1.398±0.861[a]	0.352
0.075	2.331±1.494[a]	1.638±0.848[a]	0.646

4. Conclusion

$Y^{subtrate}$ of *E. coli* was influenced by the concentration of sugar used in the study. Increased sugar concentration has resulted to higher $Y^{substrate}$. Still, *E. coli* grew more prolific in glucose than in lactose as depicted in the value of $Y^{substrate}$.

5. References

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