Investigating the Effects of Dextrose on Acetate Secretion by *Escherichia coli*

Introduction

Background

As a bacterium commonly found in the mammalian gut, *Escherichia coli* serves various functions to maintain human health. The bacterium produces various nutrients such as vitamin K and vitamin B12 which are used by their host. In addition, *E. coli* consumes oxygen through aerobic respiration which aides in the growth of other bacterium in the gut, many of which are obligate anaerobes. However, one of the most important functions of *E. coli* is to kill potentially pathogenic bacteria by outcompeting them, and thus, depriving them of nutrients (Blount, 2015).

Vibrio cholerae is a pathogen transmitted through contained water and is the cause of cholera (Rebaudet et al., 2013). Upon infection, the host will exhibit diarrhea of varying severity. Although not common in the United States, cholera pandemics routinely occur in developing countries in Asia, Africa, and Latin America. It is in these countries that treatment is frequently lacking or much too costly, leading to death from dehydration, especially for children under 5 years of age (Harris et al., 2012).

Recent studies have found that *E. coli* grown in the presence of glucose can actively protect against *V. cholerae* infection by reducing intestinal pH through the production of acetate (Anhê et al., 2020). As someone who is interested about sustainable development, particularly in developing areas, I was intrigued by this potential solution to cholera pandemics. Compared to traditional cholera treatments which are difficult to obtain and present a financial burden to many households in developing nations (Sarker et al., 2013), glucose-based oral solutions may be much more accessible. On the other hand, increase blood glucose can also lead to a variety of problems such as a compromised gut barrier which would increase the risk of infection from other pathogens (Anhê et al., 2020). Thus, I decided that I wanted to investigate the effect of glucose concentration on acetate production in *E. coli* in order to find the optimal balance to minimize glucose input while maximizing acetate output.

Since I wanted to isolate the effect of glucose on acetate production, I chose to grow my *E. coli* on M9 mineral medium as opposed to the more nutritionally complete Luria broth. In addition, as a coupled enzyme assay would have been needed to measure acetate production (Sigma Aldrich 2014), I instead chose to measure the pH change of the broth which was much more accessible option for me. Lastly, I chose to substitute glucose for dextrose within my experiment as the two compounds are chemically identical, but dextrose was available to me while glucose was not.

Research Question

What is the effect of differing w/v concentrations of dextrose solution (0%, 5%, 10%, 15%, 20%) on the secretion of acetate by *Escherichia coli* grown in M9 mineral medium as measured through change in pH?

Aim

The aim of this investigation is to determine the relationship between the concentration of dextrose solution (0%, 5%, 10%, 15%, 20%) and the amount of acetate secreted by *Escherichia coli* grown in M9 mineral medium by measuring the change in pH of the medium.

Hypothesis

As the concentration of the dextrose solution increases, the amount of acetate secreted will also increase as more and more acetate is produced through the Pta-AckA pathway (Enjalbert et al., 2017). This will cause the pH to decrease as, being an organic acid (Fuentes et al., 2013), acetate accumulation in the medium will only lead to a lower pH. However, the change in pH of the medium overall will increase as the difference between the starting and ending pH of the medium will increase.

Variables

Controlled Variables

Variable	Method of Control	Justification
Bacteria type	All *E. coli* were swabbed from the same parent sample I grew on a Luria Agar plate inoculated from a slant.	Considering the high replication rate of bacteria, *E. coli* obtained from different sources and grown in different conditions may be genetically different enough to affect the results of the experiment. Although obtaining the bacterium from the same source and growing in the same conditions will not prevent mutations, it will minimize the discrepancy between bacteria.
Growth tube size and sterility	All tubes used to grow the *E. coli* were sterile Falcon tubes obtained from the same package.	The amount of oxygen (Goldberg et al., 1994) and the presence of other organisms (Jang et al., 2017) all affect the growth of *E. coli* which would affect the amount of acetate produced in total.
M9 mineral medium composition and volume	All M9 mineral medium was prepared from the same M9 salt solution sterilized through autoclaving. Although the MgSO4 and CaCl2 solutions were added separately, the same micropipettes with new tips each time were used. 5 mL of medium was added to each Falcon tube using a pipette.	Differences in mineral medium composition and amount could lead to different growth rates of *E. coli* which would lead to different amounts of acetate produced overall.
Amount of dextrose solution	1 mL of dextrose solution was added to each batch of M9 mineral medium using the same micropipette with a new tip each time.	Discrepancies in the amount of dextrose solution added would mean that different amounts of sugar were added. As it is known that the amount of sugar affects the production of acetate, this would surely affect the results.
Inoculation method	Each Falcon tube was inoculated by 5 swirls of a metal inoculation loop with sterilization by flame in between each inoculation.	Swirling the inoculation loop a set amount of times for all Falcon tubes ensures the least discrepancy between the amount of bacteria added to each tube. Sterilizing the tube prevents contamination of other bacteria.
Temperature	Falcon tubes containing *E. coli* were placed in an incubator at 37^{o} Celsius.	Differences in temperature affect the growth of *E. coli* (Goldberg et al., 1994) which would affect the amount of acetate produced.
Duration of growth	All *E. coli* were grown overnight for ~18 hours.	More time for growth would mean more bacteria which would mean more acetate production.
pH sensor	The same Vernier pH sensor was used for all trials.	Differing sensors may have differences in readings due to battery life, sensitivity, etc.

Independent Variable
The independent variable in this investigation is the w/v concentration of dextrose solution added to the M9 mineral broth. This concentration determined by the number of grams of dextrose per 100 mL of overall solution.

Dependent Variable
The dependent variable in this investigation is the amount of acetate secreted as measured by the change in pH.

Experiment

Materials

- Distilled Water
- Miller's LB Agar Powder (3.5 g)
- Na2HPO4 • 7H2O (6.4 g)
- KH2PO4 (1.5 g)
- NaCl (0.25 g)
- NH4Cl (0.5 g)
- MgSO4 • 7H2O (2.47 g)
- Dextrose (50 g)
- CaCl2 (1.47 g)
- Autoclave
- Plastic scoopula (9)
- Blank Printer Paper (5)
- Scissors
- Sharpie
- OHAUS Electronic Balance (±0.01 g)
- 50 mL, 100 mL Graduated Cylinder (±1 mL)
- 100 x 15 mm Petri Dish (1)
- Metal Inoculation Loop (1)
- Bunsen Burner
- Lighter
- K-12 *Escherichia coli* (Slant)
- 125 mL Wheaton Graduated Glass Media Bottle (3)
- 50, 140, 250 mL Glass Beaker (2; 5; 5)
- Corning Hot Plate (1)
- 4 mL Falcon Plastic Pipette (8)
- P1000 Micropipette
- P20 Micropipette
- Box of 96 P1000 Micropipette Tips
- Box of 96 P20 Micropipette Tips
- Glass Stirring Rod (5)
- 6 x 10 Nalgene Tube Rack
- 14 mL Falcon Tube (30)
- Miller's Luria Broth (2.5 g)
- Microwave
- Hot Hand Protector
- Incubator
- Vernier pH Probe
- pH 4, pH 7 Buffer Liquid
- Box of XL KimWipes
- Wash Bottle
- Laptop with LoggerPro Software
- Bleach
- Plastic Tub (2)
- Tap Water
- Ziploc Bag (7)
- Fume Hood

Procedure[1]

1. Gather the following materials: 100 mL graduated cylinder, distilled water, tap water, Miller's LB Agar powder, 1 plastic scoopula, 1 piece of blank printer paper, autoclave, sharpie, OHAUS electronic balance, 125 mL Wheaton graduated glass media bottle, Corning hot plate.
2. Measure out 100 mL of distilled water using the graduated cylinder.
3. Rip blank printer paper in half and place one half on the OHAUS electronic balance.
4. Zero the electronic balance.
5. Measure out 3.5 grams Miller's LB Agar powder using scoopula.
6. Pour distilled water from graduated cylinder into Wheaton graduated glass media bottle.
7. Pour Miller's LB Agar powder into Wheaton graduated glass media bottle.
8. Label bottle "3.5 g agar" with sharpie.
9. Seal the bottle and shake to dissolve powder.
10. Pour tap water into autoclave until touching the bottom of the inner layer and place on hot plate.

[1] Recipe for M9 mineral medium was found on IGEM and is listed in the works cited.

11. Heat up autoclave until water starts boiling.
12. Unscrew bottle cap until loosely on and place bottle in autoclave.
13. Autoclave for 20 minutes.
14. Turn off hot plate, depressurize autoclave, remove bottle and place in fridge for ~24 hours.
15. Gather the following materials: Hot hand protector, microwave, 100 x 15 mm petri dish, sharpie, metal inoculation loop, Bunsen burner, lighter, K-12 *Escherichia coli* slant.
16. Take bottle out of fridge and microwave until agar boils over.
17. Remove agar from microwave with hot hand protector and place on table.
18. Wait ~5 minutes for agar to cool.
19. Label bottom of petri dish with "LB Agar".
20. Pour agar into petri dish to about 5 mm thickness.
21. Wait ~15 minutes for agar to cool.
22. Light Bunsen burner with lighter and adjust until flame is stable.
23. Flame inoculation loop.
24. Inoculate agar plate from slant with the metal inoculation loop by swishing loop in a zig-zag motion on the agar's surface. Be careful not to push down too hard.
25. Flame inoculation loop.
26. Turn off Bunsen burner gas supply.
27. Incubate *E. coli* at room temperature for ~72 hours.
28. Gather the following materials: Distilled water, Na2HPO4 • 7H2O, KH2PO4, NaCl, NH4Cl, MgSO4 • 7H2O, CaCl2, autoclave, plastic scoopula, blank printer paper, scissors, sharpie, OHAUS electronic balance, 100 mL graduated cylinder, 125 mL Wheaten graduated glass media bottle, 50 mL glass beaker, Corning hot plate, 4 mL Falcon plastic pipette, Box of XL KimWipes.
29. Using sharpie, label the glass beakers "1 mL CaCl2" and "10 mL MgSO4" respectively. Label the Wheaten graduated glass media bottle "100 mL M9 salts".
30. Cut up 1 sheet of blank printer paper into 8 equal squares.
31. Place one square on the OHAUS electronic balance and zero the balance.
32. Measure out 6.4 grams of Na2HPO4 • 7H2O using scoopula.
33. Repeat steps 30-31, measuring out 1.5 grams of KH2PO4, 0.25 grams of NaCl, 0.5 grams of NH4Cl, 2.47 grams of MgSO4 • 7H2O, and 1.47 grams of CaCl2 respectively.
34. Pour Na2HPO4 • 7H2O, KH2PO4, NaCl, and NH4Cl into Wheaten graduated glass media bottle.
35. Fill up bottle to 100 mL with distilled water and screw lid on tightly.
36. Shake bottle until contents are mixed.
37. Perform steps 10-13.
38. Turn off hotplate.
39. Pour MgSO4 • 7H2O into 50 mL glass beaker.
40. Using a Falcon plastic pipette, pipette out 10 mL distilled water into beaker.
41. Cover beaker with KimWipe.
42. Pour CaCl2 into 50 mL glass beaker.
43. Pipette 1 mL distilled water into beaker.
44. Cover beaker with KimWipe.
45. Let sit for ~24 hours.
46. Gather the following materials: 100 mL graduated cylinder, distilled water, tap water, Miller's Luria Broth, 1 plastic scoopula, 1 piece of blank printer paper, autoclave, sharpie, OHAUS electronic balance, 125 mL Wheaton graduated glass media bottle, Corning hot plate.
47. Repeat steps 2-14 replacing 3.5 grams of Miller's LB Agar powder with 2.5 grams of Miller's Luria Broth, and thus, labeling the bottle "2.5 g LB".
48. Gather the following materials: Distilled water, 1 piece of blank printer paper, OHAUS electronic balance, dextrose, scissors, sharpie, 50mL graduated cylinder, 140 mL glass beaker, 250 mL glass

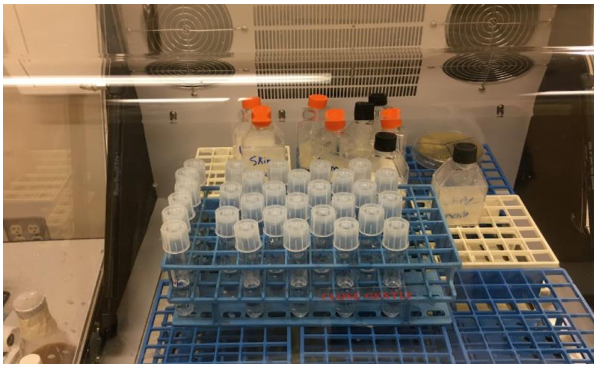
beaker, Corning hot plate, 4 mL Falcon plastic pipette, P1000 micropipette, P20 micropipette, box of 96 P1000 micropipette tips, box of 96 P20 micropipette tips, glass stirring rod, 6 x 10 Nalgene tube rack, 14 mL Falcon tube, microwave, hot hand protector.

49. Using the sharpie, label 1 each of the 140 mL glass beaker and 250 mL glass beaker with “0%”, “5%”, “10%”, “15%”, and “20%”.
50. Using the graduated cylinder, measure out and pour 20 mL of M9 salts into each of the 250 mL glass beakers.
51. Using the graduated cylinder, measure out and pour 80 mL of distilled water into each of the 250 mL glass beakers.
52. Using the P1000 micropipette, mix up the MgSO4 solution and pipette 200 μL into each of the 250 mL glass beakers. Be sure to discard pipette tips between beakers.
53. Using the P20 micropipette, mix up the CaCl2 solution and pipette 10 μL into each of the 250 mL glass beakers. Be sure to discard pipette tips between beakers.
54. Cut up 1 sheet of blank printer paper into 8 equal squares.
55. Repeat steps 30-32 4 times, measuring out 5 grams, 10 grams, 15 grams, and 20 grams of dextrose respectively.
56. Pour the 5 grams of dextrose into the 140 mL beaker labeled “5%”, pour the 10 grams of dextrose into the 140mL beaker labeled “10%”, and so and so forth until all dextrose is poured.
57. Using a graduated cylinder, fill all 5 140 mL beakers with water until the water line reads 100mL.
58. Turn on hot plate and place all 140 mL beakers onto hot plate.
59. Place a glass stirring rod into each beaker.
60. Heat for ~10 minutes (or until dextrose is dissolved) on low heat while stirring with glass rod.
61. Turn off hot plate.
62. Using the P1000 micropipette, pipette 1000 μL of 0% dextrose solution into the 250 mL beaker labeled “0%”, pipette 1000 μL of 5% dextrose solution into the 250 mL beaker labeled “5%”, and so on and so forth. Be sure to discard pipette tips between beakers.
63. Take out 30 Falcon tubes.
64. Using a sharpie, label 5 Falcon tubes with “C”, 5 with “0%”, 5 with “5%”, etc until all tubes are labeled.
65. Label 1 Falcon tube out of each category with a “U”.
66. Place the Falcon tubes in the Nalgene tube rack with each row corresponding to a category as shown in Picture 2.
67. Place 1 Falcon plastic pipette into each 250 mL beaker.
68. Mix the contents of each beaker with the plastic pipette in the beaker.
69. Using the Falcon plastic pipette in the beaker, pipette 5 mL of liquid from the beaker labeled “0%” to each tube labeled “0%”, pipette 5 mL of liquid from the beaker labeled “5%” to each tube labeled “5%”, etc. Do not use pipettes from one beaker to pipette the liquid of another beaker.
70. Repeat steps 16-18 with bottle labeled “2.5 g LB”.
71. Using a new plastic pipette, pipette 5 mL of liquid from the bottle to each tube labeled “C”.
72. Make sure that all Falcon tubes are capped.
73. Gather the following materials: Metal inoculation loop, Bunsen burner, lighter, incubator.
74. Take out the petri dish with *E. coli*.
75. Light Bunsen burner with lighter and adjust until flame is stable.
76. Flame inoculation loop.
77. Inoculate each Falcon tube not labeled with a “U” by taking a bit of *E. coli* from the petri dish with the metal inoculation loop and swirling the loop 5 times within the liquid in the Falcon tube. Be sure to close each Falcon tube and to flame the inoculation loop after each inoculation.
78. Flame inoculation loop.
79. Turn off Bunsen burner gas supply.

80. Place Nalgene tube rack in incubator and incubate for ~18 hours at 37° Celsius.
81. Gather the following materials: Bleach, plastic tub, tap water, fume hood, Ziploc bags, sharpie.
82. Create a bleach bath by filling 1 plastic tub with ~10% bleach and tap water.
83. Place the petri dish with the *E. coli* within the bleach bath.
84. Pour out all liquid from the glass beakers into Ziploc bags. Make sure to label each bag with the contents and to have a separate bag for each beaker.
85. Place bags into plastic tub.
86. Place both tubs into fume hood.
87. Gather the following materials: Vernier pH probe, pH 4, pH 7 buffer liquid, box of XL KimWipes, wash bottle, distilled water, laptop with LoggerPro software.
88. Rinse probe thoroughly with distilled water from wash bottle and calibrate using buffer liquid.
89. Take Nalgene tube rack out of incubator.
90. Take the pH of each Falcon tube and record data. Make sure to rinse and wipe the probe in between tubes.
91. Cap all of the Falcon tubes.
92. Gather the following materials: Bleach, tap water, fume hood, 250 mL beaker.
93. Create a bleach bath by filling the beaker with ~10% bleach and tap water.
94. Pour out all liquid in Falcon tubes into beaker.
95. Place all empty Falcon tubes into bleach bath with petri dish.
96. Place both baths into fume hood.

Setup

Picture 1: A picture taken on 4/4/2022 depicting the process of creating various M9 mineral mediums with differing concentrations of dextrose.



Picture 2: A picture taken on 4/6/2022 depicting the incubation of the *E. coli* in Falcon tubes containing M9 mineral medium.

Results

Raw Data

Raw Data Table: Uninoculated and inoculated pH levels (± 0.01) across varying dextrose concentrations (% ± 1)

Dextrose Concentration (% ± 1)	Trial	Uninoculated pH (± 0.01)	Inoculated pH (± 0.01)
Control	1	6.43	6.33
	2		6.67
	3		6.38
	4		6.13
0%	1	7.18	7.18
	2		7.18
	3		7.18
	4		7.18
5%	1	7.18	7.07
	2		7.08
	3		7.02
	4		7.04
10%	1	7.18	7.11
	2		7.07
	3		7.09
	4		7.02
15%	1	7.21	6.80
	2		6.92
	3		6.90
	4		6.90
20%	1	7.18	7.02
	2		7.05
	3		7.05
	4		7.08

Observations

- The murkiness of the liquid within the Falcon tubes had a direct correlation to the percentage of dextrose. The Luria Broth showed the most murkiness followed by the 20% solutions, the 15% solutions, the 10% solutions, and the 5% solutions. The 0% solutions were completely clear with no visible evidence of bacterial growth.
- The smell of the liquid within the Falcon tubes also showed a similar correlation with the Luria Broth being the most pungent and the 0% solutions having no smell at all.
- There was some precipitate at the bottom of the tubes, but there was no general trend between the percentage of dextrose and the amount of precipitate. This precipitate was mostly likely just CaCl2 that had not been fully mixed in.

Sample Calculations

1. **Calculation for Change in pH**
   $Inoculated\ pH\ -\ Uninoculated\ pH$

   Example Calculation (Control Trial 1):
   $6.33 - 6.43\ =\ -0.10$

2. **Calculation for Average Change in pH**

$$\frac{\sum Change\ in\ pH\ for\ each\ trial}{Number\ of\ trials}$$

Example Calculation (Control):

$\frac{-0.10 + 0.24 + -0.05 + -0.30}{4} \approx -0.05$ (rounded to 2 significant figures)

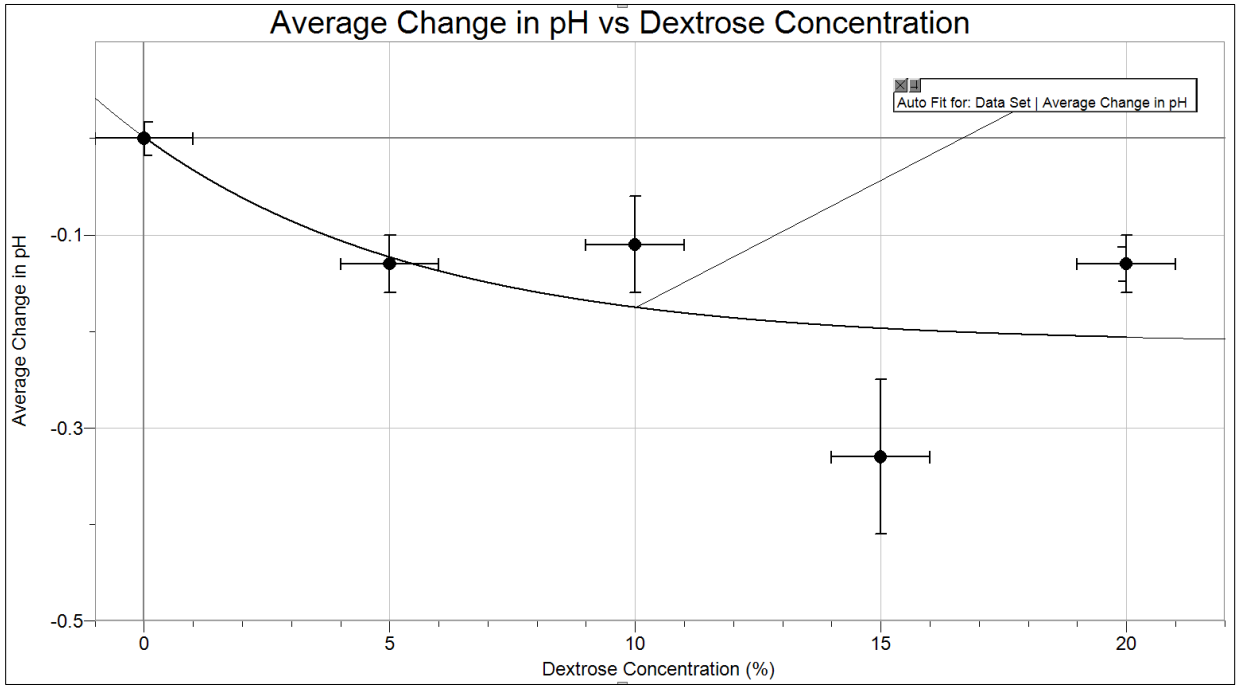
Processed Data

Processed Data Table: Change in pH (± 0.02) and average change in pH across varying dextrose concentrations (% ± 1)

Dextrose Concentration (% ± 1)	Trial	Change in pH (± 0.02)	Average Change in pH	Uncertainty of Average Change in pH
Control	1	-0.10	-0.05	±0.29
	2	0.24		
	3	-0.05		
	4	-0.30		
0%	1	0.00	0.00	±0
	2	0.00		
	3	0.00		
	4	0.00		
5%	1	-0.11	-0.13	±0.03
	2	-0.10		
	3	-0.16		
	4	-0.14		
10%	1	-0.07	-0.11	±0.05
	2	-0.11		
	3	-0.09		
	4	-0.16		
15%	1	-0.41	-0.33	±0.08
	2	-0.29		
	3	-0.31		
	4	-0.31		
20%	1	-0.16	-0.13	±0.03
	2	-0.13		
	3	-0.13		
	4	-0.10		

Graph

Graph: Average Change in pH vs. Dextrose Concentration (± 1%)



The vertical error bars were set to the uncertainty calculations listed in the processed data table. The horizontal error bars were set to 1%.

Discussion

Data Analysis

From the data, I concluded that an exponential trend was the most likely as *e* is the most common base in the natural world. Overall, it can be seen that as the concentration of dextrose increases, the average change in pH also decreases but the absolute value of the change increases. This is clearly seen when comparing the 0 pH change in the 0% dextrose concentration and the ~-0.13 pH change in the 20% dextrose concentration. However, we also notice that the pH change at 15% dextrose concentration is clearly an outlier. The reasons for this discrepancy will be discussed in *Sources of Error*. Other that that, I am fairly confident in my data, as it is consistent with B. Enjalbert's study which found that *E. coli* does not excrete acetate when not grown in excess glucose (Enjalbert et al., 2017). In this case, as no dextrose is present, there will be no acetate secretion.

Relating back to my initial aim of finding the optimal balance between glucose input and acetate output, we see that the graph starts to plateau at around 10% dextrose concentration. Any additionally increase in the concentration of dextrose after that does not lead to a significant increase in acidity. Thus, it can be concluded that the optimal amount of dextrose to stimulate acetate production is around 10 g per 100 mL.

Safety, Environmental, and Ethical Issues

Safety Issues

Most of the safety issues associated with bacteria relate to the potential to create pathogenic mutations as well as accidental cultivation of other, unsafe strains. Although *E. coli* is usually harmless, and in fact, helpful to humans, there are various strains that cause disease. Thus, all petri dishes were bleached after the experiment and all contents of the Falcon tubes were also emptied. In addition, to prevent contamination from outside

bacteria, autoclaving was used whenever possible and cases where it was not, materials were microwaved or sterilized with alcohol.

Environment Issues
As I needed to create the M9 mineral medium myself, I used a variety of chemicals. In order to ensure safety, all chemicals were placed into labeled Ziploc bags to be disposed of safely.

Ethical Issues
As I was working with bacteria, there are no severe ethical issues associated with this experiment.

Uncertainty
The uncertainty for the dextrose concentration was calculated by taking the largest uncertainty of the instruments I used – an OHAUS electric balance and a graduated cylinder. The OHAUS electric balance had an uncertainty of ±0.01 grams while the graduated cylinder had an uncertainty of 1 mL or 1%. As I was measuring sugar amounts larger than 1 gram, the graduated cylinder clearly had the larger percentage uncertainty. Thus, I set the overall uncertainty of the dextrose concentration at 1%.

The uncertainty for the pH was ±0.01 pH as the Vernier pH probe provided readings to the 2nd decimal place.

The uncertainty for the change in pH was ±0.02 pH. This was found by summing together the uncertainties of the two values that were subtracted.

The uncertainty for the average change in pH varied greatly depending on the dextrose concentration. This was determined by taking the absolute value of the largest distance between a trial result and the average.

Sources of Error
One of the largest sources of error occurred when making the M9 mineral medium for 15% dextrose. I had run out of M9 salt solution and so was only able to pour in ~10 mL. As making more M9 salt solution would have taken far to long of a time, I made up the rest of the difference with distilled water. This clearly affected the results, as it can be seen in the graph that the average change in pH for the 15% dextrose solution was much larger.

Another source of error occurred when I was pipetting 15% dextrose solution into the Falcon tubes. Before pipetting into the first Falcon tube, I forgot to mix the solution. This was crucial as there was some precipitate from the CaCl2 at the bottom of the solution, so the first Falcon tube received much less CaCl2 than the other Falcon tubes.

Lastly, as mentioned earlier, there was some precipitate from the CaCl2 at the bottom of some Falcon tubes. This may have affected the pH readings as CaCl2 is acidic.

Limitations/Extensions

Limitations
- Only 4 trials were made for each dextrose solution which means that data may not be entirely representative of the actual correlation between dextrose concentration and acetate production.
- Although measures were taken to ensure that each Falcon tube was inoculated with a similar amount of bacteria, it was impossible to ensure complete consistency.
- As I was only able to measure change in pH and was not able to directly measure acetate production, it may be that other byproducts were produced by the *E. coli* that led to the decrease in pH.
- Although these findings concluded that 10 g of glucose per 100 mL of water was the optimal ratio, this ratio is extremely high, and definitely not a viable nor healthy option for prevention and treatment of cholera.

Extensions

- A possible extension is to increase the number of dextrose solutions so that a more accurate trend line can be observed.
- Another possible extension is to perform a coupled enzyme assay in order to isolate the increase in acetate.
- Lastly, it is known that *E. coli* can also decrease acetate amount as it will reverse the Pta-AckA pathway and actually use acetate in respiration (Enjalbert et al., 2017). By increasing the amount of dextrose within the solution, an upper boundary can be found where the amount of acetate within the solution reaches an equilibrium as the *E. coli* produces and uses the same amount of acetate.

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