

**TED ANKARA COLLEGE FOUNDATION
PRIVATE HIGH SCHOOL**

To investigate the pH tolerance of the bacterium *Escherichia Coli*

BIOLOGY EXTENDED ESSAY

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ABSTRACT

The aim of this experiment was to find out the pH tolerance of *E.Coli*. Hence, in this extended essay there is a comparison of the optical density of *E.Coli* in different pH levels. These pH levels are chosen to investigate the *E.Coli* activity in acidic medium.

My research question was “How do different pH levels affect the growth of *Escherichia Coli* by measuring its optical density via spectrophotometer?”

According to the research question my hypothesis was “As the pH level of the medium increases, it will be more likely for *E.Coli* to die.”

In this experiment spectrophotometric method was used to measure the optical density of *E.Coli* in 3, 4, 5, 6, 7 and 8 pH levels. These pH levels are chosen so that experiment includes both acidic and neutral medium and the comparison of *E.Coli* activity can be made easily. Liquid broths with different pH levels were prepared and were put in bacteriological incubator for 1 day at a stable temperature. After 1 day the optic densities of these liquid broths were measured via spectrophotometer. Optical densities of these liquid broths illustrated the reproduction of the *E.Coli*. Higher optical density illustrates more reproduction of the bacteria.

The optical density is higher in 6, 7 and 8 pH levels and this means that there is more reproduction of *E.Coli* in those pH levels. On the other hand the optical density of 3, 4 and 5 pH levels were low comparing other pH levels. As a result of this experiment it can be said that *E.Coli* can live in neutral and die in acidic medium.

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INTRODUCTION

In the ninth grade, when we first started learning about kingdoms, one of them attracted my attention; kingdom bacteria. In our lessons, our teacher taught us a little bit about bacteria but I wanted to learn more because they are interesting organisms which can live in extreme media. Archaeobacteria are great examples of those organisms. Therefore, one of the reasons why I chose Biology as an extended essay topic is because I am curious of bacteria.

I love cooking so much. When I cook, I always see some advice from well-known cooks in recipes. They suggest putting some vinegar in food and they suggest washing vegetables with vinegar. I guess the reason for that is to kill bacteria live on food which can cause diseases. So I wanted to do a research on it and wanted to learn which acid level would kill the bacteria effectively. Hence, I decided to find bacteria which have easy access and harmless for doing an experiment to see its pH tolerance and the medium it lives. Therefore,, I asked my supervisor and we decided on a harmless strain of *E.Coli*.

To start with, I did a deep research on *E.Coli*. Briefly, *Escherichia Coli* is a gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia*.¹ Mostly it has harmless strains. However, it has kinds which can cause diarrhea. For example *E. coli* O157:H7 causes bloody diarrhea and can cause kidney failure and even death. It can be found in the intestines of cattle and can be transmitted through contaminated water or food, or through contact with animals or people.² The harmless strains are part of human and animal intestines which produce vitamin K₂ preventing colonization of the intestine with pathogenic bacteria.³

In addition, I also did a research on pH. pH is a measure of acidity and basicity. The solutions which have a pH less than 7 are called acids, the solutions which have a pH greater than 7 are called bases or alkaline and pure water is neutral with a pH of 7.0.⁴ pH has control over the growth of bacteria. Bacteria are divided into three according to their pH tolerance. Microorganisms which have an optimum growth below 7.0 pH are called acidophiles. Microorganisms which grow best at neutral pH are called neutrophiles and microorganism which can grow best at greater pH than 7 are called alkaliphiles.⁵

¹ From Wikipedia, the free encyclopedia, *Escherichia Coli*; http://en.wikipedia.org/wiki/Escherichia_coli

² <http://www.mayoclinic.org/diseases-conditions/e-coli/basics/definition/con-20032105>

³ Hudault S, Guignot J, Servin AL (Jul 2001). "Escherichia coli strains colonising the gastrointestinal tract protect germfree mice against *Salmonella typhimurium* infection". *Gut* 49 (1): 47–55. doi:10.1136/gut.49.1.47. PMC 1728375. PMID 11413110.

⁴ From Wikipedia, the free encyclopedia, pH; <https://en.wikipedia.org/wiki/pH>

⁵ 2008-2012 Kenneth Todar, PhD - Madison, Wisconsin, http://textbookofbacteriology.net/nutgro_4.html

In order to kill the bacteria in our food, we use acids. For example low-acid canned foods are not acidic enough to prevent the growth of these bacteria. So acids block their growth, or destroy them more rapidly.

Adding lemon juice, citric acid, or vinegar can increase the acidity of food. Most common and effective way to increase the acidity is to use vinegar. Most of the well-known cooks suggest using vinegar while cooking and washing vegetables. Vinegar is ideal since it contains acetic acid and it breaks down the coating on vegetables and destroys the bacteria.

Soaking vegetables into bleach, hydrogen peroxide and vinegar removes dirt and kills most of the bacteria, however it is not the same for *E.Coli*. It would be easy to kill *E.Coli* in a vinegar-water solution but since the bacterium sticks to the vegetable it becomes harder to kill. When *E.Coli* sticks to the vegetable it produces a substance called biofilm. This substance helps the bacterium to stick everything it latches onto, keeps it from being washed away and protects it from chemicals that could kill them in water solution. Briefly, vinegar is not effective in the same way in water and on vegetable. *E.Coli* also has another special feature. It penetrates into the interior tissues of the vegetables, where no sanitizer can reach them. Hence, it becomes harder to kill 100% of the bacteria and just one of them is enough to make us sick.⁶

Also I did a research on spectrophotometric method which I would use for my experiment. There are some ways for measuring pH tolerance but spectrophotometric method is the easiest and the most reliable method. In spectrophotometric method a sample of each pH level is used. Optical density of each sample is measured in this method.

E.Coli is a bacterium which has strains. Some of these strains can cause sickness and even death. I want to learn about its pH tolerance so that I can take precautions while I cook. Therefore,, my research question will be "How do different pH levels affect the growth of *Escherichia Coli* by measuring its optical density via spectrophotometer?"

⁶"How to Kill E. Coli on Vegetables." Quick and Dirty Tips

<http://www.quickanddirtytips.com/health-fitness/prevention/how-to-kill-e-coli-on-vegetables?page=all>

HYPOTHESIS

As I search, the bacteria on food are generally neutrophiles. This means that those bacteria can only live in neutral medium. As I mentioned in the “Introduction” part of the extended essay neutrophiles can live in pH levels like 6-7 and die in pH levels lower and upper than 6-7 pH levels. Therefore,, it can be said that little amount of acid would be enough to kill them.

There are some experiments done on neutrophiles for investigating their pH tolerance. For instance there is an experiment on *E.Coli* in 2008 in California science fair by Andrew T. Schilling. As a result of this experiment it is found that *E.Coli* can't live in pH levels like 4-5 and it is seen that it reproduces in neutral medium. According to the experiment acid is toxic to *E.Coli*.⁷ There is also another experiment which is done on neutrophiles. This experiment also suggests that if the concentration of acid increase, the growth of bacteria decreases. That experiment used the bacteria *Micrococcus luteus* and *Serratia marcescens*. In the experiment it is found that these bacteria can show optimum growth in pH level 8.⁸

It is possible to kill other neutrophiles than *E.Coli* with weak acids but as I mentioned in the “Introduction” part of the extended essay it is harder to kill *E.Coli*. It produces a substance called biofilm and this substance helps *E.Coli* to stick on everything, protects it from chemicals which have the potential to kill it and keeps it from being washed away. As a result it weak acids aren't effective to *E.Coli* ad becomes hard kill *E.Coli*. Hence, in my experiment I aimed to find its pH tolerance to kill it and take precautions. It is believed that *E.Coli* is neutrophile. Therefore, it can be hypothesized that as the pH level of the medium increases, it will be more likely for *E.Coli* to die.

⁷ <https://www.usc.edu/CSSF/History/2008/Projects/J1429.pdf>

⁸ http://www.all-science-fair-projects.com/print_project_1108_107

METHOD DEVELOPMENT & PLANNING

After deciding to work on *E.Coli* I talked to my mother's biologist friend. One of her friends who is the dean of microbiology department in Ankara University, Murat Özsarı, told us that they had harmless strain of *E.Coli*. Then I went to his office and we talked about my extended essay and which method would be more appropriate for my experiment. I learnt that I could use the laboratory and his assistant Haydar Kutlu could help me with my experiment.

After that I met him and told him about my experiment. He said that there were some ways to measure pH tolerance of *E.Coli*. One of the methods was to put bacteria in solid broth and drop pH solutions on them and measure the diameter of zone of inhibition. Unfortunately that method was not reliable and it was hard to record data. Another method he suggested was, measuring the growth of *E.Coli* in different pH levels in liquid broth by measuring its optical density by the help of spectrophotometer. Since it is the most reliable and easiest method I decided to do the experiment with the spectrophotometric method.

Before I started doing the experiment, I did a little research about the spectrophotometric method. Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. Spectrophotometry uses photometers that can measure a light beam's intensity as a function of its colour (wavelength) known as spectrophotometers. A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions.⁹ According to this method we prepared the pH levels and the bacteria in liquid broth.

In the experiment I decided to prepare liquid broths. The reason for me to do this is that it would be hard with solid broth. If I used solid broth, it would be hard to arrange the pH levels and record data, count the number of bacteria reproduced. In the liquid broth method, arranging the pH levels was very easy. Also it was easy to add the homogenized bacteria and it was manageable to collect data via spectrophotometric method.

In the experiment the hygienic conditions of the medium should be maintained while pouring nutrient broth in the test tubes, otherwise, the bacteria on air can enter both the nutrient broth in the test tubes and the nutrient broth which will be used in the experiments in the future. This can cause random errors in the experiment and the data collected would be wrong. So, the preparation of the test tubes were done near fire to prevent bacteria on air to enter the test tubes.

I decided to prepare 3, 4, 5, 6, 7 and 8 pH levels. Since it is believed that *E.Coli* lives in neutral medium, I involved 6, 7 and 8 pH levels. Also I prepared 3, 4 and 5 pH because I believed that these

⁹ From Wikipedia, the free encyclopedia; <https://en.wikipedia.org/wiki/Spectrophotometry>

pH levels would help me to see the growth of bacteria and to see its activity in acidic levels, so that I can choose which acid level I should use to take precautions.

Since it is hard to add the same number of bacteria I decided to take one colony of bacteria and homogenize it in nutrient broth. As a result I had nutrient broth with homogenized bacteria in it. I added 10µl of homogenized *E.Coli* to every pH level. So it can be said that equal number of *E.Coli* were put in every pH level. This method is easier, more reliable and manageable than solid broth method because in solid broth method, the number of bacteria before reproduction wouldn't be the same in each trial.

For data collection I put the test tubes in bacteriological incubator, which had the temperature of 37°C, for one day. I put them for one day because it would be enough for bacteria to reproduce. Also the temperature of the bacteriological incubator was 37°C since it is average temperature for *E.Coli* to live.¹⁰ For example, the results of an experiment done on *E.Coli* suggest that the optimum temperature for *E.Coli* to reproduce is 37°C which is body temperature. Temperatures upper and lower than that limits the growth of *E.Coli*.¹¹

**<http://anamariacaputo.com/download/molecularcellbiology3.pdf>

¹⁰ <http://study.com/academy/lesson/growth-requirements-of-e-coli-and-auxotrophs.html#lesson>

¹¹ <http://anamariacaputo.com/download/molecularcellbiology3.pdf>

MATERIAL LIST

X8 20mL Vacuumed test tubes

45mL Nutrient broth

30mL 0.1M HCl

30mL 0.1M NaOH

X1 10 µL Micropipette

X1 200 µL Micropipette

X7 Spectrophotometer cuvettes

Colony of *Escherichia Coli* (from EMB)

Bacteriological incubator

Biochrom WPA Biowave II UV/Visible Spectrophotometer

Hanna Instruments pH meter



Figure 1: Micropipette



Figure 2: Spectrophotometer cuvettes



Figure 4: Spectrophotometer



Figure 3: Preparation of pH levels and pH meter



Figure 5: E.Coli on EMB

METHOD

A. Preparation of the pH Levels

1. Put 5mL nutrient broth in 7, 20mL vacuumed test tubes. Do this step near fire to prevent bacteria in air enter to nutrient broth.
2. The pH of nutrient broth is around 7. Therefore, add NaOH in order to obtain pH level 8 and HCl to obtain 3, 4, 5 and 6 pH levels.
3. Also prepare a control group.

B. Homogenization of *E.Coli*

1. Take one colony of *E.Coli* from EMB.
2. Put 10mL of nutrient broth in a 20mL vacuumed test tube.
3. Homogenize one colony of *E.Coli* in nutrient broth.

C. Adding Homogenized *E.Coli* in Each pH Level

1. Via 10µL micropipette take 10µL of homogenized *E.Coli*.
2. Put 10µL of homogenized *E.Coli* in every test tube with different pH levels.

D. Data Collection

1. Place all of the tubes and the control group in bacteriological incubator, which has the temperature of 37°C, for one day.
2. After one day take all the tubes.
3. Set the wavelength of the spectrophotometer to 610nm.
4. Via 200 µL micropipette take 200 µL sample from each test tube and put it in spectrophotometer cuvette.
5. Set the spectrophotometer's optical density by the control group's turbidity.
6. Measure all the pH levels' optical density.
7. Record the data.

DATA COLLECTION AND PROCESSING

pH Level of Nutrient Broth	Trials	Optical Density*	Volume of Nutrient Broth in each test tube (mL)(±0.5)	Volume of Homogenized E.Coli in each test tube (µL)(±1)	Volume of Sample Taken(µL)(±1)	Temperature of Incubator (°C)(±0.5)	Time of Experiment (day)
3	1	0.001	5.0	10	200	37.0	1
	2	0.002					
	3	0					
	4	0					
	5	0.001					
4	1	0.005	5.0	10	200	37.0	1
	2	0.007					
	3	0.006					
	4	0.005					
	5	0.006					
5	1	0.013	5.0	10	200	37.0	1
	2	0.010					
	3	0.011					
	4	0.012					
	5	0.010					
6	1	0.826	5.0	10	200	37.0	1
	2	0.825					
	3	0.821					
	4	0.820					
	5	0.819					
7	1	1.116	5.0	10	200	37.0	1
	2	1.118					
	3	1.116					
	4	1.112					
	5	1.114					
8	1	1.358	5.0	10	200	37.0	1
	2	1.358					
	3	1.360					
	4	1.359					
	5	1.356					

Table1: The table above shows the optical density of *E.Coli* in each pH level and the controlled variables such as temperature of incubator, volume of each sample, volume of nutrient broth in each

test tube, volume of homogenized *E.Coli* in each test tube and the length of the experiment. *There is no available unit for optical density therefore, in table above its unit and uncertainty is not stated¹².

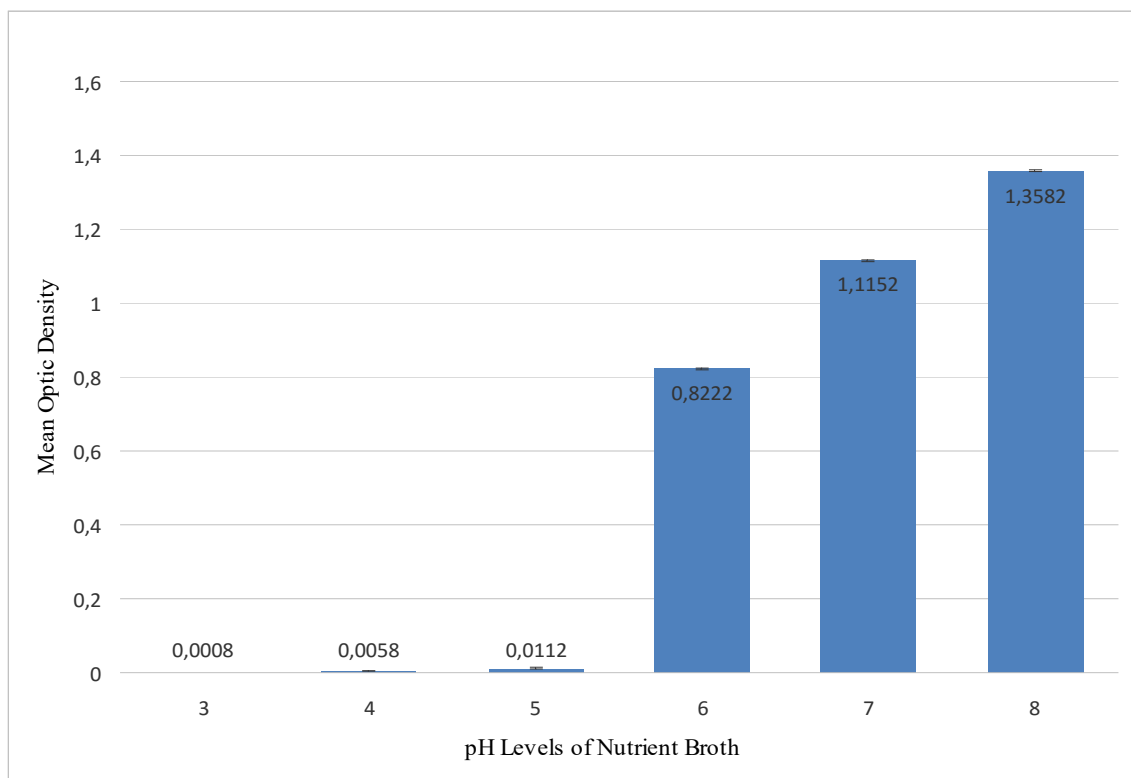
Trials/ pH	Optical Density					
	3	4	5	6	7	8
1	0.001	0.005	0.013	0.826	1.116	1.358
2	0.002	0.007	0.010	0.825	1.118	1.358
3	0	0.006	0.011	0.821	1.116	1.360
4	0	0.005	0.012	0.820	1.112	1.359
5	0.001	0.006	0.010	0.819	1.114	1.356
Mean	0.0008	0.0058	0.0112	0.8222	11.152	13.582
Mode	0.001	0.005	0.010		1.116	1.158
Median	0.001	0.005	0.011	0.821	1.116	1.358
Range	0.002	0.003	0.003	0.007	0.006	0.004
Variance	0.00000067	0.00000067	0.0000000167	0.00000970	0.00000520	0.00000220
Standard Deviation	0.00083666	0.00083666	0.00130384	0.0031145	0.0022804	0.0014832
Standard Error	0.02892508	0.02892508	0.03610873	0.0558075	0.047753	0.0385129
T	0.05	0.05	0.05	0.05	0.05	0.05
95% Confidence Interval	0.00073335	0.00073335	0.00114285	0.0027299	0.0019988	0.0013001

Table2: The table above shows the statistical analysis for 3, 4, 5, 6, 7 and 8 pH levels of nutrient broth.

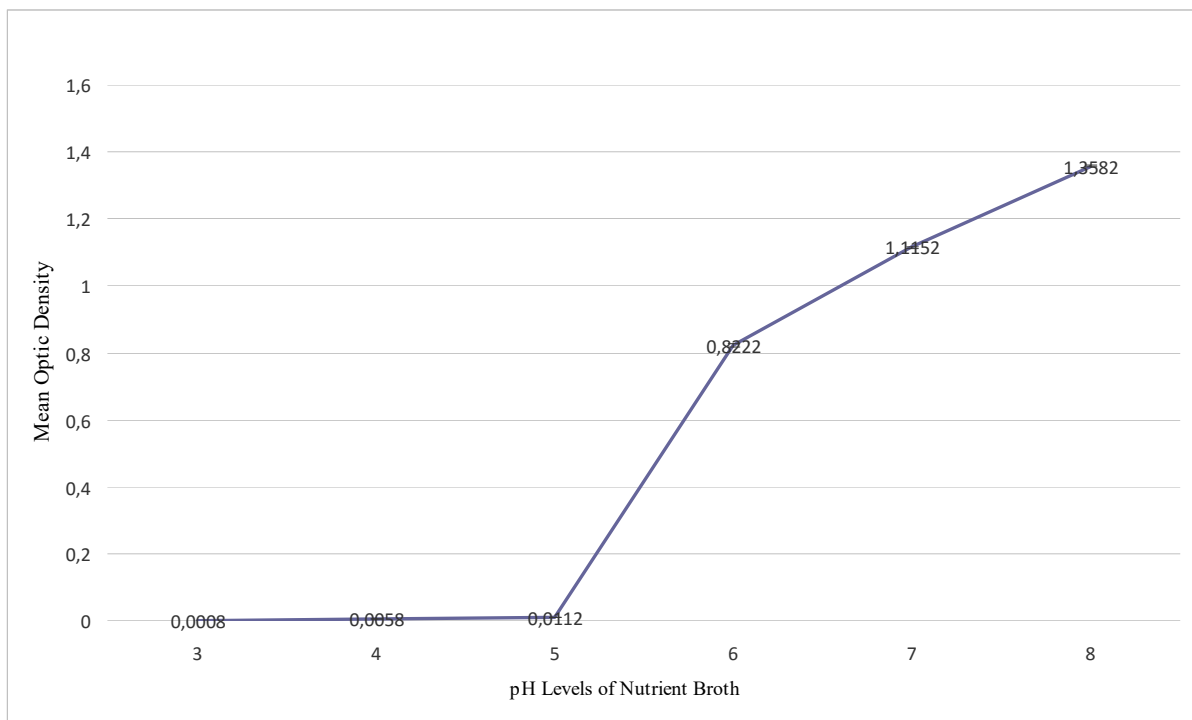
¹² How to Make Your Next Paper Scientifically Effective". *J. Phys. Chem. Lett.* (4): 1578–1581. 2013. doi:10.1021/jz4006916

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	9,6739	5	1,93478	574687	4,2E-60	2,62065
Within Groups	8,1E-05	24	3,4E-06			
Total	9,67399	29				

Table 3: ANOVA test: From the table above it can be seen that the P- value (in bold) is smaller than 0.05. Therefore, there is a meaningful difference between groups.



Graph 1: The bar graph above is the optical density vs. pH levels of nutrient broth graph according to mean value of optical densities of each pH level and with error bars according to 95% confidence interval. *In the bar graph error bars according to 95% confidence interval are added but since they are so small numbers they are very small.



Graph 2: The line graph above shows the optical density of every nutrient broth with different pH levels after putting the bacteria in the bacteriological incubator under 37°C and same CO₂ concentration.* In the line graph above the error bars are added according to 95% interval, however they are so small so, they are not evident.

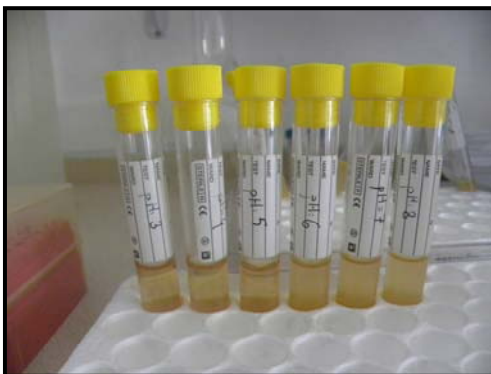


Figure 6: The test tubes after one day of incubation

CONCLUSION

Escherichia Coli is a gram- negative, facultatively anaerobic bacterium. It has both harmless types and it also has strains that can cause diarrhea. It can be transmitted through contaminated water or food, or through contact with animals or people. In order to prevent it, using acids like vinegar are recommended while cooking. Since I love cooking, I wanted to find out which acid levels kill *E.Coli* so that I can take precautions.

In this experiment my aim was to find out the pH tolerance of *Escherichia Coli* since it is going to help me while I cook. For my experiment I used spectrophotometric method which was the easiest and the most reliable method. In this method I homogenized bacteria in several nutrient broth which had different pH levels; 3, 4, 5, 6, 7, 8 and after 24 hours I measured their optical density via spectrophotometer.

In my experiment I saw that the optical density of *E.Coli* was the highest in pH level 8. In pH level 3, the most acidic medium I used, the optical density was nearly 0, the data were 0.001, 0.002, 0, 0, 0.001 in five trials, in order and the mean was 0.0008. In pH level 4 the optic densities were a little bit higher. It was 0.005, 0.007, 0.006, 0.005, and 0.003. The mean of pH level 4 was 0.0058. In pH level 5 the optical densities were 0.013, 0.010, 0.011, 0.012, 0.010 and the mean was 0.0112. In pH level 6 the optical density of *E.Coli* were 0.826, 0.0825, 0.821, 0.820, 0.819 and the mean was 0.8222 which were higher values since the pH level increased and nearly became neutral medium. In pH level 7, neutral medium, the optical densities were 1.116, 1.118, 1.116, 1.112, and 1.114 in five trials, in order. The mean of pH 7 was 1.1152 which was higher than other pH levels. In pH level 8 the data were 1.358, 1.358, 1.360, 1.359, and 1.356 and the mean was 1.13582 . As a result I saw that *E.Coli* can live best around neutral medium. When the pH level decreases the optical density of *E.Coli* decreases since the medium becomes acidic. Standard deviation of pH 3 was 0.00083666, pH 4 was 0,00083666, pH 5 was 0,00130384, pH 6 was 0,0031145, pH 7 was 0,0022804 and pH 8 was 0,0014832. 95% confidence interval of pH 3 and 4 was same and was 0,00073335. 95% confidence interval of pH level 5 was 0,00114285, pH level 6 was 0,0027299, pH level 7 was 0,0019988 and pH level 8 was 0,0013001. The errors bars on graphs are drawn according to 95% confidence intervals. The error bars on graphs were too little therefore,, it can be said that the data are reliable. Also the P-value from the ANOVA (Analysis of Variance) test is $4,2E-60$. It is smaller than 0.05 which means that there is a significant difference between pH groups.

From both bar and line graphs the pH levels which *E.Coli* can live best is clear. In 3, 4 and 5 pH levels the optical density is lower than 6, 7 and 8 pH levels. On the graph there is a sudden increase in the optical density. Also on the line graph between pH levels 5 and 6 there is an increase in the slope of the line and the optical density. The mean optical density of pH level 3 is nearly 0 which means the number of *E.Coli* is small. In pH level 4 and 5 it increases. However, in pH level 6 there is a huge increase in mean optical density, value of 0.8222.

When comparing the data obtained with the data recorded from other experiments, they are similar. For examples the experiment given in “Hypothesis” section of the extended essay, which were done on *E.Coli* and *Micrococcus luteus* and *Serratia marcescens*, also suggests that neutrophiles can live

better at neutral media and can't live in acidic medium. Every experiment suggests that *E.Coli* lives in pH levels around 6-7 and die in acidic medium. In my experiment my data also suggests that the optimum pH for *E.Coli* is 6 and 7 pH levels. In 3, 4 and 5 ph levels *E.Coli* nearly don't reproduce. As a result the data recorded is compatible with other examples done.

EVALUATION

The experiment is done generally in a more professional way. It wasn't like the experiments that we did in school. It was more fun. All the equipment that I used was professional. For instance, the equipment I used for measuring pH level. In school we use pH test strips but in my experiment I used pH meter which was more professional, easier to use and easier to measure the pH level.

Although it was a successful experiment, there were limitations. One of those limitations was about sanitary. While taking nutrient broth there is a possibility of bacteria in air stuck on it. I tried to be careful while doing it. I did it near fire which provides a hot medium with high temperature which bacteria can't withstand and bacteria in air to die but that doesn't exterminate the possibility of bacteria being stuck on it.

Another limitation was about arranging pH levels. The nutrient broth has a pH level of 7. Therefore, for arranging other pH levels I used HCl, for preparing acidic medium, acid and NaOH, for preparing basic medium. For pH levels of 3, 4, 5 and 6, I added HCl in the nutrient broth since adding HCl lowers the pH level. For basic medium, pH level of 8, I added NaOH in the nutrient broth since it increases the pH level. For example for pH level 3 I added HCl until I saw the value 3 on the pH meter. The pH level may seem like it is 3 but it was not directly 3. There were some uncertainties.

As a result of this experiment I learnt the pH tolerance of *E.Coli*. The data and the result of this experiment will help me while I cook. Since I learnt the life conditions of *E.Coli* I will be able to take precautions for not getting ill because of *E.Coli*. I learnt that to kill bacteria on vegetables, such as *E.Coli*, an acidic medium needed to be created. Vinegar, citric acid or lemon juice can be used because their pH level is low and they are strong acids. Therefore, washing vegetables without vinegar or lemon juice won't be helpful since rinsing don't kill all the bacteria. This means that every time I use vegetables, I will either use vinegar or lemon juice while washing them.

Also while doing the experiment I did lots of research. During my research I learnt new information about bacteria. I gained knowledge about other species. For instance, I learnt that *Clostridium Botulinum*, can reproduce in canned food when canned foods have not been heated effectively during canning. Since their acidity level is low and the bacteria produces toxins that can cause botulism and food poisoning. Also I learnt that it is a neutrophil and can live best at 6-7 pH levels.

Another advantage of this experiment for me was to learn new laboratory techniques, which will benefit me a lot in the future, because in the university I want to study Molecular Biology and Genetics. I learnt some different techniques to do one experiment in several ways. I am going to spend too much time in laboratories while studying in the university and also after leaving the university. This experiment and extended essay became a great practice for me before starting university. I am so grateful that I had the opportunity to work in a laboratory like professionals and learn new techniques.

Results of my experiment can be helpful for all the people around the world who cook. Considering the data I obtained from my experiment, they can take precautions and would consume cleaner food. As a result, food poisoning which is caused by *E.Coli* would lessen. Some people's lives can be saved.

I heard that in canned food industry there are some methods to stop bacteria reproducing on food from my cousin who is a food engineer. These methods contain canning the food in high temperature like 116-130°C. There is also another method which prevents canned food to absorb air. If those methods are not done effectively enough then bacteria on canned food can reproduce and become toxic to people if eaten. Therefore, some acids are used to stop bacteria reproduction in case those methods are not effective enough¹³. Results of my experiment also showed that acidic medium can kill neutrophile bacteria. Therefore, it can be said that if the results of my experiment are considered, it would be useful to all canned food industry.

¹³ <http://aggie-horticulture.tamu.edu/food-technology/food-processing-entrepreneurs/microbiology-of-food/>

APPENDICES

Appendix 1

Symbols and Abbreviations

pH: Power of hydrogen

EMB: Eosin Methylene- Blue Lactose Sucrose Agar

NaOH: Sodium hydroxide

HCl: Hydrochloric acid

M: Molarity

mL: milli liter

nm: nano metre

µL: micro liter

Appendix 2

Formulas Used In Statistical Analysis

a. Arithmetic Mean:

$$\bar{x} = \frac{1}{N} \sum_{i=1}^N x_i. \quad 14$$

b. Median: Median is the number which is in the middle when a series of numbers are in ascending order.

c. Mode: The most repeated number in a series of numbers.

¹⁴ Weisstein, Eric W. "Mean." From MathWorld--A Wolfram Web Resource. <http://mathworld.wolfram.com/Mean.html>

d. Variance¹⁵:

$\sigma^2 = \langle (X - \mu)^2 \rangle$, μ : population mean $\langle X \rangle$: expectation value of X.

e. Standard Deviation¹⁶: The square root of variance.

$$\sigma = \sqrt{\langle x^2 \rangle - \langle x \rangle^2} \quad \mu = \bar{x} = \langle x \rangle: \text{the mean}$$

f. Standard Error¹⁷: Defined as the square root of the estimated error variance $\hat{\sigma}^2$ of the quantity.

$$s_e = \sqrt{\hat{\sigma}^2}$$

g. 95% Confidence Interval¹⁸: A confidence interval is an interval in which a measurement or trial falls corresponding to a given probability. Usually, the confidence interval of interest is symmetrically placed around the mean, so a 50% confidence interval for a symmetric probability density function would be the interval $[-a, a]$ such that

$$\frac{1}{2} = \int_{-a}^a P(x) dx.$$

¹⁵ Weisstein, Eric W. "Variance." From MathWorld--A Wolfram Web Resource. <http://mathworld.wolfram.com/Variance.html>

¹⁶ Weisstein, Eric W. "Standard Deviation." Retrieved from: MathWorld--A Wolfram Web Resource. <http://mathworld.wolfram.com/StandardDeviation.html>

¹⁷ Weisstein, Eric W. "Standard Error." Retrieved from: MathWorld--A Wolfram Web Resource. <http://mathworld.wolfram.com/StandardError.html>

¹⁸ Weisstein, Eric W. "Confidence Interval." Retrieved from: MathWorld--A Wolfram Web Resource. <http://mathworld.wolfram.com/ConfidenceInterval.html>

Appendix 3

Spectrophotometric Method

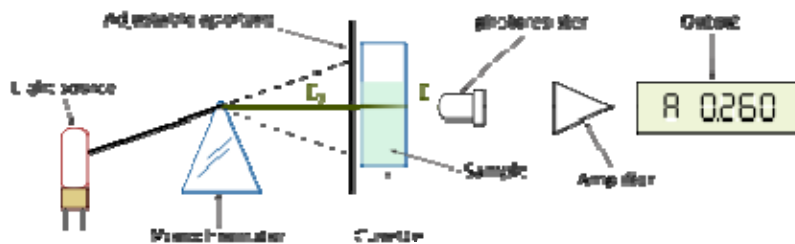


Figure 7: Spectrophotometry

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear range of absorption or reflectance measurement.¹⁹

The sequence of events in a modern spectrophotometer is as follows:

1. The light source is imaged upon the sample
2. A fraction of the light is transmitted or reflected from the sample
3. The light from the sample is imaged upon the entrance slit of the monochromator
4. The monochromator separates the wavelengths of light and focuses each of them onto the photodetector sequentially.

¹⁹ From Wikipedia, the free encyclopedia; <https://en.wikipedia.org/wiki/Spectrophotometry>

BIBLIOGRAPHY

1. From Wikipedia, the free encyclopedia, *Escherichia Coli*;
http://en.wikipedia.org/wiki/Escherichia_coli
2. "E. Coli." - Mayo Clinic. N.p., n.d. Web. 10 Jan. 2016.
<http://www.mayoclinic.org/diseases-conditions/e-coli/basics/definition/con-20032105>
3. Hudault S, Guignot J, Servin AL (Jul 2001). "Escherichia coli strains colonising the gastrointestinal tract protect germfree mice against Salmonella typhimurium infection". *Gut* 49 (1): 47–55. doi:10.1136/gut.49.1.47. PMC 1728375. PMID 11413110.
4. From Wikipedia, the free encyclopedia, pH; <https://en.wikipedia.org/wiki/PH>
5. 2008-2012 Kenneth Todar, PhD - Madison, Wisconsin,
http://textbookofbacteriology.net/nutgro_4.html
6. "How to Kill E. Coli on Vegetables." Quick and Dirty Tips. N.p., n.d. Web. 10 Jan. 2015
<http://www.quickanddirtytips.com/health-fitness/prevention/how-to-kill-e-coli-on-vegetables?page=all>
7. Experiment Done On *E.Coli*
<https://www.usc.edu/CSSF/History/2008/Projects/J1429.pdf>
8. From Wikipedia, the free encyclopedia; <https://en.wikipedia.org/wiki/Spectrophotometry>
9. Growth Conditions of *E.Coli*
<http://study.com/academy/lesson/growth-requirements-of-e-coli-and-auxotrophs.html#lesson>
10. How to Make Your Next Paper Scientifically Effective". *J. Phys. Chem. Lett.* (4): 1578–1581. 2013.
11. Weisstein, Eric W. "Standard Deviation." Retrieved from: MathWorld--A Wolfram Web Resource. <http://mathworld.wolfram.com/StandardDeviation.html>
12. Weisstein, Eric W. "Standard Error." Retrieved from: MathWorld--A Wolfram Web Resource. <http://mathworld.wolfram.com/StandardError.html>
13. Weisstein, Eric W. "Confidence Interval." Retrieved from: MathWorld--A Wolfram Web Resource. <http://mathworld.wolfram.com/ConfidenceInterval.html>
14. Weisstein, Eric W. "Variance." From MathWorld--A Wolfram Web Resource. <http://mathworld.wolfram.com/Variance.html>

15. Weisstein, Eric W. "Mean." From MathWorld--A Wolfram Web Resource. <http://mathworld.wolfram.com/Mean.html>

16. <http://aggie-horticulture.tamu.edu/food-technology/food-processing-entrepreneurs/microbiology-of-food/>

17. http://www.all-science-fair-projects.com/print_project_1108_107

18. <http://anamariacaputo.com/download/molecularcellbiology3.pdf>