Cambridge Assessment International Education

Cambridge IGCSE

Cambridge O Level

Cambridge Upper Secondary Science Competition regional winner

How do different concentrations of acidic liquids affect microorganism growth – an investigation into natural cleaning solutions.

Judge's comments:

This is a very thorough research report based on a real life problem relevant to the group's environment.

Faced with a genuine problem the group completed some empirical research and designed an appropriate experimental technique. This was technically accurate and delivered competently, with a good range of clear observations recorded in detail. Potential sources of error were itemized and analyzed, such that feedback into experimental technique improved the final design.

A full set of data were collected and analyzed.

The group took into account the economic impact of each choice of subject solution.

Investigation

"How do different concentrations of acidic liquids affect microorganism growth?"

Aim: To investigate whether different concentration levels of acidic liquids will affect the growth of microorganism, to encourage the use of natural and environmentally friendly acidic liquid cleaning solutions.

Objective/World link: Introduce healthy, legal, preservatives for foods and natural cleaning products throughout Indonesia, Asia and the rest of the globe, in order to help improve the health of the locals as well as the tourists. It is also important to make sure that this concept is accessible to all people, and is fairly cheap so that people are more inclined to use healthier methods. For at the moment we are risking contaminating the environment and the health of the people when using chemical cleaning products and unnatural preservatives/additives.

Research Question: How do different concentrations of acidic liquids affect the growth of microorganisms?

Background Research:

This experiment was an investigation of the overall growth of microorganisms in varying acidic environments. Microorganisms are microscopic organisms especially bacterium, virus, or fungus.[1] There are 5 main classifications of microbes which include: bacteria, fungi, algae, protozoa and viruses. The 2 types of microorganisms prone to develop in this experiments environment are bacteria and fungi. Bacteria are single-celled microbes, which reproduce by dividing into two.[2] They essentially grow under warm, moist, protein-rich environments that is pH neutral or low acid conditions, with some exceptions. Those being that several forms of bacteria can also thrive under high acidity and/or very salty settings. Fungi is a broad, diverse group of organisms which don't need to photosynthesis. There are two groups of fungi mould and mushrooms (filamentous fungi) as well as yeast. The optimal environment is often slightly acidic with low moisture. However,depending on the type fungi itself preferred surroundings will be different. This why the experiment is conducted in a dark cabinet. It is also a realistic comparison to how food is usually stored for preservation and therefore gives accurate environmental characteristics for the research of this experiment.

The topic of food preservation and growth of microorganism is important all over the world, and especially in Asia. Approximately 50% of travellers going to Indonesia suffer from gastrointestinal infection within 2 weeks of being there. This is because of excess bacteria and toxins that are transferred from contaminated food and water and poor cooking hygiene. The main reason for contaminated food is because of the poor storage and unsanitary tools/utensils. An additional issue is the use of non-food grade additives in foods - especially in street foods. Chemicals such as boric acid and formaldehyde have been found to be used as food preservatives. It is important to introduce healthy, legal, preservatives for foods throughout Indonesia, to help improve the health of the locals and the tourists. It is also important to make sure that this concept is available to all people,and isn't too expensive so that people are more inclined to use healthier methods.[3] Not only can natural preservatives be used in shops or restaurants,it also allows for tourists to have a viable option if necessary.[4] Other than Indonesia the findings from this investigation can benefit other parts of the globe as well. The results of this experiment could also be applied to the use of substances such as acid water as a method of cleaning or preservation, as it evaluates the effectiveness of such a product, and it is important to note that using natural substances allows for them to be used as food preservation as well as cleaning products.[5] In summary, the objective of this investigation is to introduce healthy, legal, preservatives for foods and natural cleaning products throughout Indonesia, Asia and the rest of the globe, in

1 2 https://www.1000sciencefajrprojects.com/Biology/Study-of-Bacteria-Growth-in-Varying-Acidic-Environments.php http://www.ijsrp.org/research-paper-0413/ijsrp-p4014.pdf

3 https://www.healthguidance.org/entry/9987/1/dangers-of-food-additives-and-preservatives.html

4 http://medind.nic.in/jal/t09/i4/jalt09i4p419.pdf

5 https://cnx.org/contents/NxduIcMv@3/The-Effects-of-pH-on-Microbial-Growth

order to help improve the health of the locals as well as the tourists. It is also important to make sure that this concept is accessible to all people,and is fairly cheap so that people are more inclined to use healthier methods. For at the moment we are risking contaminating the environment and the health of the people when using chemical cleaning products and unnatural preservatives/additives.

This is because they are the most readily available materials for people in Indonesia as well as other parts of the world, and are fairly cheap, which is an important factor for poorer areas which lack sterility/cleanliness - and therefore presents a reasonable method for natural food preservation.[6]

There are environmental risks of using unnatural cleaning products. Many chemical cleaning products take a long time to degrade into harmless products, andin the process, they often contaminate the environment. In fact, some products do not even break down at all, and instead, persist in the environment.[7] Another issue with this is that it could enter the food chain,and be eaten by aquatic animals. This can then mean that it is consumed by humans. When chemical cleaning products are consumed or absorbed through the skin, it can cause harmful effects, and even lead to cancer if a high amount is consumed.

Alongside the environmental risks of chemical cleaning products, there are hazards to the use of additives/chemical preservatives. According to international scientific research publications, there are medical concerns such that they can cause different allergies and conditions such as hyperactivity and Attention Deficit Disorder, asthma, hay fever and certain reactions such as rashes, vomiting, headache, tight chest, hives and worsening of eczema for some people.[8] A specific example is the sulfites that are common preservatives used in various fruits, that may cause side effects in the form of headaches, palpitations, allergies, as well as cancer. After consuming certain foods causing allergies that can be noticed, instead of instantly exhibiting indications some people develop the symptoms of allergy a day or two later, thus it is difficult to know what is causing the problem. People consume a variety of foods so it is difficult to find out the exact substance which causes allergy. [9]For this reason, people have to go on an elimination diet. They stop eating all foods that might be problematic and introduce one at a time to see if side reaction occurs. Side reactions of these preservatives can be immediate or build up in the body over time. Only in recent years have researchers seriously considered the physical impact of these additives over the long term.

There were multiple substances used as part of this experiment, all of which manipulate the food differently in order to preserve it. There are many options available all over the world, but sodium chloride, vinegar, and lime were used specifically. This is because they are the most readily available materials for people in Indonesia as well as other parts of the world, and are fairly cheap, which is an important factor for poorer areas which lack sterility/cleanliness - and therefore presents a reasonable method for natural food preservation.

Table salt (sodium chloride) is an effective preservative. Sodium chloride draws water out of cells via the process of osmosis. This means that the water goes across the cell membrane to equalise the concentration of sodium chloride on all the sides of the cell. If there is a concentration of 20% sodium chloride on the cell, it will ensure that all the water is drawn out of the cell and it is unable to grow or reproduce. Using only a small amount of table salt changes the process to fermentation.[10] This is the process of maintaining a consistent amount of organisms in a portion of food to turn a starch, sugar, or carbohydrate into an alcohol or acid.

Another natural preservative is vinegar. Low concentrations of acetic acid (an active ingredient in vinegar) can help to treat biofilms(microorganisms attached to a surface) on physical wounds, by preventing bacterial

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6 https://www.thoughtco.com/why-does-salt-work-as-preservative-607428 https://www.sciencedaily.com/releases/2015/09/150915105208.htm https://www.leaf.tv/articles/lemon-juice-as-a-preservative-to-improve-shelf-life/ https://www.lonelyplanet.com/indonesia/health

7 https://www.iamat.org/country/indonesia/food-and-water-safety# http://seafast.ipb.ac.id/article/the-need-of-comunicating.pdf

8 http://resources.schoolscience.co.uk/SGM/sgmmicrobes3.html

9 https://www.alkaviva.com/uses-for-acid-water

10 https://www.redandblack.com/news/acidic-water-can-kill-bacteria/article_38e31b49-780b-5d7c-9b32-290f81c9ad8b.html acid water for cleaning https://www.sciencebuddies.org/science-fair-projects/references/interpreting-agar-plates

colonisation.[11] The use of a low concentration vinegar is much more effective than a higher concentration, and therefore it is possible that it can be used as treatments for food and wounds. It was shown that vinegar at concentrations of 0.16-0.3% could completely eradicate the pre-formed colonies,and prevent the growth of all further strains.

Finally, lime juice can also be used as a preservative. Lime juice contains ascorbic acid and citric acid which are natural antioxidants and antibiotics. Lime juice is similar to sodium chloride in that it draws out the water to balance the pH factor and ensure that there are no microorganisms growing in food. The acids in limes also affect the oxygen molecules and prevent them from interacting with the molecules on the surface of the food. As a result, the chemical reactions slow down and the food goes badly more slowly.

Hypothesis: The higher the concentration of each type of liquid, the lower amount of microorganisms grown by surface area. Based on background research, sodium chloride is the most effective food preservative in comparison to lime juice and vinegar.

Variables:

Independent Variable Different acidic liquids and their concentration	1. Vinegar (25%) 2. Lime juice (Key Lime Juice) 3. Salt (sodium chloride) The concentration of each liquid will be changed by adding 20 ml, 15 ml, 10 ml, 5 ml and 0 ml of water into each sample. (final solution of each liquid concentration will be 25ml) The ratios of substance : water for each liquid ● 5:20 ● 10:15 ● 15:10 ● 20:5 ● 25:0
Dependent Variable The amount of microorganisms grown (surface area)	The amount of microorganisms on each petri-dish will be measured by calculating its surface area. - The surface area is measured in the units cm^2
Controlled Variable 1. Mass of chicken 2. Time for the microorganisms to form 3. Environment in which the petri-dishes are placed 4. Size of petri dish 5. Material of petri dish 6. Sterilizing equipment 7. Same type of limes 8. Same percentage of vinegar acidity 9. Same type of salt 10. Type of water	1. Each chicken sample should weigh a mass of exactly 3 grams using an accurate scale. This is to ensure an equal amount of chicken is exposed to the petri dish. 2. The microorganisms should be given 4 days to grow in order to visually see the microorganisms. The microorganisms need to be visible, in order to be measured. After 4 days, the measuring process of the microorganisms will begin and continue for a span of 7 days. 3. All petri dishes should be kept in a dark cabinet at room temperature, this will ensure that no environmental change will affect the growth of microorganisms.

[11] https://www.greenchoices.org/green-living/cleaning/environmental-impacts

	4. The same size petri dishes should be used to ensure that all the samples of the raw chicken in different concentrations are exposed to the same amount of agar agar. 5. All the petri dishes should be glass to ensure reliability in results. 6. All equipment should be sterilized with rubbing alcohol (80%) before usage. This is done in order to prevent other bacteria interfering with the experiment. 7. The same type of limes should be used for the lime concentrations (key limes) 8. Each vinegar concentration, should be the same acidity (25%) 9. For each salt concentration, table salt was used to ensure reliability 10. Boiled tap water was used for the water to be sterilized and clean.
Uncontrolled Variable 1. The dirt and bacteria in the air surrounding the chicken. 2. Temperature of the different liquids 3. Condensation on the petri dishes	1. When conducting the procedure, bacteria/dust from surrounding could come in contact with the agar dish or the chicken. Causing some unreliability in the results. 2. The temperature of the different concentration liquids could have an affect on the data collected. As this is a factor which affects growth of microorganisms. 3. condensation occurring on the petri dishes due to changes in the outside temperature could affect the growth of the microorganisms. Changing in the microorganisms environment, could affect some of the data's reliability.

Apparatus:

- 48 equal pieces of raw chicken (3g each)
- 150 ml lime juice
- 150 ml of white vinegar
- Table salt (Sodium Chloride)
- Distilled water
- Agar powder
- 48 Petri dishes
- Rubbing alcohol
- Forceps
- Kettle
- Syringe
- Stirring rod
- Beakers
- Measuring cylinder

- Disposable gloves
- Weight scale
- Gridded paper
- Labelling sticker
- Scissors
- Light box or lamp stand

Method :

Preparation

1. Collect all apparatus listed in the materials needed.
2. Separate the 48 petri dishes into 3 groups of 15 and 1 group of 3. Meaning 5 for each trial, having 3 trials for each concentration.
    a. 15 petri dishes for vinegar
    b. 15 petri dishes for salt
    c. 15 petri dishes for lime
    d. 3 petri dishes for water
3. Label each petri dish lid based on their concentration category using masking tape.
4. Sterilize all 48 petri dishes using rubbing alcohol and a cotton pad.
5. Boil enough water in a kettle order to make an agar-agar solution to fill all petri dish.
6. Add in 3 tablespoons of agar powder into the kettle.
7. Stir the agar solution in the kettle for 2 minutes.
8. Pour the agar solution into a beaker
9. Syringe 20 ml of agar solution into each petri dish. Ensure to close petri dish lids once filled with the agar solution to prevent any entry of dust and bacteria.
10. Prepare 48 samples of chicken, weigh each sample to be 3 grams each.
11. Place the chicken in a bowl and seal it with cling wrap.
12. Place in a fridge until used.

Making the concentrations

1. Prepare 16 beakers for each concentration
    a. 5 beakers for vinegar concentrations
    b. 5 beakers for salt concentrations
    c. 5 beakers for lime concentrations
    d. 1 beaker for water
2. Label each beaker based on their concentration category using masking tape.
3. Juice the limes to collect 75 ml of lime juice into a measuring cylinder
4. Separate the lime juice with water between 5 beakers in these ratios
    a. 5 ml of lime juice and 20 ml of water into 1 beaker
    b. 10 ml of lime juice and 15 ml of water into 1 beaker
    c. 15 ml of lime juice and 10 ml of water into 1 beaker
    d. 20 ml of lime juice and 5 ml of water into 1 beaker
    e. 25 ml of lime juice and 0 ml of water into 1 beaker
5. Repeat with vinegar and salt (grams) concentrations.

Procedure

1. Set up the apparatus into sections based on the liquid
2. Sterilize all equipments before use

3. Dip the chicken sample into the liquids concentration.
4. Swab the chicken sample onto the petri dish for 10 seconds. Ensuring the whole petri dish is covered with the raw chicken and solution. (do not leave the chicken in the petri dish)
    a. Repeat this procedure for each liquid and its different concentrations. Having 3 trials per concentration.
    b. Ensure to close petri dish once the chicken is swabbed
5. Place all petri dishes in the same environment.
6. Record the results after 4 days have passed in order for the bacteria to start being seen visibly. (continue recording for 7 days)
7. Place the petri dish on a light box, putting gridded paper (1x1cm) under the petri dish in order to measure how much surface area (cm^2) of microorganisms have grown.
    a. Measuring and observing the size of the microorganism by using the 1x1 square on the grid paper. (As 1 microorganism/dot would have to be the size of the 1x1 square, a microorganism that takes up half the 1x1 square will be considered as 0.5. Adding up all the microorganisms will give you the surface area of the bacteria that has grown.)
    b. Do not open the lid off of the petri dish as the exposure to the different environment will affect the results
    c. Counting all the microorganisms present then dividing the number by 2, in order to give the surface area of the number of bacteria that has grown.
    d. Ensure the same person is collecting/measuring the data for every sample.
8. Collect data into a table.
9. After a week has passed process the data by finding the average of the three trials for each day.
    a. For example, find the average of sodium chloride for day 1: trial 1, trial 2 and trial 3
    b. The average from that day will then be used in the graph
10. After all data has been processed, place into a line graph.
11. Analyse the data further on.

Safety and Ethical considerations

1. **Treat living microorganisms as if they are possible pathogens.**
    - Microorganisms are not pathogenic to humans, and are rarely shown to cause illnesses. However, in a rare occasion, some microorganisms that are not pathogenic may become pathogens. Therefore, it is important to treat all microorganisms as if they are pathogens. Students with low immune systems or illnesses should also consult with an instructor or medical advisor before conducting the experiment.
2. **Sterilize and clean the materials, equipment, and work areas before and after use.**
    - A disinfectant such as Alcohol solution 80% should be used to sterilize all the materials and equipment which are used in the microorganism growth process.
    - Disinfect all waste materials before discarding them.
    - Clean up spills that can possibly contain microorganisms with care by covering them with paper towels soaked in a disinfectant (Alcohol solution 80%) and then place the soaked towel over the spill and let it sit for a few minutes to kill unwanted bacteria.
    - Disinfectants can be highly flammable and its important to be cautious when using a disinfectant around a flame or high heat source.
    - Disinfectants can be dangerous when splashed on eyes. Therefore, it is important to know where the nearest medical station/sink is located before conducting the experiment.
3. **Make sure unwanted bacteria is removed before and after the procedure.**
    - Wash your hands with soap before and after conducting the experiment to remove unwanted bacteria
4. **Do not consume anything in the lab or leave food in the lab where the microorganisms are stored.**

- Never eat or drink in the lab and keep your fingers out of your mouth to avoid getting possible disease from the microorganisms.

5. **Label clearly**
   - Make sure all of the apparatus used that may be hazardous are labeled clearly with a proper warning.
6. **Wear the proper attire during the whole experiment.**
   - Wear a labcoat to protect your clothing from being completely exposed by microorganisms.
   - Wear goggles to protect eyes from hazardous substances.

Observations	
Table Salt	- Microorganisms were varied, trial 1 of the concentration 5:20, trial 2 and 3 of the concentrations 20:5 had large dark green, almost black microorganisms and the rest of the tests had small, white microorganisms - Growth of microorganisms was slow and the most microorganisms grown was an average of 7.2cm^2 using the concentration 20:5 - Ideal to use salt 25:0 as the average microorganisms grown within a 7 day span was 2.7cm^2 - The large green microorganisms grew separate from each other and the small white organisms grew in clusters
Vinegar	- Microorganisms were small and white, not much variation between colour and size for each trial - Barely visible for the first 3 days - Growth of microorganisms was at a steady pace with the most microorganisms grown was an average of 17.7cm^2 of both the concentrations 20:5 and 25:0 - Grew in clusters rather than spread out over the entire petri dish
Lime	- Microorganisms varied from small to large and all microorganisms were dark green - Growth of microorganisms was varied, the growth of concentrations using the most lime was very rapid - 25:0 had grown from an average of 1.67cm^2 to 9cm^2 - The tests using the most lime (25:0) had the largest, most prominent microorganisms grown as there was more of the fresh lime juice which does not have a large shelf life outside of the refrigerator
Water	- Microorganisms were varied, trial 1 had a large almost black microorganism grown and trials 2 and 3 had small white microorganisms - Grew rapidly everyday starting from an average of 2.98cm^2 to 21.5cm^2

Observation Table:

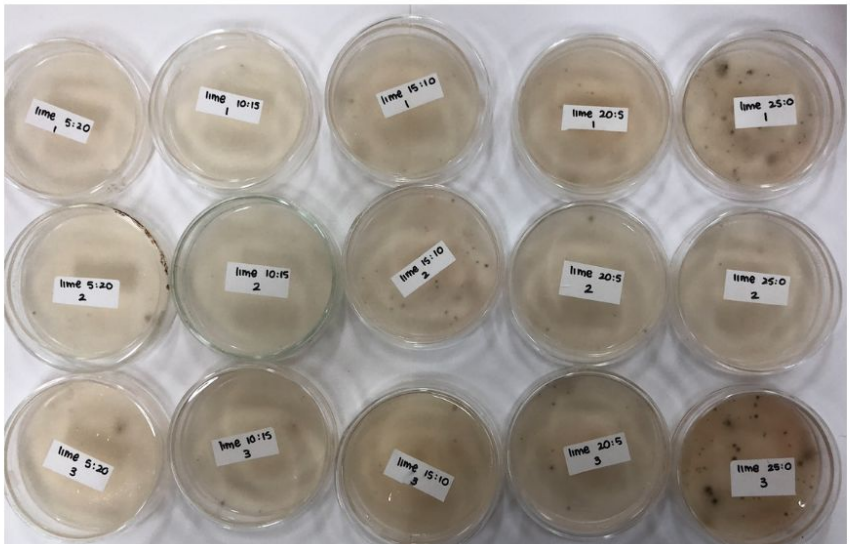
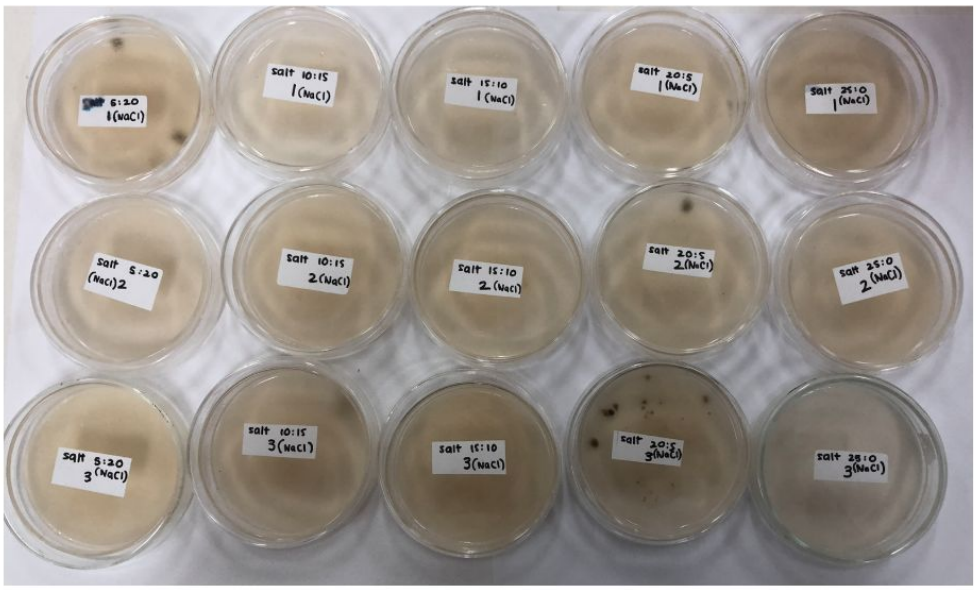
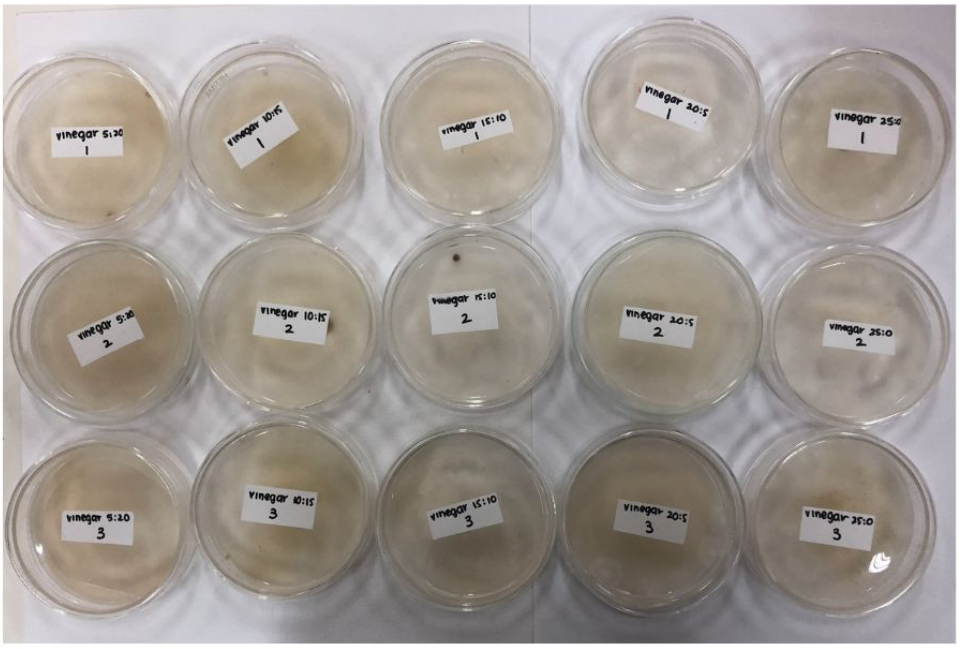
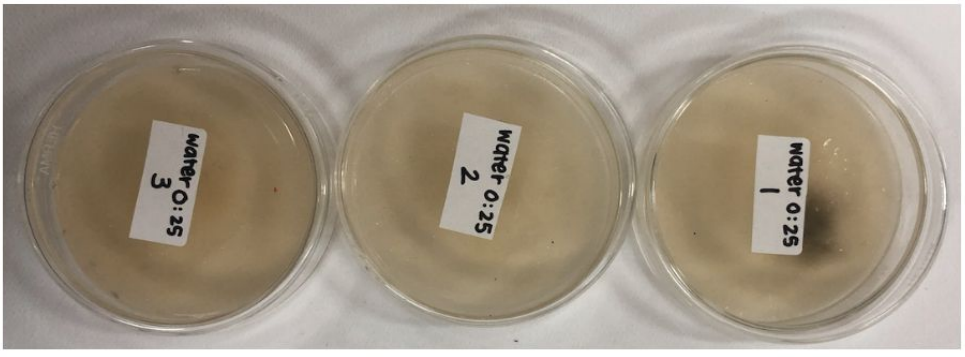
Concentrations	**Observations on last day (day 10)**
Lime	

Table Salt	
Vinegar	
Water	

Raw Data :

WATER -TRIAL 1							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
0:25	7.5	9	10	15	16.5	19	22

WATER -TRIAL 2							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
0:25	0.75	3.5	7.5	8.3	11.5	12	15

WATER -TRIAL 3							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
0:25	0.5	3.5	5.5	8.5	10.5	15	19

LIME - TRIAL 1							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
5:20	0	0	0.5	0.5	1	1	2
10:15	0	0.5	0.25	1	1	2	3
15:10	0.25	1	4	4	6	6.5	8
20:5	1.5	1.8	1.9	2	3	3.5	12.5
25:0	2	3	3	6	8	9	9.5

LIME -TRIAL 2							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
5:20	0.25	0.29	1.5	2	3	3.5	4
10:15	0	0.25	1	1.2	1.4	1.5	2.5
15:10	1	1.5	1.6	2	5	6	6.5
20:5	0.25	0.5	1	1.5	2.5	2.8	3.5
25:0	0.5	0.68	1	2	4	5	5.5

LIME -TRIAL 3							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
5:20	0	0.5	1.5	1.5	2.5	3	3
10:15	0.5	0.5	1.5	2	3.5	4	5.5
15:10	0.5	1	1	2	3.5	5	6.5
20:5	1	1.5	2.5	2.8	2.9	3	4
25:0	2.5	3	3	6	11	11	12

VINEGAR -TRIAL 1							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
5:20	6	9	11	11	12.5	14	14
10:15	8	9.5	9.7	9.9	10	10	10
15:10	9	11	14	15	16.3	17	19
20:5	4	7	13	13.5	15	15.5	16
25:0	12	13	13.5	14	16	16.5	18

VINEGAR -TRIAL 2							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
5:20	5	5.5	8	9	9.5	11	11.5
10:15	5.5	7	11	12	12.5	13	16
15:10	6.5	7	12	12.3	12.5	12.6	13
20:5	3	8	15	16	18	19	23
25:0	11	11.7	13.5	14	14.9	16.5	17

VINEGAR-TRIAL 3							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
5:20	4	4	5	5.5	7	7	8
10:15	2	3.5	7	7	8.5	8.5	9.5
15:10	2.5	5	5.5	6.5	7	7	7
20:5	2	2.5	3	8.5	12	12	14
25:0	8.5	11	24	11	12	17	18

TABLE SALT (SODIUM CHLORIDE) -TRIAL 1							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
5:20	1	1.5	4	5	6	7	9
10:15	2	2	2	3	4	4.5	6.5
15:10	0	0.5	2	3	4.5	4.5	7
20:5	1	1.5	2.5	4	4.5	5.5	6.5
25:0	0.5	0.5	1	1	1.5	2	2

TABLE SALT (SODIUM CHLORIDE) -TRIAL 2							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
5:20	0	0.5	1	2	2.5	2.6	3
10:15	0	0	0.5	0.75	1	1.5	2
15:10	0.5	1	2	2.5	3.5	3.7	4.5
20:5	1	1.5	3	4.5	4.7	6	6.5
25:0	0.25	0.5	1	1	1.5	1.5	2

TABLE SALT (SODIUM CHLORIDE) -TRIAL 3							
CONCENTRATIONS RATIO/ML	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
5:20	0.5	0.5	0.5	2	2	2.5	3
10:15	0.5	1	2	3	3	4	4.5
15:10	0	0.5	1	2	4.5	4.5	6
20:5	2	2	4	6.5	7	8.5	8.5
25:0	0	0	1.5	2	2	3.5	4

Processed Data :

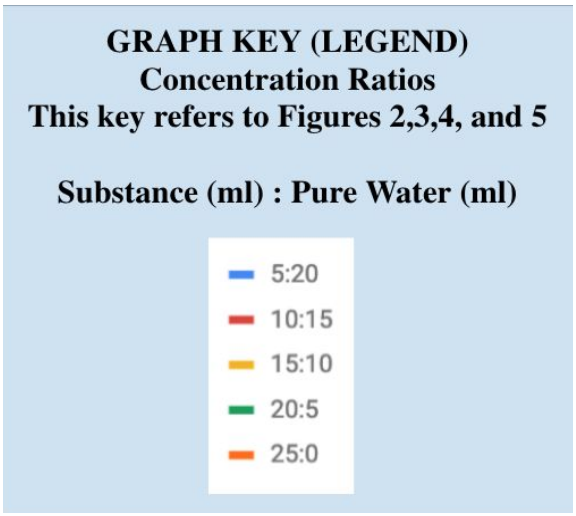
WATER							
CONCENTRATION RATIO/ml	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0:25	2.92	6.3	8.67	10	13.2	17.3	21.5

LIME							
CONCENTRATION RATIO/ml	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
5:20	0.25	0.83	1.17	1.33	1.83	2.17	3
10:15	0.17	0.33	1	1.33	1.83	2.5	3.67
15:10	0.58	1.17	2.17	2.5	4.83	5.83	7
20:5	0.92	1.17	1.67	1.83	2.67	3	6.33
25:0	1.67	2.17	2.33	2.88	4.67	8	9

VINEGAR							
CONCENTRATION RATIO/ml	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
5:20	5	6.2	8	8.5	9.7	11.2	14
10:15	5.2	6.7	9.2	9.2	10	10.5	11.8
15:10	6	7.7	11.2	11.5	11.8	12.2	13
20:5	3	5.8	10	12.5	15	15.5	17.7
25:0	10.5	11	13	14	16	16.7	17.7

SODIUM CHLORIDE							
CONCENTRATION RATIO/ml	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
5:20	0.5	0.8	1.7	2.7	3.5	4	5
10:15	0.8	1	1.5	2.3	2.7	3.2	4.3
15:10	0.2	0.7	1.7	2.5	4.2	4.5	5.8
20:5	1.3	1.7	3	3.5	5.3	6.7	7.2
25:0	0.3	0.7	1.2	1.3	1.7	2.3	2.7

Graphs :



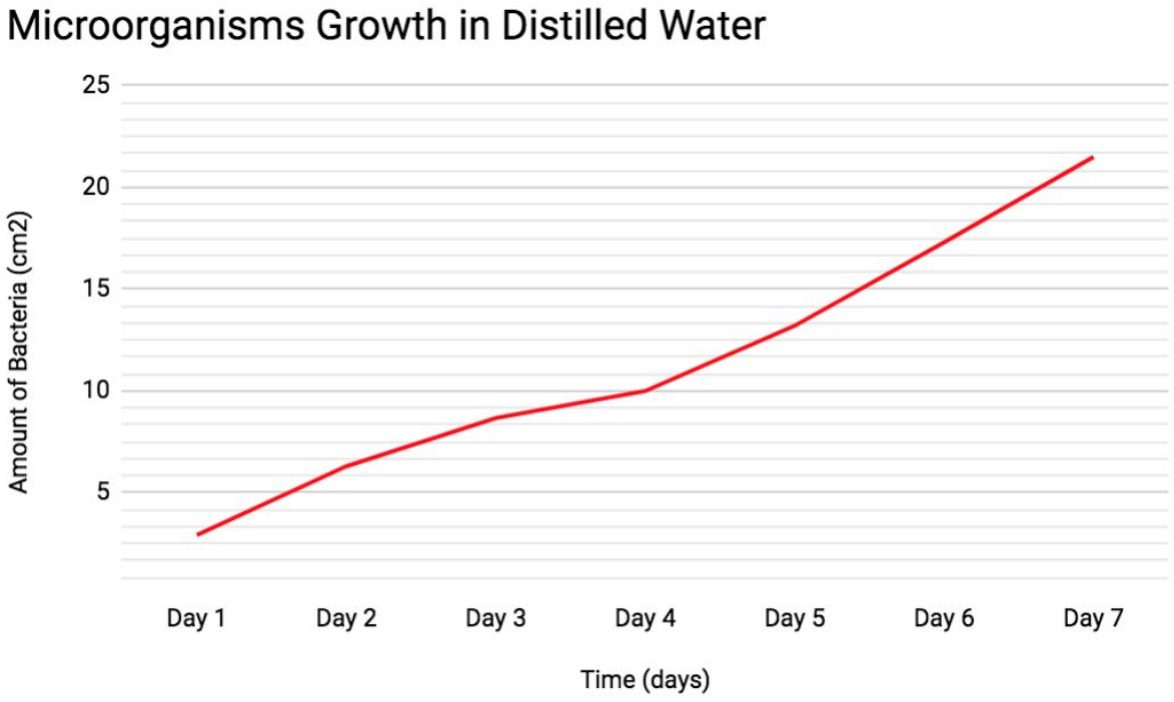


Figure 1

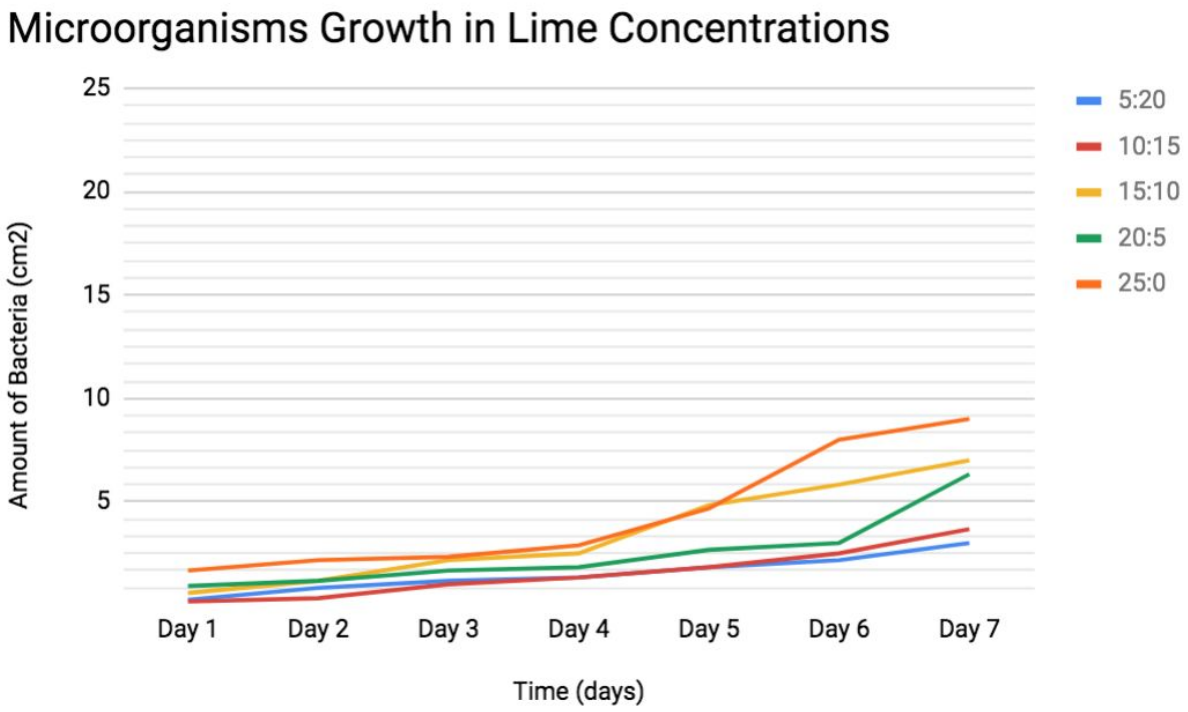


Figure 2

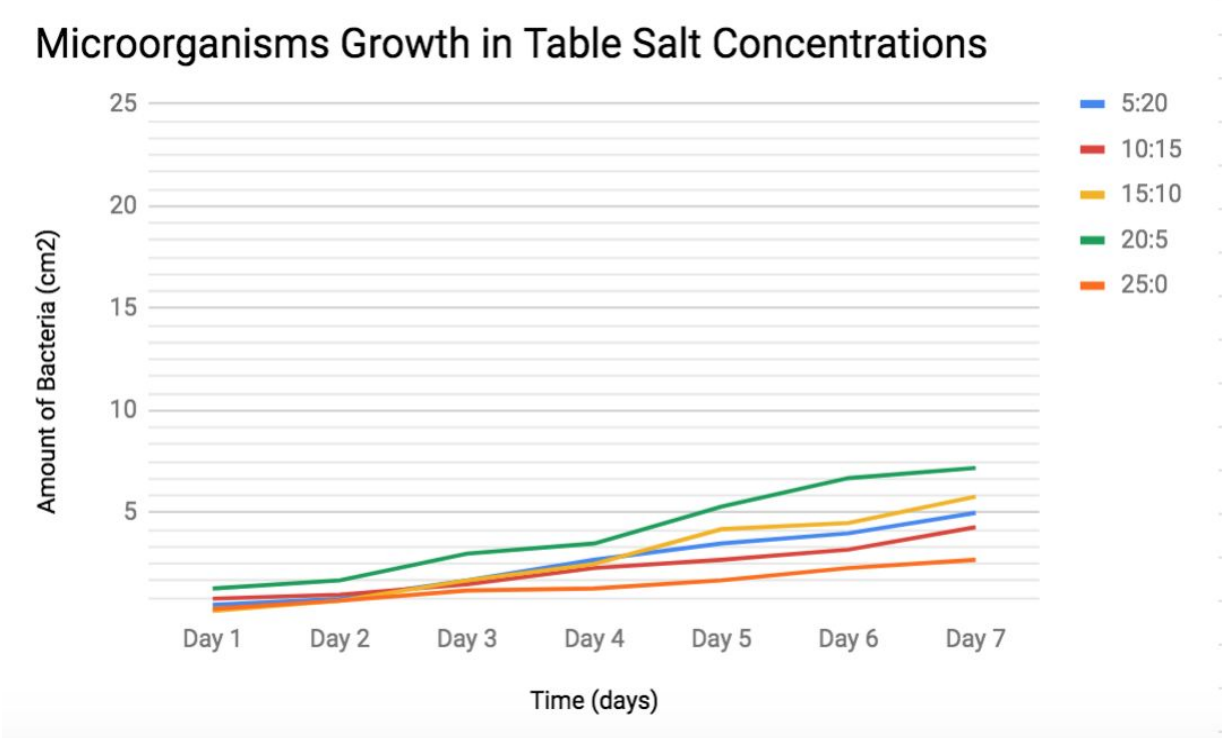


Figure 3

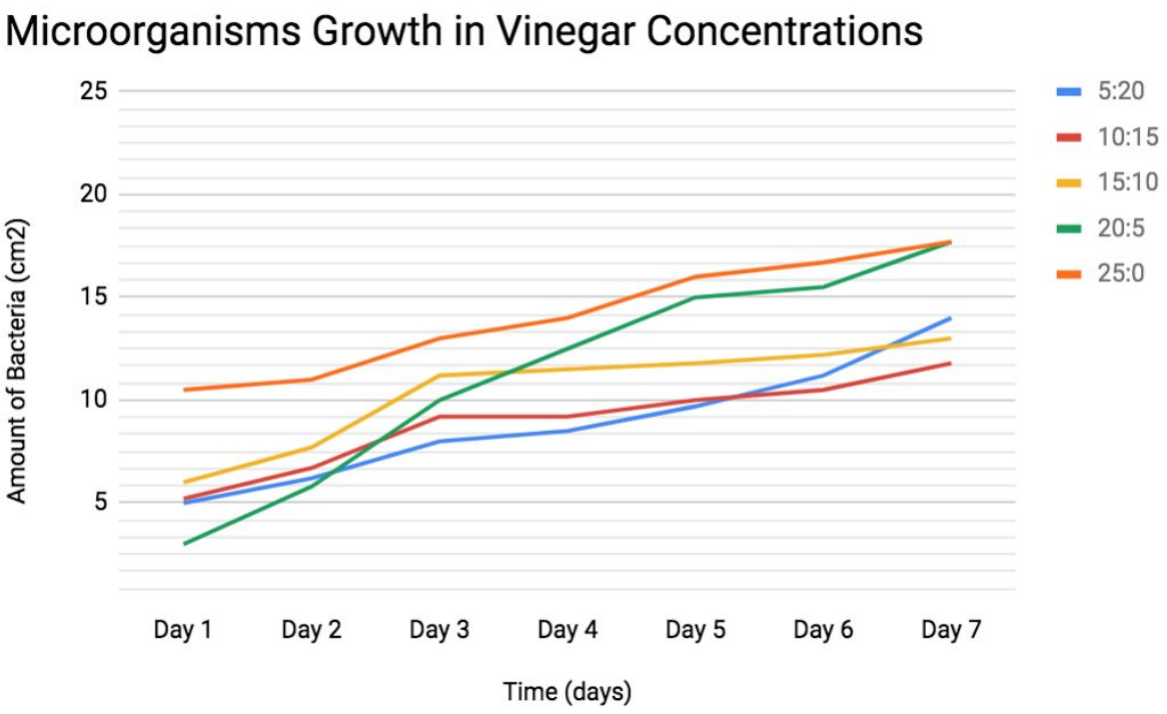


Figure 4

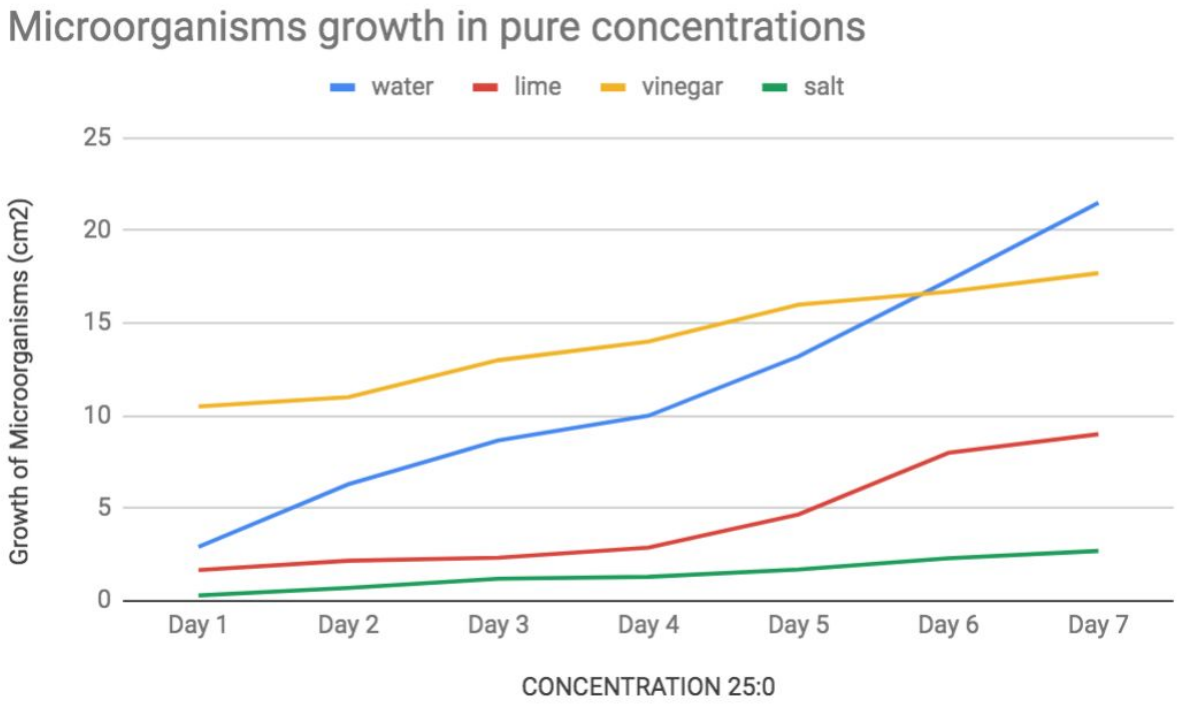


Figure 5

Conclusion:

This experiment had numerous descriptive results on how effective certain concentrations of different substances were for restricting the growth of microorganisms. Firstly, in regards to the most ineffective substance as a whole, that would be the vinegar. The comprehensive amount of microorganisms for the vinegar solutions was a higher result than that of all the other substances - excluding the controlled substance, water which had a mean surface area of 21.5cm^2 on Day 7. Continuing, regarding the prevention of the growth of microorganisms, the most effective substance was Sodium Chloride. The results accumulated for Sodium Chloride exhibit that the mean surface area of microorganisms on the final day of the experiment across all concentrations, was 5.0cm^2. Whilst the data for lime and vinegar manifest that the mean surface area on day 7 were 5.8cm^2 and 14.84cm^2 respectively.

In the first few days of data collection, the lime juice solution appeared to be the most successful overall with a surface area average of 1.67cm^2 across all of the concentrations by Day 3. However, the final results do not reflect this because of an increased growth of microorganisms especially in the last 3 days. The reason for this is that lime juice only remains fresh for 2-3 days in the fridge, and after this point can no longer be used as a preservative as it only increases the amount of microorganisms growing.

In addition to the documentation gathered concerning the efficiency of limiting microorganism growth, for the four different substances (including water) as a whole, another clear result is that throughout the range of substances, the most ineffective concentration was the highest (25:0), ultimately. It consistently had the highest level of microorganism growth over the seven days that the experiment was carried out for, across each of the substances. However, an anomaly was found in the data and is perceptible in the graphs, the results demonstrate that the data collected for sodium chloride was anomalous, because highest concentration was in fact the most effective at restricting microorganism germination.

The results of this experiment show that there are a variety of options for sustainable preservatives or cleaning products. The fact that there are this many options means that people can have the optimal option for their situation. For example, the most effective solution would be a vinegar solution of concentration of 15:10, but the fact that it is more expensive means that people would be less likely to use that option. Instead they could use a lime solution at 25:0 concentration which would have a similar effect with a lower cost. Another option would also be a sodium chloride solution of 10:15 concentration which also had a similar result. Perhaps the results of a natural solution wouldn't be as immediately successful as a chemical-based solution, however, there would be the opportunity to adjust the solution in order for it to have the highest success rate. There are also many options for whatever circumstances - such as cost, and availability.

Evaluation:

EVALUATION AND EXPERIMENTAL ERRORS		
Source of error	**Weakness/strengths**	**Suggested Improvements**
<u>Equipment and usage</u> 1. Balance to measure the mass of the chicken 2. Syringe to measure and keep the volume of agar-agar in each petri dish constant. 3. Ruler to measure the surface	1. All 46 pieces of chicken were measured precisely to 3 grams to ensure fair and reliable results 2. 20 ml of agar-agar was poured into each petri dish by using a syringe. The syringe ensured that the agar-agar was poured into	1. Very low error, however a more precise balance could have been used to implement the experiment further. Additionally, the surface area of the chicken samples could also be controlled to improve.

area of the petri-dishes'.	each petri dish slowly, making sure nothing will spill and create an impact in the results. 3. The surface area of the petri-dishes were measured carefully by using the formula πr^2. However, reading a ruler from the wrong angle or choosing the wrong marker to represent the experiment (parallax error) may have possibly impacted the results.	2. No improvements necessary, however human error such as a parallax error is possible and should be minimised. 3. No improvements necessary, however human error such as a parallax error is possible and should be reduced.
<u>Independent Variable</u> 1. The range of liquids tested 2. The range of concentrations for each liquid. 3. Number of trials conducted for each concentration.	1. There were 4 different liquids which were tested (water, lime, salt, and vinegar) this is a sufficient amount to analyse comparisons and see differences between the growth of microorganisms. 2. The concentrations tested for each liquid is a wide enough range to evaluate which ratio is best for cleaning microorganisms. 3. Only 48 petri dishes were available, and used in order to repeat the experiment exactly 3 times for reliable results	1. No improvements necessary, however it could be possible to add more liquids if the experiment were to be conducted in the future. 2. No improvements necessary, an extension would be to add an even larger variety of concentrations. 3. 5 trials should have taken place to provide more accurate and reliable results. To improve, more petri dishes should be provided. `
<u>Dependent variable</u> 1. Method of calculating the surface area of microorganisms	1. The surface area of microorganisms covering each petri dish were collected by placing the petri dishes on top of a light box. Gridded paper (1x1 cm) was then placed under the petri dish in order to measure and Place the petri dish on a light box, putting gridded paper (1x1cm) under the petri dish in order to measure how much surface area (cm^2) of microorganisms have grown. Occasionally, the micro organisms were scattered around the petri-dish and it was hard to count how much covered each square on the gridded paper. Additionally, fogging/condensation occurring in the petri dishes made it hard and distracting to identify the microorganisms.	1. An alternative way to measure the amount of microorganisms would be to measure the change in mass of the petri-dishes using an accurate balance/scale.

Controlled Variables 1. Mass of chicken 2. Time given for the microorganisms to form 3. Environment in which the chicken is placed 4. Same type of limes 5. Amount of alcohol used for sterilizing the equipment. 6. Same type of salt 7. Type of water 8. The method of converting table salt millilitres to grams 9. Acidity of vinegar	1. Each chicken sample was exactly 3 grams per piece and rubbed onto the agar -agar on each petri-dish the same number of times. 2. The petri-dishes were left for 4 days, which is the time needed to visually see the microorganisms. After 4 days, the recording process of the microorganisms begun. This process took place for a span of 7 days. 3. All petri dishes were stored in a cupboard to ensure that they are kept in the same temperature and are unexposed from direct sunlight. 4. It is important that the species of lime (key-lime) is kept constant throughout the experiment, as different types of lime have different acidity levels, which could have an overall impact with the microorganism growth. 5. All equipment was sterilized using rubbing alcohol (80%) to ensure that no bacteria interferes with the experiment. 6. Table salt/sodium chloride (NaCl) was the only salt used throughout the experiment for a fair test. 7. Boiled water, was the only type of water used throughout the whole investigation. This may have provided unintended oversight and made the results less relevant to people who might use tap water or dirtier water for personal	1. No improvements necessary, however an alternative option would be to control the surface area as well as the mass of the chicken samples. 2. In the future the microorganisms could be recorded for more than 7 days for a more detailed analysis. 3. It is difficult to keep the temperature controlled as the temperature of the cupboard is constantly changing. An improvement would be to place the petri dishes in a temperature controlled unit to ensure that the test remains fair. 4. No improvements necessary, however as an alternative option different species of limes, perhaps wild limes, or lemons as an option for countries in which lemons are more readily available at a better price (such as Australia) could be tested. 5. In the future different Alcohol concentrations could be used to analyse its impact on microorganism growth. 6. No improvements necessary, however as an alternative option, different variations of salt (kosher salt, sea salt, etc) could be used to analyse whether it will have an impact on the growth of microorganisms. 7. In the future, the type of water could act as an additional independent variable to the experiment. The experiment can be focused on how different types of water (distilled, boiled, cold, tap, etc.) affect microorganism growth. 8. The correct method of converting salt milliliters to grams should be used in future experiments: -To calculate table salt conversions from millilitres to grams, the density of salt must be found first, to guide us with the

	use. 8. There was confusion in the way table salt should be converted from ml to grams. It was assumed that because 1 millilitres of water converts to 1 gram, the same calculations could be used with table salt. However this is not the case. 9. The same type of vinegar (25%) was used throughout each experiment for fair results.	calculations. Milliliters is a volume unit and grams is a mass unit. To convert from millilitres to grams we multiply the measurement in ml by the density of table salt which is unknown until measured. The approximate average density of table salt is 1.2-2.16 g/cm³ (however this varies with the brand of salt used). In reality 1 ml of salt is not equivalent to 1 gram of salt (this is only water's conversion) Instead 1 gram of salt is equal to 1 ml multiplied by the density of table salt. 9. In the future, the vinegar acidity could act as an additional independent variable to the experiment for a deeper analysis.
<u>Uncontrolled variables</u> 1. The dirt and bacteria in the air surrounding the chicken.	1. When conducting the procedure, bacteria/dust from surrounding could come in contact with the agar dish or the chicken. Causing some unreliability in the results.	1. The petri dishes could be placed in a more secure location, when the microorganisms were grown. For example, storing them in a cupboard that was moisture and dirt free would be effective as it will ensure that excess bacteria will not contaminate the petri dish. Additionally, the petri dishes could have been sealed for the entire duration of the experiment, so that they weren't accidently opened and exposed to bacteria in the air.

Team Reflection

In these past few weeks, our group has managed to work cohesively, and successfully carry out our experiment. The purpose of our group project was to not only investigate how different acidic liquids affected the growth of microorganisms, but it was also to encourage others to deepen their understandings on the importance of hygiene and even more significantly their health; in terms of unnatural preservatives and chemical cleaning products.

The participation of this investigation has implemented many skillful lessons within our team, such skills included problem solving, observing situations, communication, inferring theories and forming conclusions. These different skill sets were used throughout the process of the experiment in many different forms. Additionally, this experiment helped us as learners to grasp the main idea of working together and collaborating, which was what pulled these skills into action. Not only were these skill sets useful throughout the investigation, but will also be valuable for us individuals in future opportunities. As this shows, that the experiment not only showed us the significance of its original objective but taught us many skills that can also be useful later on in life such as work environments or group projects in the future of schooling.

The project was reasonably well planned out, and we all put in effort towards the final project. We believe that it was organised well, and that we weren't rushed before it was presented. The process of the experiment as a whole was well thought out from the beginning, so as to make it clear how the work would progress. The team worked well together, and we made sure that no one was doing all of the work themselves. The only issues that were found throughout the experiment were easily fixable mistakes to do with the tests and investigation itself. These issues were also easily solved because of how we worked together well and without disagreement. However, we only gathered outside of school once, and work could have possibly been completed more efficiently if we had multiple meetings. Also, we had to rely on teachers for a few aspects - such as materials and a space for the experiment. If it were possible to remain more independent, that would definitely be an improvement. An additional improvement that could be made is making personal jobs and deadlines clear and certain so that there would be no one falling behind with their workload. This would visually be made clear by a table containing important information such as dates and tasks. Once a deadline had been reached all members of the group can evaluate work and discuss about improvements and additional ideas. In the case that a group member does struggle with a section of work we can easily identify this concern and offer our support as a group should. We would be able to resolve issues more efficiently in our written investigation and work towards a group with even better effective communication and teamwork skills.

As mentioned before, we tested four different liquids (vinegar, lime juice, distilled water and salt water). These are all fairly cheap and easily accessible but in order to further develop our understanding of the benefits of using natural products we could also test chemically made cleaning products. This will allow us to advance our original research question. As testing the efficiency of chemically based cleaning products will allow for deeper analysis. As a compare and contrast would be able to take place. This sort of data will be used to see if natural cleaning products can work just as well to chemical cleaning products.

Overall at the end of the project, what matters most is what we have learnt from our team work experiences. The experience of working together throughout this experiment, has brought in difficult situations which have tested us in terms of making decisions under pressure, and coming up with innovative solutions. As a result of this, we have achieved a strong outcome which not only enhances our knowledge, but also empowers our group as a whole. This investigation taught us several skills for instances inside and outside of school environments, and helped to improve our investigation skills generally. The opportunity that we had to experience an

individualised project was an experience which will ultimately help us going forward, and allow for our future projects to have more depth, understanding, and creative thinking.

Individual reflections

Team member 1

The Cambridge Upper Secondary Science Competition was a huge opportunity which I had the chance to participate in. The process of the investigation taught the team many lessons, as well as challenging us with critical thinking and preparation. All team members were cooperative and worked efficiently throughout the experiment, showing responsibility and dedication through their hard work in all aspects. The team put in strong enthusiasm and effort into the experiment, which made the overall experience well enjoyed for us students. As well as, raising awareness and gaining knowledge on the ongoing problem of unnatural preservative usage in daily lives. Not only did our experiment help further understanding of the topic, but allowed us to be present and helpful in ongoing problems in our community. The tragic Earthquake which occurred in Lombok was what really inspired us to find a solution for such a worldwide issue. As the citizens of Lombok were suffering from the cleanliness of their surroundings and general hygiene, which caused many illnesses to arise. Investigating these cheap and sustainable alternatives, in order to improve the citizen's hygiene throughout this crisis was something our team was highly passionate about. Overall, this was a significant experience to remember as I was able to work on a serious and important matter with a great team.

Team member 2

After completing the Cambridge Upper Secondary Science Competition investigation and developing a dedication towards the purpose of our experiment I have learned several valuable lessons and I'm sure that I can say the same for my fellow group members. From inquiring, collaborating, resolving, and experimenting, we have attained skills that will not only help us at the present time but in many years to come; thinking innovatively, creatively and communicating successfully. As a team all members were positively involved, we functioned well with one another, supported in addition to learned from each other and in result grew because of our teamwork. When faced with challenges the dependability of our team was what essentially guided us to overcome these setbacks. Our team was always devoted to completing this piece of work to the best of our ability, and this is what inspired all team members to work effectively. With researching the best suitable acidic liquid in addition to concentration, our team will be able to aid in local and global problems of chemical cleaning and unnatural preservatives; considering high availability and cheap prices as important factors. In less economically developed countries such as Indonesia, there are concerns regarding hygiene and health. As a group, we are passionate about how our findings can influence communities and people around the world. We aspire that our conclusions can impact the world for the better: help people, teach people and allow people to thrive under better circumstances. Honestly, I believe our team is able to accomplish this. In the most devastating situations of natural disaster and slum livelihood, cleanliness, as well as the preservation of food, may not always be a possibility. Yet our team hopes that our conclusions are capable of providing new cheap and accessible solutions. In summary, reflecting back on teamwork, it is evident that our cooperativeness and enthusiasm has been entangled into our work. I am very fortunate and humbly able to say that this investigation not only benefits my group and me but possibly everyone around the globe.

Team member 3

I am very grateful to had received the opportunity to partake in The Cambridge Upper Secondary Science Competition. This remarkable experience has taught both my group and I critical skills which will be used in our future experiences. Teamwork was a crucial aspect of the completion of this assignment, and it may well be argued that all group members worked together accordingly. I enjoyed cooperating with all my team members as we all helped each other throughout this learning process by coming up with new ideas collaboratively, and by enlightening each others creativity. One important accomplishment which I am personally very proud of, was the passion and determination all group members shared which I believe is the main reason we all worked amazingly well. A fundamental aspect which helped us choose our experiments topic, was the recent natural disasters which have affected our local community. We detected the impact these recent natural disasters delivered into our local community and decided that one significant issue which our local community and many other people around the world face, is the lack of knowledge of the importance of proper sterilization and how it could affect overall health and hygiene. Therefore, we decided to deepen our knowledge about this social situation even further by performing this investigation, in order to then each others about this situation. Overall, it is easy to say that we all worked cohesively as a group and all gained knowledge which will be valuable for us in the future.

Team member 4

I found that participating in the Cambridge Upper Secondary Science Competition was an unforgettable experience for both myself personally, as well as my group. Throughout the time we spent tirelessly working on this project, we managed to learn many lessons and pick up many new habits which will in turn help us in our future of schooling and working. The tasks we had taught us how to communicate our ideas clearly and effectively in order to come to the best possible solution - especially in the brainstorming phase near the beginning of the investigation. We also had the opportunity to become practiced at teamwork and compromise in the instances where we needed to come forward and make sure that everything was working well for the experiment and the group. I believe that the team worked incredibly well together, and we were cooperative, effective team mates. Everyone was dedicated to making this project the best that it could be, and that made it so that we could work without major issues within the group. There was always a feeling of enthusiasm and passion about the matter at hand.

Another aspect of this project was how it affected the world around us. For our team, this was one of the most important parts of the investigation. We took the time to make sure that we were doing something that could be beneficial for not only Indonesia, but for people worldwide. When coming across the issue of hygiene, we knew that this was a subject that isn't thought of enough, and is definitely one of the most important factors to a person's health and lifestyle. By finding accessible ways for people in less desirable situations to have clean food, it ensures that people are living a more comfortable life, and as people with so much privilege, we wanted to make sure that we weren't taking what we have for granted. In conclusion, the project as a whole was a great success and I am honored to have been a part of such an incredible opportunity, which helps not only me, but could also help others around the world.

Team member 5

My group and I had the wonderful opportunity to partake in the Cambridge Upper Secondary Science Competition and after completing the investigation it is safe to say that we all learnt important lessons and skills for our future. Teamwork was a very important factor to completing the investigation as we all overcame obstacles that may seem challenging together as a group. When figuring out what to make our investigation about we made it crucial that the main element of our investigation was to ensure that it impacted the world by being more environmentally-friendly.

Hygiene in third world countries isn't as of an important aspect compared to food and water with low income individuals and most cheap cleaning products used everyday contain chemicals which are harmful to our health. As a way to improve this we used cheap and easily accessible items that work and benefit the health of others. To sum up, this investigation went smoothly and has definitely taught me many important life lessons that are useful later on in life.

Team member 6

To reflect upon the process of our experiment, I would say that our group worked tremendously well together, we completed the experiment without any conflicts or disputes. In addition, the burden of work was split up quite evenly between the six of us, everyone contributed and did their part to the best of their ability. I am proud of our team for putting in the extra effort to come to school on weekends to collect raw data, this really manifested our commitment and dedication. If we received the opportunity to go through this whole process again, I would suggest that we spend more time rehearsing our presentation as a group to further solidify the fluency of the presentation and get the opportunity to give or receive feedback on out individual parts. Personally, I thoroughly enjoyed the process because I managed to retain plenty of new knowledge, I also gained more experience with writing a lab report which will help me in the future. Overall, I would say, I am overjoyed with the out come of this process, a large part of this would be because or team was able to work cohesively and effectively communicate.