Oxidizing, Non-oxidizing And Surface Active Agents, Which Is The Most Effective

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Oxidizing Agents

Oxidizing agents kill pathogens by oxidizing them, when an oxidizing agent comes in contact with a pathogen it penetrates the cell wall, when inside the cell, it releases molecules called reactive oxygen species (ROS). These highly reactive molecules damage the pathogen's cell structure, they can react with and damage the proteins, lipids, nucleic acids and DNA of the pathogen, making it unable to replicate itself. This causes the pathogen to die. Oxidizing agents can be really effective against bacteria, fungi and viruses but tend to be less effective against spores.

Non-oxidizing Agents

Non-oxidizing agents can kill pathogens by interfering with their cell walls and cell membranes, disrupting cellular functions and ruining the stability of the cell. The cell wall or membrane is made up of macromolecules (proteins, lipids etc.), they act as a support structure for the cell. When non-oxidizing agents interact with these macromolecules they change the physical properties and charge of the membrane. This will either kill or inactivate the pathogen.

Surface Active Agents

Surface active agents have several ways to kill or deactivate a pathogen. The most common way is by disrupting the cell membrane. Surface active agents interact with macromolecules to cause disruptions in the cell, which leads to the cellular contents leaking, killing the pathogen. Surface active agents can also disrupt the cellular functions of microbial cells by interfering with their metabolism. For example, they can stop enzymes and the production of energy in the cell, causing it to die. The last way surface active agents can kill pathogens is by preventing them from performing important functions like replicating DNA and protein synthesis (making proteins).

Active Ingredients Used

Type	Ingredients	Chemical Formula
Oxidizing	Alkyl (67%)	CnH2n+1
	Hydrogen peroxide (1.0%)	H2O2
Non-oxidizing	Acetic acid (10.0%)	CH3COOH
	Citric acid (5.0%)	C6H8O7
Surface active	Myristyl glucoside (3.0%)	C33G65O12
	Caprylyl glucoside (2.5%)	C16H32O9

Physical And Chemical Properties Of The Agents

Oxidizing	Non-oxidizing	Surface active
- High electronegativity, which means they hold more electron cloud density around the central atoms - Highly reactive, they react immediately with reducing agents - Accept electrons from other substances - Colourless but have a strong smell - Can be corrosive in high concentrations - Can release energy and heat in oxidizing reactions - Release electrons during chemical reactions, making them more stable	- Give electrons to other substances - Can create coloured compounds when they react with some substances - Oxidize if they become too unstable - Can reduce many substances (accept electrons from them) - They don't have oxygen atoms so they are non-flammable and non-volatile (they don't evaporate rapidly in room temperature conditions) - Can be naturally occurring or synthetic	- They have a hydrophobic and a hydrophilic end , the hydrophilic end is attracted to water molecules which the hydrophobic end is attracted to nonpolar molecules - Have the ability to reduce surface tension between a liquid and a solid or a liquid and a liquid - Have the ability to lower the surface tension of water, making it easier to wet surfaces -Ability to form a monolayer at a liquid to liquid interface and form micelles in solutions that are aqueous (explained below) - They are nonionic

Types Of Pathogenic Bacteria That Could Have Grown

Lactobacillus species: This species is associated with the fermentation process and can be found on human mucous membranes, skin and in our digestive system.

Micrococcus species: This species can be located in the environment, the soil and water.

Hypothesis

I believe that oxidizing agents will be more effective at killing pathogens than the other agents because they are more reactive. This makes me believe that they will be more effective at killing pathogens since they have to react (oxidize) with the pathogen in order to kill it. I also believe this because they are the most commonly found disinfectants in the market, they must have to be good if they are most commonly being sold.

Variables

Manipulated variable: The disinfectant used

Responding variable: The number of bacteria that is killed by the disinfector.

Controlled variable: Temperature of the incubator, Amount of disinfectant used on slides, Amount of soy broth in each tube , Amount the filter paper is put on the solution and Where the bacteria comes from (surface used to collect bacteria).

Uncontrolled Variable: Concentration of the disinfectants, how quick the bacteria grew, the amount of bacteria that grew.

Material

» 6 disinfectants
» Test Tubes (121)
» Peptone water (800 ml)
» Ethanol (for cleaning equipment)
» Tryptic Soy Broth (250 ml)
» Test Tube holders (7)
» Sterile Swabs (22)
» Droppers (112)
» Slides (21)
» Coffee Filter Paper
» Petri Dishes pre-filled with nutrient agar (22)
» 7 plastic containers
» Soldering iron
» 40 w bulb
» Cooler
» Thermometer
» Masks, gloves, apron and hand sanitizer

Procedure

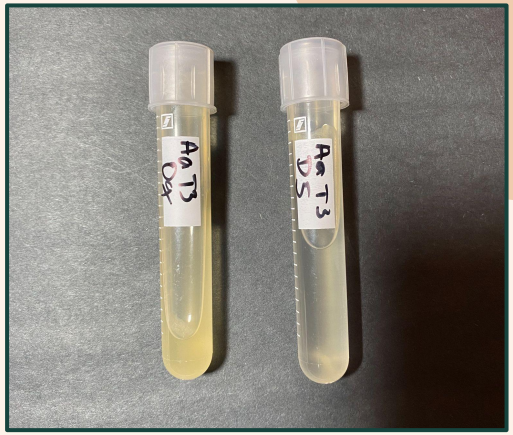
- Label the Petri dishes
- Make a homemade incubator (put a lightbulb in a cooler)
- Prepare the surface for placing slides
- Print the labels for test tube holders, trials and active ingredients
- Take out a sterile swab and swab the shoe mat, sink and door knobs
- # Make 7 lines in the petri dish in a zig-zag pattern with a swab
- Rotate the petri disk and make 7 more lines
- Incubate the petri dish for 3 days
- Make test tube holders
- Use a sanitized toothpick to transfer the bacterial colonies into a test tube
- Place test tube in test tube holder
- Fill the test tube with 10 ml of peptone water
- Shake the test tube and let it sit for 2 hours,making a bacterial solution

Procedure

- Sanitize the slides with ethanol to be used for pouring the bacterial culture solution from the test tube
- Place the slides on a sanitized wooden board covered with non-pores plastic wrap
- Cut filter paper to be x2 the size of the slides
- Place the three slides onto the wooden board equally spaced for each disinfectant plus one for control
- Pour 0.25 ml of the bacterial mixture with a dropper and let it air dry
- Pour 1 ml of each of the disinfectants into 1 tube and label each tube with the name of the active ingredient
- Dip a filter paper in 1ml of disinfectant and put it over slides for all three slides
- Let the filter paper sit for 5 mins
- Prepare 3 test tubes with 10 ml of tryptic soy broth for each active ingredient
- Put one slide in each tube and label it T1 T2 T3 for all three trials

Procedure

- In the first serial dilution D1, take 1ml of original bacterial culture solution from the test tube containing a slide dipped in tryptic soy broth and add it into a test tube filled with 9 ml of peptone water.
- In the second serial dilution D2, take 1ml from the D1 test tube and add it into a test tube filled with 9 ml of peptone water.
- In the third serial dilution D3, take 1ml from the D2 test tube and add it into a test tube filled with 9 ml of peptone water.
- In the fourth serial dilution D4, take 1ml from the D3 test tube and add it into a test tube filled with 9 ml of peptone water.
- In the fifth serial dilution D5, take 1ml from the D4 test tube and add it into a test tube filled with 9 ml of peptone water.
- Now, repeat the same process for all the three trials T1 T2 T3
- Take all three D5 test tubes. You can clearly notice the color change in the first test tube and the last D5 test tube. It has become a very light colour after 5 serial dilutions.
- Incubate all of the 5th dilution test tubes for 12 hours under temperature 27-32 C.
- Repeat the same procedure for all the disinfectants



Procedure

- Label the petri dish with the name of the active ingredient and the trial number
- Take a test tube out of the incubator and use a dropper to take 1ml of the solution and drop it into the petri dish in a zig zag pattern
- Repeat the same steps for all 15 test tubes.
- In the end, you will have 21 Petri dishes (3 trials for all 7)
- Incubate the Petri dishes for 2 days and take observation every day
- Notice the odour changes, number of colonies, and colony morphologies
- Put bleach in the Petri dishes to kill the bacteria at the end of the experiment

One Page Procedure

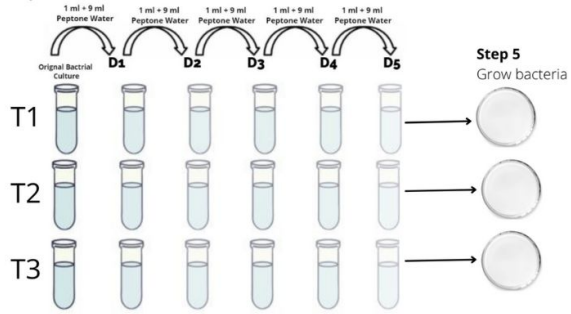
Step1
Grow bacterias for 3 days and transfer colonies to test tube

Step2
colony samples +sterilized water

Step3
Take 0.25 liquid from the test tube and put it on all slides. Let it dry. Put 1 ml of disinfectant on a coffee filter paper and cover slides for 5 minutes.

	C	A	HP	CA	AA	MG	CG
T1							
T2							
T3							

Step4 **Serial Dilution of Bacterial Culture**



Step 5
Grow bacteria

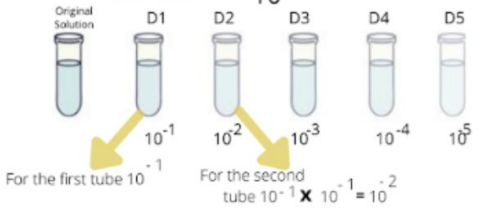
Step 6
Repeat the all serial dilution steps for control and all disinfectants

Calculation Formula

The serial dilution I have used in my experiment is called a ten-fold dilution, this is because I started with 10 ml of bacterial culture.

$$\text{Dilution Factor} = \frac{\text{Volume of the sample}}{\text{Volume of the sample + Diluent}}$$

$$= \frac{1\text{ ml}}{1\text{ ml} + 9\text{ml}} = \frac{1}{10} = 0.1 = 10^{-1}$$

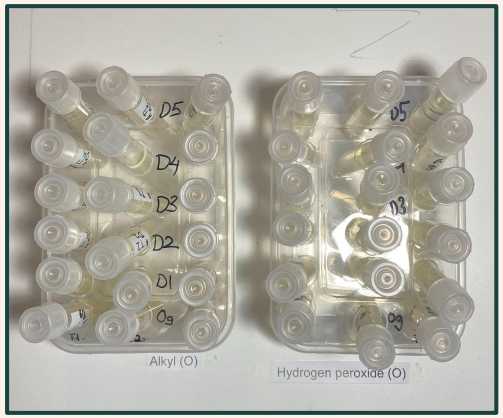


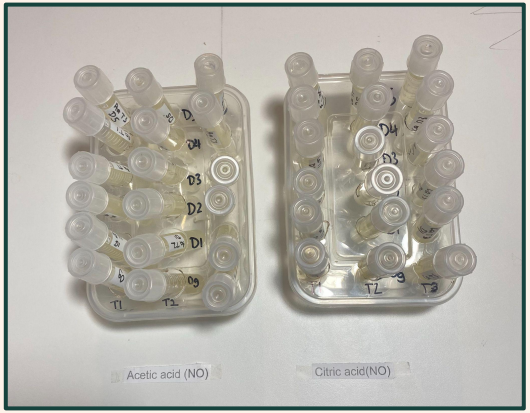
Every time there is an addition to the next tube the dilution factor would change

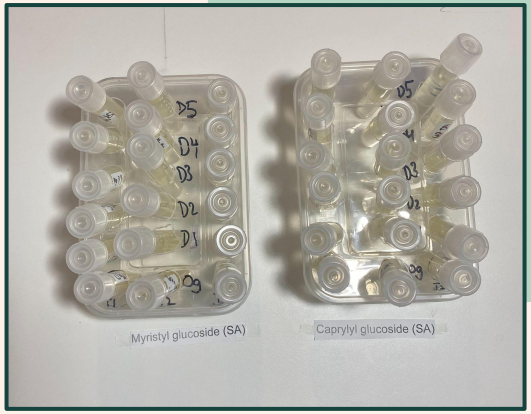
$$\text{Formula} = \frac{\text{CFU}}{\text{Dilution Factor}} \times \text{Volume of Sample}$$

Example: For the 8 colonies after serial dilution it would be (8 X 10^5) X 1 if we haven't applied serial dillution.

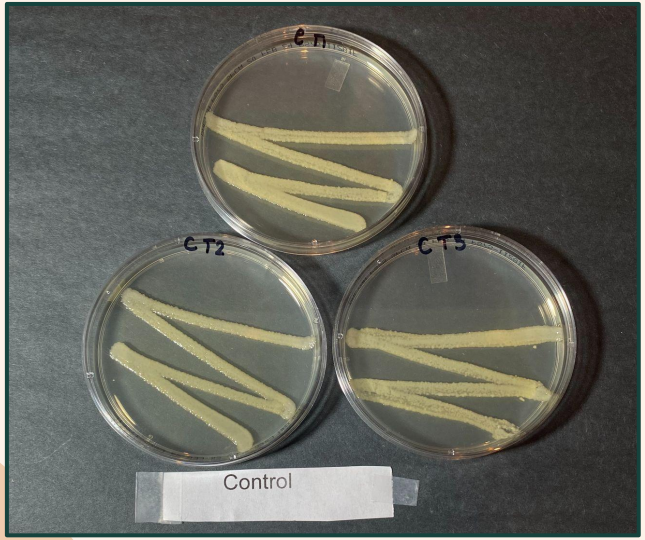
Test Tubes After Dilution





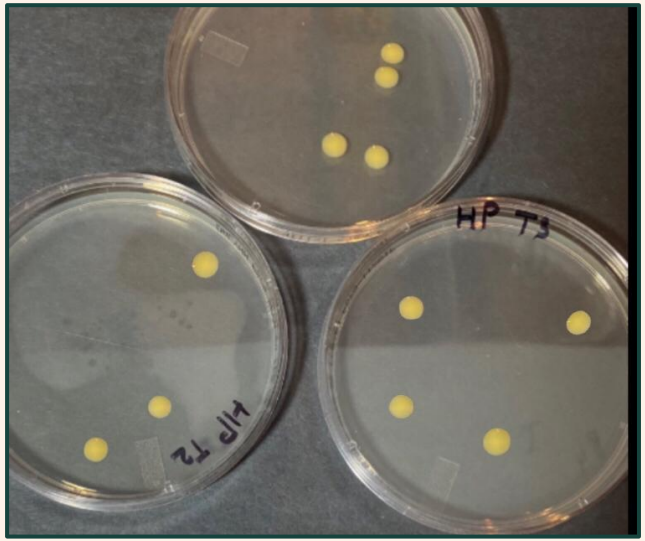


Results (Control)

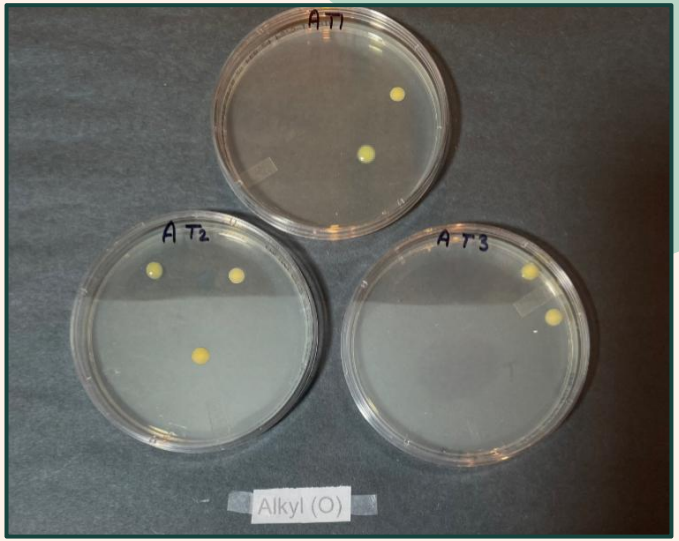


Way too many colonies grew, anything over 330 is considered “uncountable” after serial dilution.

Results (Oxidizing)

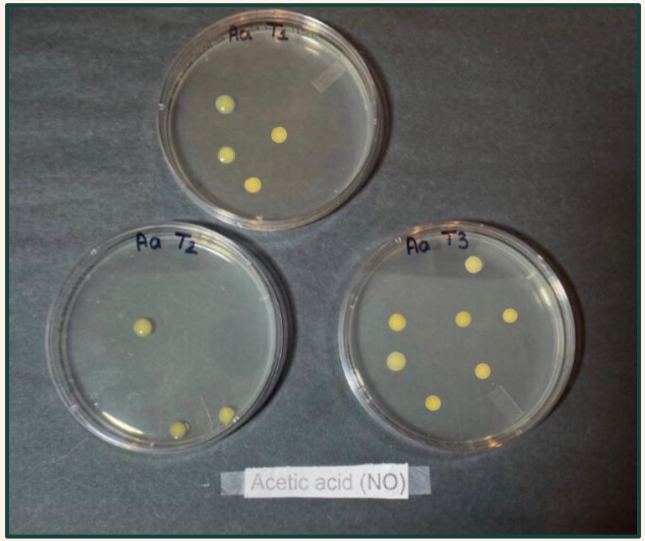


T1- 5
T2- 3
T3- 4
Avg- 4

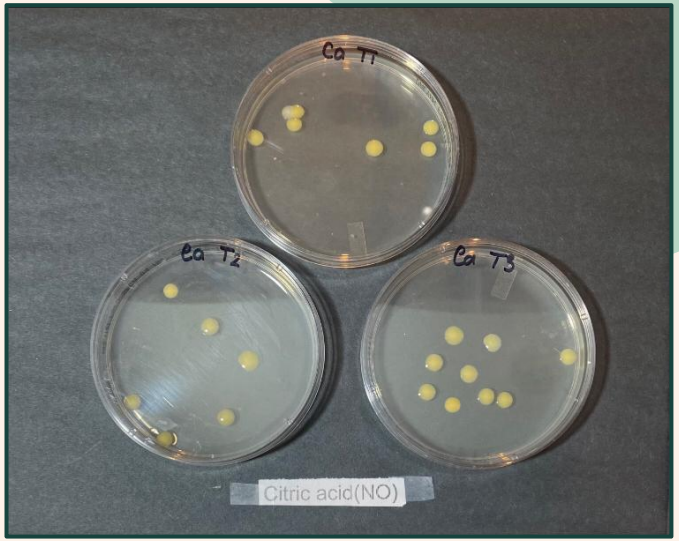


T1- 2
T2- 3
T3- 3
Avg- 3

Results (Non-oxidizing)

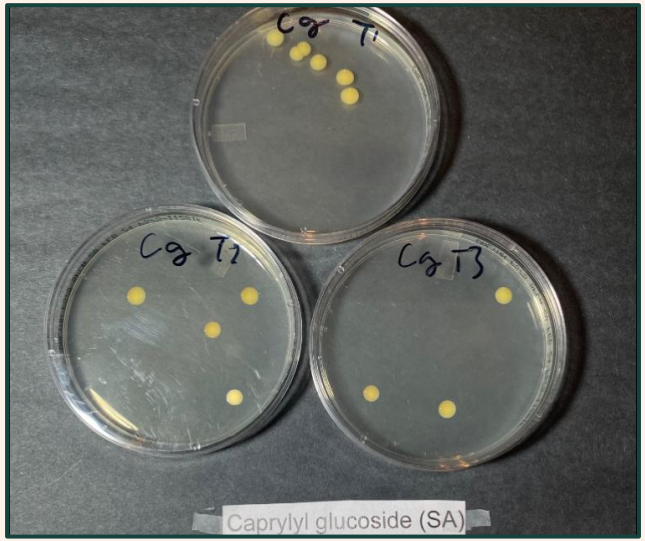


T1- 7
T2- 6
T3- 7
Avg- 7

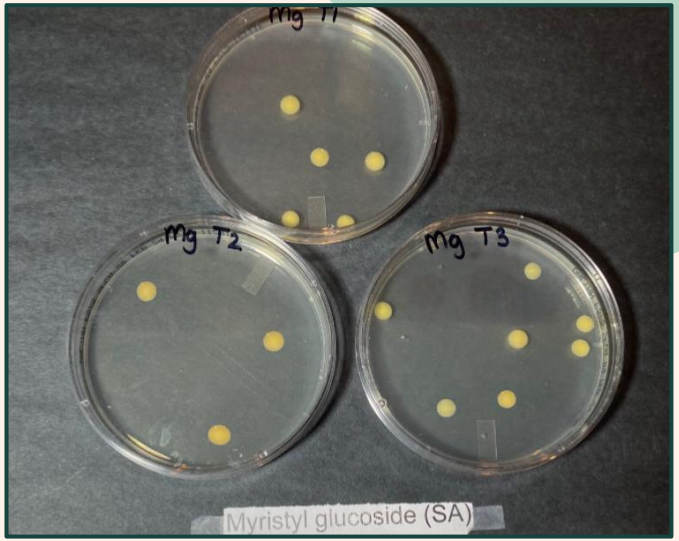


T1- 8
T2- 6
T3- 9
Avg- 8

Results (Surface Active)

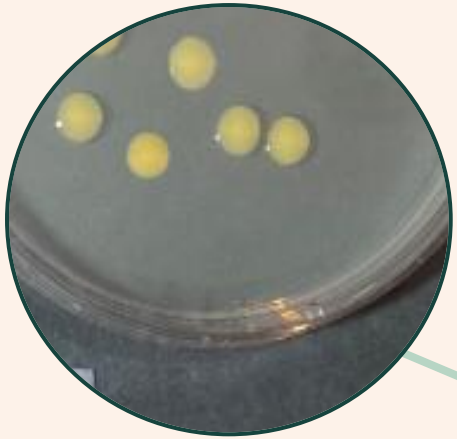


T1- 6
T2- 4
T3- 3
Avg- 5



T1- 5
T2- 3
T3- 7
Avg- 5

Colony Morphology

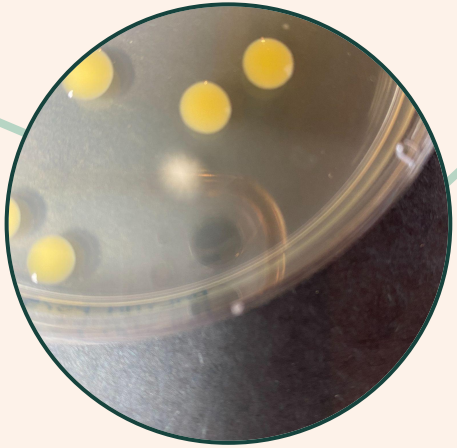


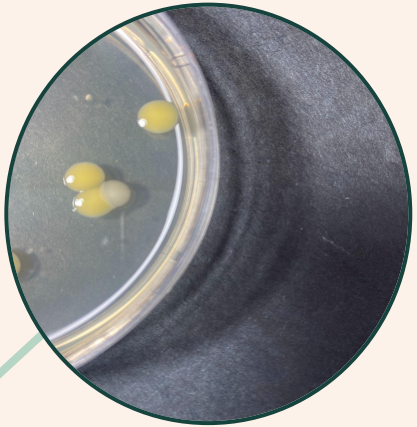
Coloured Colonies

Yellow colonies that looked shiny, round and raised

Filamentous Colony

Dusty white, Irregular, flat and filamentous





Merged Colonies

White and yellow, merged and raised

Data

Type	Active Ingredient	Average # Of Colonies
	Control	All colonies were merged
Oxidizing	Alkyl	3
	Hydrogen peroxide	4
Non-oxidizing	Acetic acid	7
	Citric acid	8
Surface-active	Myristyl glucoside	5
	Caprylyl glucoside	5

Data

Active Ingredient	Serial Dilution Calculation	Colonies Without Dilution
Alkyl	$(3 \times 10^5) \times 1$	300000
Hydrogen peroxide	$(4 \times 10^5) \times 1$	400000
Acetic acid	$(7 \times 10^5) \times 1$	700000
Citric acid	$(8 \times 10^5) \times 1$	800000
Myristyl glucoside	$(5 \times 10^5) \times 1$	500000
Caprylyl glucoside	$(5 \times 10^5) \times 1$	500000

Results


Which Type Of Agent Is The Best
Number of Colonies Without Dilution
800000
600000
400000
200000
0
Alkyl
Hydrogen peroxide
Acetic acid
Citric acid
Myristyl glucoside
Caprylyl glucoside
Name of Agents

Sources of Error

- Bacteria could have entered the petri dish or test tube when it was opened, slightly changing the results. If they were made of glass I could have flamed the rim every time I opened them. This is also the reason I didn't make my own Petri dishes, cooking your own agar can cause contamination, altering the results of the project.

- As much as I tried to control the temperature of my homemade incubator there were times when the temperature was the same all the time.

Future Project

If I got another chance to do this experiment I would use a spectrophotometer to get a more accurate count of the colonies. A spectrophotometer is an instrument that measures the amount of light absorbed by a solution, this can be used to get an accurate count of bacteria as cells absorb light of a specific wavelength. This count also helps count the microscopic colonies that couldn't be counted.

Applications

Using the information from this project you know that oxidizing agents are better at killing pathogens, this information can be used in many fields such as:

1. Disinfecting surfaces: Oxidizing agents can be used to disinfect surfaces.

2. Water purification: Oxidizing agents can be used to kill microorganisms in water, making the water safer to drink.

3. Waste Treatment: oxidizing agents can be used to break down organic waste, making it easier to dispose of.

Conclusion

My project's purpose was to find out which type of agent (oxidizing, non-oxidizing or surface active) was the most effective at killing pathogens. To make the results more clear I used serial dilution to lessen the colonies that grew so they would be easier to count.

The results showed that the oxidizing agents were the most effective, followed by surface active agents and then non-oxidizing agents. This means the oxidizing agents killed the most pathogens, making them the most effective. This makes my hypothesis right.

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Thank You