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

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Timetable:

Date	Activity
Dec 21- 27, 2023	Background research
Jan 1, 2024	Changed topic
Jan 1-8, 2024	Adding to background research
Jan 4, 2024	Growing bacterial in first petri dish
Jan 5, 2024	Buy / Order material
Jan 7, 2024	Make test tube holders
Jan 7, 2024	Transfer bacterial colonies to test tubes
Jan 7, 2024	Print our labels
Jan 8, 2024	Serial dilution
Jan 9, 2024	Transfer the bacteria to Petri dishes
Jan 10, 2024	Observation day 1
Jan 11, 2024	Buy more slides and redo serial dilution
Jan 12, 2024	Observation day 1

Why I chose this topic:

In grade 7, I did a similar project where I compared eco-friendly active ingredients to traditional active ingredients, this year I wanted to do something with bacteria again so I looked back into my logbook and found out that disinfectants can be categorized into categories, oxidizing, non-oxidizing, surface active etc. The category depends on how they kill the pathogen. This year instead of comparing different active ingredients I chose to compare which way of killing a pathogen would be more effective.

Background Research:

What is the difference between cleaners, sanitizers and disinfectants?

Cleansers are designed to clean surfaces, removing dirt and other visible impurities, they do not necessarily have antibacterial properties. Sanitizers are designed to reduce the amount of pathogens on a surface, they don't always kill them all. Disinfectants are designed to kill pathogens on a surface, being more effective than sanitizers.

What are pathogens?

Pathogens are microorganisms that can cause disease, illness and infections in humans and animals, bacteria and viruses are well-known examples. Pathogens can enter the body in many ways, once they are inside they can multiply, causing health issues.

What is the active ingredient in a disinfectant?

The active ingredient in a disinfectant is the chemical that is responsible for killing or inactivating pathogens, doing this by disrupting the pathogen's cell structure. Other ingredients with various purposes usually aid active ingredients in disinfectants.

What is the difference between bacteria and a virus?

Bacteria and viruses are both pathogens. They are both self-replicating cells but bacteria are generally larger in size and can live both inside and outside of the human body. Viruses, on the other hand, require hosts to survive. Bacteria can replicate on

their own as viruses need to infect cells and use those cells in order to replicate themselves. Bacteria are responsible for infectious diseases while viruses are responsible for infections.

Types of pathogenic bacteria that could have grew:

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.

Which active ingredients fall under which category?

Oxidizing- Halogens, chlorine, iodine, sodium hypochlorite, **alkyl,** bromine, chlorine dioxide, peracetic acid and **hydrogen peroxide**.

Non-oxidizing- Quaternary ammonium compounds, amphoterics, biguanides, and **acid anionics (citric acid and acetic acid are acid anionics**).

Surface active- **Non-ionic surfactants(myristyl glucoside and caprylyl glucoside are non-ionic surfactants)**, ionic surfactants, emulsifiers and biocids.

What are macromolecules in a pathogen?

When smaller molecules come together to make up a larger molecule, the larger molecule is known as a macromolecule. Each macromolecule has different responsibilities and rules. Pathogens contain macromolecules such as proteins, lipids, nucleic acids and carbohydrates. They are important for the survival, growth and activity of a pathogen. Proteins act as structural support for cells, enzymes and hormones. Proteins are made up of amino acids (NH2-CHR-COOH) linked together like a chain. Carbohydrates are molecules made up of carbon, hydrogen and oxygen. They provide energy to organisms. Lipids play many roles in a cell, they help form the cell

membrane and cell walls and are also responsible for many cellular processes, such as cell division, transporting molecules etc. Nucleic acids carry genetic information, they are responsible for passing down traits to offspring.

Physical and Chemical properties:

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	- 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	- They have a hydrophobic and a hydrophilic end , the hydrophilic end is attracted to water molecules which the hydrophobic end is attracted to non polar 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

them more stable	occurring or synthetic	monolayer at a liquid to liquid interface and form micelles in solutions that are aqueous (explained below) - They are nonionic

What are non polar molecules?

Molecules that don't have a positive or a negative net charge are known as non-polar, because they don't have a charge separation between their atoms and they don't dissolve in water. Water molecules are polar because they have a positive charge on one end and a negative charge on the other end.

What does it mean to form a monolayer at a liquid-to-liquid interface and to form micelles in aqueous solutions?

Surface active agents can form thin one-molecule layers between the boundaries of two different liquids, this can help reduce the surface tension between them, making it easier for them to mix.

Surface active agents can form small groups of molecules that self-assemble in a sphere-like structure, these are called micelles. They have a hydrophobic shell but the insides are hydrophilic. MIcelles are useful for releasing hydrophobic things into water, making them more soluble.

What is the difference between oxidizing, non-oxidizing agents and surface active agents (active ingredients)?

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 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 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).

What is a good temperature to grow bacteria in nutrient agar and tryptic soy broth (TSB) ?

The temperature at which bacteria grows the most in tryptic soy broth and nutrient agar is between 20°-37°C.

What is bacterial culture?

Bacterial culture is a group of bacteria allowed to grow under regulated laboratory conditions for research purposes.

Which culture medium is used to grow bacteria and why?

The nutrient agar plates and tryptic soy broth are used in my experiment. Nutrient agar and tryptic soy broth are used to grow bacteria because these are media that have many nutrients essential for bacteria to grow.

What is serial dilution and why is it used?

A serial dilution is the stepwise dilution of a substance in a solution. In serial dilution, the density of cells is reduced in each step. It helps to easily count the number of colonies in the sample.

How should you dispose of a petri dish with bacteria in it?

You should hold the petri dish over a sink and open it. You will need to pour a small amount of bleach onto the petri dish (wear gloves). After that put the petri dish into a zip lock bag and dispose of it in the trash.

Active ingredients used in my experiment:

Type	Brand	Ingredients
Oxidizing	Lysol	Alkyl (67%)
Oxidizing	Lysol	Hydrogen peroxide (1.0%)
Non-oxidizing	Allen	Acetic acid (10.0%)
Non-oxidizing	Eco	Citric acid (5.0%)
Surface active	Attitude	Myristyl glucoside (3.0%)
Surface active	Purell	Caprylyl glucoside (2.5%)

Active Ingredient	Chemical Formula
Alkyl	CnH2n+1

Hydrogen peroxide	H2O2
Acetic acid	CH3COOH
Citric acid	C6H8O7
Myristyl glucoside	C33G65O12
Caprylyl glucoside	C16H32O9

Short Forms Used:

T1- Trial 1
T2- Trial 2
T3- Trial 3
HP- Hydrogen peroxide
A- Alkyl
CA- Citric Acid
AA- Acetic Acid
MG- Myristyl glucoside
CG- Caprylyl glucoside
O- Oxidizing
NO- Non-oxidizing
SA- Surface active
Og- Original
D1- Dilution 1
D2- Dilution 2
D3- Dilution 3
D4- Dilution 4
D5- Dilution 5

Testable question:

Are oxidizing or non-oxidizing active ingredients more effective at killing pathogens in disinfectants?

Purpose:

The purpose of my experiment is to find out which category of active ingredients is the most effective at killing pathogens

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 inorder 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.

Materials:

» 6 disinfectants (2 oxidizing, 2 non-oxidizing and 2 surface active)
» 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
- 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
- We will not use any disinfectant on the control slides.
- 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
- Once all of the 3 slides are in tubes start serial dilution
- 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 the disinfectants plus 1 control
- At the end of serial dilution for all disinfectants, you will have 7x3=21 test tubes for incubation.

- 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

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.

Observations:

First Petri Dish-

Day 1:
- No bacterial colonies
- Incubator smelled bad
- Foggy lid

Day 2:
- Colonies Started Forming
- Milky white and transparent colonies
- Many were merged
- Small colonies
- Incubator smelled bad

Day 3:
- Colonies got bigger
- Darker in colour
- Some had a yellowish tint
- More merged colonies

Serial dilution:
- Bacterial solution got lighter each time it got diluted
- Fifth dilution test tube was the lightest

Petri dishes after dilution-

Day 1 (Jan 10, 2023) :

- The Petri dishes grew way too many colonies, they were all merged in a line and even an app wouldn't pick up on them since they were all merged. I will discard all the Petri dishes and will be starting over tomorrow from the slides, instead of growing new bacteria I'll use some from one of the control petri dishes to make my bacterial solution.

Serial dilution (Jan 11):

I had enough slides left over to experiment again but I had to buy more Petri dishes, peptone water, TSB, droppers and sterile swabs. I made my bacterial solution by 2:00 pm and started my serial dilution around 4:10 pm. I checked my incubator's temperature more often and tried to have it at the perfect temperature so my bacteria would grow quicker because my project was late.

Observations (petri dishes)-

Day 1 (Jan 12):

Type	**Active Ingredient**	**# Of Colonies**
	Control	All colonies were growing merged
Oxidizing	Alkyl	T1- 0 T2- 2 T3- 1
Oxidizing	Hydrogen peroxide	T1- 3 T2- 2 T3- 2
Non-oxidizing	Acetic acid	T1- 4 T2- 5 T3- 4
Non-oxidizing	Citric acid	T1- 6 T2- 3

		T3- 5
Surface-active	Myristyl glucoside	T1- 3 T2- 1 T3- 5
Surface-active	Caprylyl glucoside	T1- 4 T2- 3 T3- 2

Day 2 (Jan 13):

Type	**Active Ingredient**	**# Of Colonies**
	Control	All colonies were growing merged
Oxidizing	Alkyl	T1- 2 T2- 3 T3- 3 Avg- 3
Oxidizing	Hydrogen peroxide	T1- 5 T2- 3 T3- 4 Avg- 4
Non-oxidizing	Acetic acid	T1- 7 T2- 6 T3- 7 Avg- 7
Non-oxidizing	Citric acid	T1- 8 T2- 6 T3- 9 Avg- 8

Surface-active	Myristyl glucoside	T1- 5 T2- 3 T3- 7 Avg- 5
Surface-active	Caprylyl glucoside	T1- 6 T2- 4 T3- 3 Avg- 5

Data:

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

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

For the first tube, the dilution factor is = 10^{-1} (1 ml added to 9 ml)

For the second tube, the dilution factor is 10^{-1}= (1ml added to 9 ml)

Total dilution factor = previous dilution × dilution of next tube

= total dilution of $10^{-1} \times 10^{-1} = 10^{-2}$

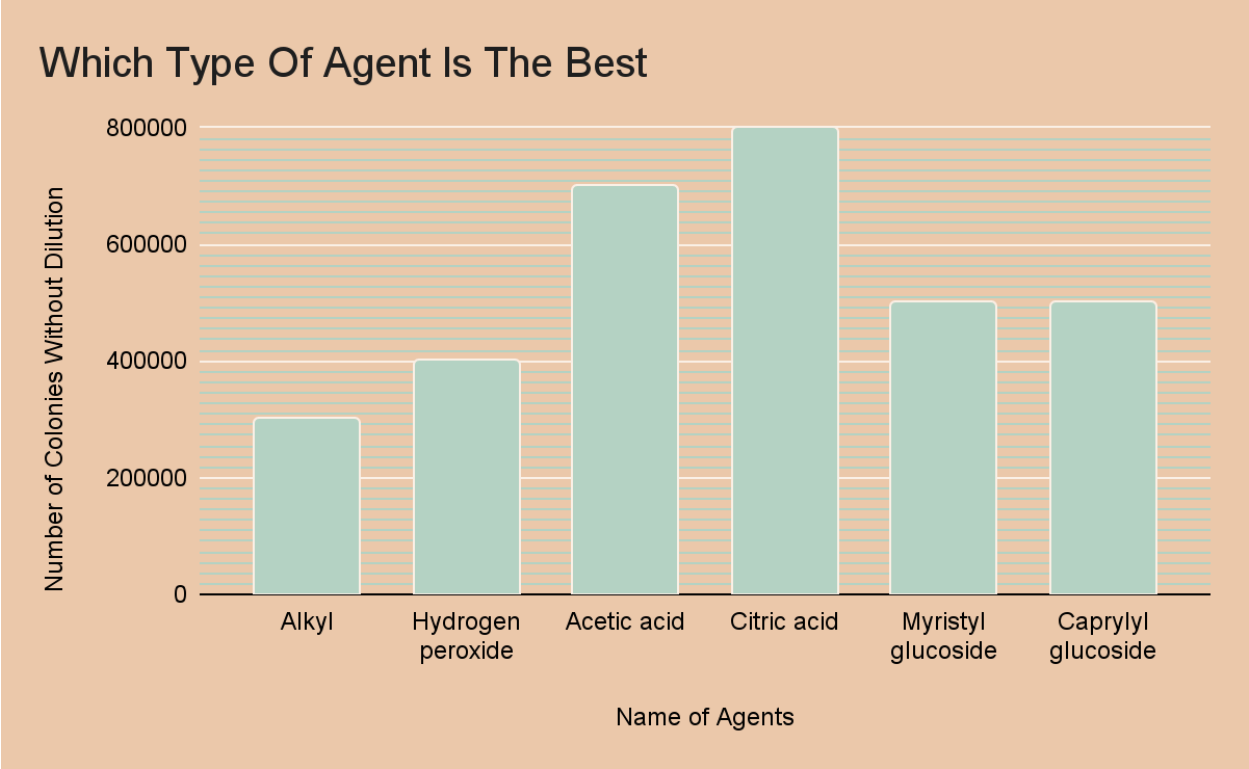
Every time there is one addition to the next test tube that would result in 10^{-3}, 10^{-4} and 10^{-5}. We can apply the formula for ten-fold serial dilution

Serial Dilution calculation formula

= (CFU/Dilution Factor) x Volume of Sample

Example: For the 8 colonies after serial dilution in a controlled petri dish, it would be $(8x10^{5})x1= 800000$

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



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.

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.

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.

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