Study of Oxidative Stress on Growth and Survivability Comparison in between Laboratory Microorganisms and Industrial Wastewater Microorganisms

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A thesis submitted to the Department of Mathematics and Natural Science in partial fulfillment of the requirements for the degree of
Bachelors of Science in Biotechnology

Mathematics and Natural Science
BRAC University
July, 2023

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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Abstract

Oxidative stress is one of the most common occurrence on microorganisms whether they are in natural environments, or chemically stressed environments, which typically causes natural cell apoptosis in microbes. ROS or Reactive Oxygen Species is a massive signifier of oxidative stress generation in bacteria apart from their natural aerobic metabolism. However, bacterial organisms have embedded antioxidant properties which can sufficiently tolerate toxic stress levels. Microorganisms contained in chemically stressed environments are highly likely to tolerate hostile environments of stress for survivability, whereas conventional laboratory microorganisms are expected to be less tolerant. There are oxidant reagents available which profoundly generate ROS species responsible for hindering bacterial growth rate at any given circumstances. The purpose of this research is to reinforce ROS generation in both primarily chemically stressed, and non-stressed laboratory bacterial culture samples through external oxidant sources and sketch a thorough comparative analysis between the growth and survivability rates of the mentioned different strains of the similar microbe. Chemically stressed microbes have been accumulated through the collection of semi chemically treated wastewater from the drainage system of manufacturing industries and their correspondent laboratory strains have been simultaneously cultured alongside them. Oxidative stress was induced through external oxidants into all the microorganism through drop spread assay. The results have partially shown as per expectation, however there have been a gigantic amount of unnatural and unexpected scenarios. An assumable level of errors and limitations, and study gaps have been discussed to demonstrate the diversity of results.

Acknowledgement

We would like to show our cordial gratitude towards Dr. Iftekhar Bin Naser, Associate professor, Department of Mathematics and Natural Science, BRAC University, for his enormous support and constant guidance throughout our research. He has patiently instructed us to reach the maximum efficacy and accuracy of our research objective by eradicating errors and loopholes. Sir has not only provided us with probable solutions to each problem during the research, but also helped us establish a better understanding of an effective research approach for future ventures.

We sincerely thank Iftekhar Sir for adequately assisting us amidst his hectic program schedules and helping us become more knowledgeable and skillful on our area of work.

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List of Acronyms

ROS Reactive oxygen species

CFU Colony- forming unit

VIBRIO 1 A strain that belongs to Vibrio genus

VIBRIO 2 A strain that belongs to Vibrio genus

Chapter: 01

INTRODUCTION

1. Overview of Oxidative stress generation on Microbes

Oxidative stress is a disorder that can develop when the body produces an excessive amount of the dangerous chemicals known as free radicals but not enough antioxidants to eliminate them. Basically, ROS are free radicals which are reactive chemicals that eventually harm biological components like DNA, protein and lipids. This process can injure normal cellular functions and contribute to different illnesses. Moreover, everybody has its natural defense mechanism which counterbalances ROS and reduces oxidative stress. The generation of ROS exceeds the body's antioxidant capacity also resulting in cellular damage and potential health concerns due to oxidative stress.

Oxidative stress can also happen when there is an imbalance between generation of reactive oxygen species (ROS) and the ability of the bacterial cell to detoxify or repair the damage which is caused by molecules. However, biological components like proteins, lipids and DNA can be damaged by superoxide radicals (O2-) and hydrogen peroxide (H_2O_2) which are extremely reactive oxygen species. Bacterial ROS are created as byproducts of normal metabolic processes or as a response to environmental stressors such as antibiotic exposure or host immunological responses. Enzymes such as superoxide dismutase, catalase, and peroxidases are among the bacterial defensive mechanisms against oxidative stress. These enzymes help to neutralize ROS or convert them into less harmful molecules. However, oxidative stress occurs when the production of ROS overwhelms the detoxifying systems of the bacterial cell.

To protect cells from oxidative stress, bacteria enhance the expression of multiple genes, including the SoxRS, OxyR, and PerR regulons. Although cells can tolerate a certain amount of free radicals, large quantities of ROS cause oxidation of many biomolecules.RNA oxidation can cause structural and functional changes in virtually all RNA species, including mRNA, rRNA, tRNA, and sRNA, resulting in translational mistakes that are harmful to cell viability. Bacteria, on the other hand, have evolved RNA quality control systems that employ RNA-binding proteins such as MutT/Nudix family members and the ribonuclease PNPase to remove oxidized RNA.

Bacteria encounter a significant challenge from oxidative stress, and the consequences can be detrimental to their survival and growth. Understanding and targeting oxidative stress responses in bacteria has health, agriculture, and environmental science implications. Bacterial metabolic health is inextricably tied to oxidative stress. Metabolism is the set of chemical

events that occur within cells to sustain life, and it includes processes such as energy production, nutrient use, and the synthesis of essential molecules. Oxidative stress can alter bacterial metabolism in a variety of ways.

- **Energy production:** Oxidative stress can impair bacterial cells' capacity to generate energy. ROS have the ability to disrupt enzymes involved in cellular respiration, such as those in the electron transport chain, which generates ATP. This can lead to decreased energy output and metabolic problems.
- **Nutrient utilization:** Bacteria absorb resources for growth and survival by using different metabolic pathways. By interfering with essential enzymes and transporters involved in nutrition absorption and utilization, oxidative stress can disrupt these pathways. This disruption can impair the bacterium's ability to absorb essential nutrients and negatively impact its overall metabolic health.
- **Redox Balance:** Bacteria maintain a balance between oxidizing and reducing activities in their cells. Oxidative stress disrupts this balance by generating an excess of oxidizing activities. This disruption may affect the activity of enzymes and metabolic pathways that rely on specific redox states, resulting in metabolic imbalances and dysfunction.

Furthermore, oxidative stress in bacteria might affect the production of essential molecules. ROS can damage enzymes that are involved in the synthesis of amino acids, nucleotides, and other cellular components. Growth and proliferation can be hampered as a result of diminished synthesis of critical macromolecules. There are also other stress response pathways that affect bacterial metabolism, and oxidative stress can activate them, affecting bacterial metabolism.

When it is required to eliminate unneeded or aberrant cells, a procedure known as apoptosis is performed. This method involves activating an internal regulated suicide program, followed by a sequence of biochemical events that result in cell death. It has, however, been found in bacteria. As a bacterial response to external stimuli, apoptosis may be necessary not just for the bacterium but also for the host. Through several mechanisms, oxidative stress can also cause apoptosis.

- **Reactive Oxygen Species (ROS) production:** ROS generation is the primary cause of apoptosis in bacteria. In essence, oxidative stress increases the generation of reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide. Excessive ROS levels can disrupt biological components such as lipids, proteins, and DNA.

- **Mitochondrial dysfunction:** ROS can directly attack mitochondria, resulting in mitochondrial dysfunction. This flaw disrupts the electron transport chain, resulting in lower ATP production. Furthermore, it promotes apoptosis by stimulating the release of pro-apoptotic substances from mitochondria, such as cytochrome c.
- **Activation of apoptotic pathways:** One of the apoptotic signaling systems that might be initiated by oxidative stress is the intrinsic route. This pathway leads to mitochondrial membrane permeabilization and apoptosis by activating pro-apoptotic proteins (such as Bax and Bak) and suppressing anti-apoptotic proteins (such as Bcl-2 and Bcl-xL).
- **DNA damage:** Stand breakage, base alterations, cross-linking and DNA damage can occur due to ROS. However, when the DNA damage gets severe it can interpret cell cycle and activate DNA repair mechanisms. The cell initiates apoptosis as a defense mechanism when the DNA damage is not repairable which helps to prevent the spread of genetic abnormalities. Nevertheless, aerobic conditions can also be a reason for oxidative stress to occur as it is a suitable environment for them to function.
- **Aerobic conditions:** There are some bacterias which are most likely to develop in an oxygen rich environment for that reason they tend to face oxidative stress as a result of ROS production.
- **Host immune system:** Bacteria that infect host species must battle with the host immune system's defense mechanisms, which include the production of ROS by immune cells. This immune reaction exposes the bacterium to significant oxidative damage.
- **Environmental toxins:** Certain contaminants or environmental chemicals can cause oxidative stress in bacteria. These toxins can either directly cause ROS or interfere with bacterial antioxidant defenses, resulting in increased oxidative stress.
- **Antibiotics:** Some antibacterial medications work by inducing oxidative stress in microorganisms. These drugs can generate ROS or impair bacterial antioxidant mechanisms, making bacteria more susceptible to oxidative damage.

1.2 Literature Review

Oxidative stress is a condition that occurs when the body develops an excess of the harmful molecules known as free radicals but not enough antioxidants to remove them. ROS (reactive oxygen species) are emerging as critical components of the bacterial response to lethal stress. There are also some naturally occurring strains, some of them are superoxide, hydrogen peroxide, and hydroxyl radical. However, hydrogen peroxide and hydroxyl radicals are the most useful ones which are being used as ROS. Hydrogen peroxide, which can also be created via superoxide dismutation, acts as a substrate for the generation of hydroxyl radicals via Fenton chemistry. However, from our research we have seen how ROS surpasses the body's antioxidant capacity, causing cellular damage and potential health issues from oxidative stress. We have also used superoxide radicals (O2-) and hydrogen peroxide (H_2O_2) which also proved to damage biological components like lipids, proteins and DNA. Basically, from the literature we have seen If hydroxyl radical accumulation is not managed, this oxidative process can kill cells because hydroxyl radical destroys nucleic acids, carbonylated proteins, and peroxide lipids. Bacteria have defensive proteins that can detoxify ROS (SodA, SodB, SodC, AhpCF, KatG, KatE) and defend against damage (e.g., SoxRS, OxyRS, and SOS regulons). When under extreme stress, bacteria may use ROS to self-destruct. Indeed, no protein-based mechanism for hydroxyl radical detoxification has been found.

ROS played a role in quick killing but not in growth inhibition as determined by MIC or in gradual death associated with long incubation durations. Sublethal superoxide generation or the absence of superoxide dismutases lowered rather than boosted antimicrobial-mediated death. In addition to the known destructive activity, superoxide appears to have a defensive effect. Moreover, in the research we have seen how ROS can damage enzymes which are involved in the synthesis of nucleotides and other cellular components and also can make change in the growth of macromolecules. We have also found how ROS can affect the natural bacterial metabolism.When it is necessary to destroy unnecessary or abnormal cells, apoptosis is undertaken. This procedure includes triggering an internal suicide program, which is then followed by a series of biochemical reactions that culminate in cell death.

Several recent studies have called into doubt the role of ROS in antimicrobial-mediated death. One illustrates cases in which ROS buildup and cell death are incompatible, while another highlights that the effect of iron/iron-sulfur clusters on antibacterial killing is mostly dependent on drug uptake, with little function for ROS in lethality. However, the Collins group mentioned different technical issues, measured lethal actions and also the factors that affect bacterial

growth. Furthermore, to overcome such issues it is also critical to distinguish between factors that influence the creation of primary damage and those that influence the response to that damage. Drug uptake, efflux, and target interactions, for example, influence direct lesion development and cell death, although they differ in principle from the cellular reaction to the lesion, i.e. the ROS cascade and secondary damage. In our research we have used some compounds which help to create a balance in oxidative relations and increased oxidative stress. The oxidants which have been used are, Hydrogen peroxide H_2O_2, Potassium dichromate $K_2Cr_2O_7$, Sodium Hypochlorite NaOCl. Basically, these were used for the bacterias to cope with the stress, Potassium dichromate-induced oxidative stress may result in electron transport chain blockage and decreased ATP generation, resulting in metabolic crisis and bacterial cell death. The material affects the type, concentration, period of exposure, and stage at which bacteria quit living.

Multiple methods are accessible to polymorphonuclear leukocytes (PMN) or neutrophils for killing ingested microorganisms. Almost all of them contain H_2O_2, showing the importance of this reactive oxygen intermediate in microbicidal action. Following bacterial ingestion by PMN, H_2O_2 is generated by the respiratory burst, which consumes O2 and produces H_2O_2 from O2.-. Within phagocytic vacuoles, H_2O_2 is deposited intracellularly near bacteria, where it can react with the MPO- H_2O_2-halide system to create deadly hypochlorous acid (HOCl) and/or perhaps singlet oxygen (1O2). In the research H_2O_2 were playing both negative and positive parts. They produced ROS which eventually included hydroxyl radicals (OH•) and superoxide anions (O2•-). Whereas, the hydroxyl radicals produced by hydrogen peroxide, can damage bacterial DNA directly. Furthermore, that resulted in mutation and broken DNA strands.

1.2.1 Responsible ROS Generating Oxidants

There are some compounds which can produce oxidation relations by receiving electrons from other substances which eventually helps to increase oxidative stress; these are known as oxidants. Some of the oxidants are Hydrogen peroxide H_2O_2, Potassium dichromate $K_2Cr_2O_7$, Sodium Hypochlorite NaOCl.

Hydrogen peroxide (H_2O_2):

Depending on the dosage and the bacteria's ability to resist oxidative stress, hydrogen peroxide (H_2O_2) can have both positive and negative effects on bacteria.

- **ROS Production:** Hydrogen peroxide can generate reactive oxygen species (ROS) within bacterial cells. ROS are very reactive molecules that can disrupt proteins, DNA, and lipids. They include hydroxyl radicals (OH•) and superoxide anions (O2•-).
- **Oxidative Stress:** High levels of hydrogen peroxide can cause oxidative stress in bacteria. Hydrogen peroxide-generated ROS can overwhelm the cellular antioxidant defense systems, causing biomolecule damage and dysfunction.
- **DNA Damage:** ROS, such as hydroxyl radicals produced by hydrogen peroxide, can damage bacterial DNA directly. This damage can result in mutations, DNA strand breaks, and other genetic alterations, all of which can have an impact on the bacteria's survival and function.
- **Cell Membrane Damage:** When hydrogen peroxide interacts with lipids in bacterial cell membranes, lipid peroxidation occurs. This process alters the cell's integrity and permeability by disrupting the structure and function of the membrane.
- **Adaptation and Resistance:** Sublethal hydrogen peroxide concentrations can induce bacterial adaptability. This can activate antioxidant defense mechanisms and DNA repair activities, improving the bacteria's ability to withstand oxidative stress and survive future hydrogen peroxide exposures.

Moreover, different bacterias have various limits of tolerance to H_2O_2 also, some bacteria can detoxify them.

Therefore, the point at which bacteria stop surviving during H_2O_2 stress is highly dependent on the specific metabolic pathways and cellular processes involved in H_2O_2 detoxification and the ability of the bacteria to cope with the stress.

Potassium dichromate ($K_2Cr_2O_7$**):**

The chemical potassium dichromate ($K_2Cr_2O_7$contains the extremely reactive hexavalent chromium (Cr(VI)) ion. It is well known that Cr(VI) compounds, such as potassium dichromate, cause oxidative stress in bacteria.

- **ROS Generation:** The redox reactions of the Cr(VI) ion can produce ROS such hydroxyl radicals (OH•) and superoxide anions (O2•-). Many biological components are at risk of oxidative damage as a result of these ROS.
- **Oxidative Stress:** Potassium dichromate exposure causes bacterial oxidative stress. The produced ROS can overwhelm the bacteria's antioxidant defense systems, resulting

in an imbalance between ROS production and elimination. Oxidative stress can damage proteins, DNA, lipids, and other biological components.

- **DNA Damage:** Potassium dichromate-generated ROS can directly target bacterial DNA, causing strand breakage, base oxidation, and other DNA abnormalities. This DNA damage can result in mutations, genomic instability, and replication and transcription issues.
- **Membrane Damage:** By interacting with lipids in bacterial cell membranes, potassium dichromate has the ability to trigger lipid peroxidation. This process compromises the integrity and function of the cell membrane, resulting in increased membrane permeability and impaired cellular homeostasis.
- **Cell Death:** Bacteria can die from severe oxidative damage caused by prolonged or high potassium dichromate concentrations. The accumulation of oxidative damage, combined with slowed cellular activities, can finally lead to the loss of bacterial viability.

Some of the bacterias can have resistance mechanism from the impact of Cr(VI)

In conclusion, oxidative stress brought on by potassium dichromate might result in the blockage of the electron transport chain and decreased ATP synthesis, which in turn causes metabolic crisis and bacterial cell death. The species, concentration, length of exposure, and stage at which bacteria stop living are all affected by the substance.

Sodium Hypochlorite (NaOCl):

There are some antibacterial qualities, NaOCl is a potent oxidizing agent that is often employed as a disinfectant.

- **Oxidative Damage:** Sodium hypochlorite can induce oxidative damage to bacterial cells by producing reactive oxygen species (ROS) such as hypochlorous acid (HOCl) and hydroxyl radicals (OH•). These ROS can react with and destroy biological components like proteins, DNA, and lipids, resulting in cellular dysfunction and death.
- **Protein Denaturation:** NaOCl has the ability to oxidize and denature proteins in bacterial cells. Protein structure and function disruption can result in the inactivation of critical enzymes and other proteins essential for bacterial survival and growth.

- **DNA Damage:** ROS generated by NaOCl can directly damage bacterial DNA. This can result in DNA strand breaks, base alterations, and other genetic changes, all of which can interfere with bacterial reproduction, gene expression, and overall cellular function.
- **Membrane Disruption:** Sodium hypochlorite can harm bacterial cell membranes. It has the potential to alter the lipid bilayer structure, leading to increased permeability and loss of cellular substance. This disruption has the potential to cause bacterial cell death.
- **Resistance Development:** Bacterial resistance mechanisms may arise as a result of prolonged or repeated exposure to sublethal NaOCl concentrations. Some bacteria can adapt and evolve detoxification or neutralization activities, reducing NaOCl's disinfection potency over time.

It's important to note that the susceptibility of different bacterial species to NaOCl varies. Some bacteria have built-in resistance mechanisms that enable them to survive the impacts of NaOCl, whereas others are more vulnerable. The concentration and duration of NaOCl exposure, as well as other environmental conditions, can all affect its efficiency and the consequent oxidative stress in bacteria

In conclusion, the precise stage at which bacteria can no longer survive in the presence of NaOCl depends on a number of variables, but the electron transport chain is a crucial pathway that can be impacted. By oxidizing electron carriers and interfering with ATP synthesis, NaOCl can interfere with this route, causing a reduction in cellular metabolism and ultimately cell death.

1.2.2 Oxidative mechanisms of the Oxidants

H_2O_2

Bacteria can be harmful to hydrogen peroxide (H_2O_2) by having their DNA, proteins, and lipids damaged. But bacteria have developed a number of methods to detoxify H_2O_2 and keep redox equilibrium in their cells. The catalase-peroxidase pathway is the metabolic mechanism utilized by bacteria for H_2O_2 detoxification.

In this pathway, the enzyme peroxidase employs reducing equivalents like NADH to convert H_2O_2 to water, whereas the enzyme catalase transforms H_2O_2 to water and oxygen. In the bacterial detoxification of H_2O_2, both enzymes are active.

Bacteria may undergo oxidative stress if the H_2O_2 stress is too great for them to detoxify, which can result in cell death and damage to biological components. The precise stage at which bacteria stop surviving under H_2O_2 stress depends on a number of variables, including the intensity and duration of the stress, the particular bacterial species, and how well the bacteria are able to remove H_2O_2 from their systems.

In general, if the H_2O_2 stress is extreme enough to overcome the bacterial detoxification mechanisms, the bacteria may go into an oxidative stress state, which can cause damage to vital biological components like the cell membrane, DNA, and proteins. This harm may eventually cause cell death and stop bacterial growth and survival.

Therefore, the point at which bacteria stop surviving during H_2O_2 stress is highly dependent on the specific metabolic pathways and cellular processes involved in H_2O_2 detoxification and the ability of the bacteria to cope with the stress.

$K_2Cr_2O_7$

Potassium dichromate is a hazardous substance that can cause bacteria to experience oxidative stress by producing reactive oxygen species (ROS), which can harm cellular elements like DNA, proteins, and lipids. Bacteria exposed to potassium dichromate must be able to repair ROS-caused damage and keep their metabolic equilibrium in order to survive.

The electron transport chain (ETC) is the metabolic pathway that is most significantly impacted by oxidative stress brought on by potassium dichromate. By moving electrons from donors like NADH and FADH2 to acceptors like oxygen or other electron carriers, the ETC is in charge of producing ATP, the primary energy unit of the cell. As a consequence of this procedure, the ETC produces ROS, which are typically neutralized by antioxidant defenses like catalase and superoxide dismutase.

Potassium dichromate exposure can overwhelm the antioxidant defenses in bacteria, causing oxidative damage to the ETC's constituent parts and an impairment of electron transfer. This may result in a drop in ATP synthesis, a metabolic crisis, and ultimately cell death.

Species and potassium dichromate concentration can both affect the specific stage at which bacteria can no longer survive in its presence. However, on general, bacteria with stronger antioxidant defenses and higher levels of oxidative stress resistance are more likely to endure longer than those with weaker defenses. Furthermore, the length of exposure to potassium dichromate influences both the severity of oxidative damage and the capacity of bacteria to repair it.

In conclusion, oxidative stress brought on by potassium dichromate might result in the blockage of the electron transport chain and decreased ATP synthesis, which in turn causes metabolic crisis and bacterial cell death. The species, concentration, length of exposure, and stage at which bacteria stop living are all affected by the substance.

NaOCl

Strong oxidizer sodium hypochlorite (NaOCl) can put bacteria under a lot of stress by destroying their cell membrane and upsetting their metabolic processes. The amount of sodium hypochlorite present, the type of bacteria present, and the length of exposure all affect how long it takes for bacteria to stop living in its presence.

NaOCl primarily affects cells by oxidizing proteins, lipids, and nucleic acids, which can result in the loss of vital biological activities and eventual cell death. The electron transport chain (ETC), which produces ATP (adenosine triphosphate), the main energy source for cellular functions, is one important metabolic pathway that NaOCl can disrupt.

The synthesis of ATP is accomplished by the ETC, which consists of a sequence of redox processes that move electrons from electron donors to electron acceptors. By oxidizing the electron carriers, such as NADH and FADH2, that are required for the ETC to operate effectively, NaOCl might cause the ETC to malfunction. A decrease in ATP synthesis and a subsequent decline in cellular metabolism may result from this interruption.

NaOCl can also harm the cell membrane, impairing the integrity of the cell and allowing vital components to flow out. This leakage has the potential to further obstruct metabolic pathways and impair cellular function.

In conclusion, the precise stage at which bacteria can no longer survive in the presence of NaOCl depends on a number of variables, but the electron transport chain is a crucial pathway that can be impacted. By oxidizing electron carriers and interfering with ATP synthesis, NaOCl

can interfere with this route, causing a reduction in cellular metabolism and ultimately cell death.

1.3. OBJECTIVE

The discussions above reflect the interlink between ROS generating oxidants and the antioxidant properties enhancing microbial survivability in stressed environment. By combining the literature reviewed section with these factors, the ideal objective of this research is to understand the survivability and growth rates of microbes while introduced to stress levels of chosen oxidants and demonstrate a comparative analytical study between two different strains of the same microbe: a primarily stressed strain and its correspondent laboratory non-stressed strain. The theme of this study includes collecting samples from specific locations which ensure the presence of certain microbes under moderate stress levels. Furthermore, the microbes are isolated and identified. Hence, their correspondent laboratory microbial strains are collected to represent the comparative analytical study.

Chapter: 02

Methods

2.1 Selection of Deferential Media

Nutrient Agar:

A type of growth media called nutrient agar is used to cultivate a wide range of microorganisms, including bacteria. The following are some of the characteristics of nutrient agar and how bacteria develop on it:

A complex mixture of nutrients called nutritional agar gives bacteria a range of carbon and nitrogen sources, as well as minerals and vitamins, to support their growth. A simple to use and make solid medium is nutrient agar. Additionally, it is reasonably priced when compared to other forms of growth media. The pH of nutrient agar is between 7.2 and 7.4, which is ideal for the growth of the majority of bacteria. Inhibitors or extra nutrients can be added to nutrient agar to encourage or hinder the growth of particular bacterial species.

Nutrient agar allows bacteria to thrive by allowing them to take up nutrients from the medium and use them for metabolism and energy. On the agar's surface, the bacteria will gather into colonies, which are represented as discrete, observable spots or patches. Temperature, oxygen concentrations, pH, and the particular nutrients present in the medium are only a few of the variables that may have an impact on the development rate and visual appearance of bacterial colonies in nutrient agar.

Nutrient agar is an all-purpose growth medium that is often used to cultivate and study a range of microorganisms.

HiChrome Agar:

A form of differential and selective agar used for the isolation and identification of different types of bacteria is called HiChrome agar. It is intended to distinguish between various microbe species based on how well they can break down particular nutrients in the agar.

Peptones, yeast extract, carbohydrates, and chromogenic substrates are all present in the agar. Chromogenic substrates are substances that have no color until they are broken down by particular enzymes produced by particular microorganisms. The colorful chemical that is released as the substrate is digested causes a unique colony to appear on the agar.

Depending on their metabolic activity, several types of bacteria will build colonies on HiChrome agar that are different colors. This makes it simple to distinguish between the bacteria. Salmonella creates pink colonies, whereas E. coli produces blue colonies.

HiChrome agar's selective qualities result from the addition of certain antibiotics in the agar, which prevent the growth of some bacteria while promoting the development of others. HiChrome agar, for instance, contains the antibiotic vancomycin to prevent the growth of gram-positive bacteria while allowing gram-negative bacteria to proliferate.

Bacteria must first be put onto the surface of HiChrome agar for it to grow on it. The agar is then incubated at the right temperature and under the right circumstances for the particular bacteria that is being tested. The chromogenic substrates and nutrients in the agar are metabolized by the bacteria as they expand, creating observable colonies that can be utilized for identification.

SS Agar:

Salmonella and Shigella species are isolated and distinguished from clinical and environmental samples using the selective and differentiating agar medium known as SS agar (Salmonella-Shigella agar). These are a few of SS agar's characteristics:

Selective: Due to the presence of crystal violet and bile salts, which impede the development of the majority of other bacteria, SS agar is selective for Salmonella and Shigella.

Differential: Lactose and sucrose, which some bacteria may ferment, are found in SS agar, making it differential. The agar turns yellow instead of green when bacteria ferment lactose or sucrose, producing acid in the process.

Indicator: The neutral red indicator found in SS agar becomes red when the agar's pH falls below 6.8 as a result of acid formation.

Depending on their metabolic properties, bacteria may display various growth patterns when inoculated on SS agar.

Non-lactose fermenters Shigella and Salmonella species both produce colorless colonies on SS agar. However, additional traits like their shape, motility, and the release of hydrogen sulfide

gas can be used to distinguish them. Shigella does not create black colonies like Salmonella does because Shigella does not produce hydrogen sulfide gas. Additionally, lactose fermenters like E. coli, which form yellow colonies, can thrive on SS agar. However, their capacity to ferment lactose as well as their shape allow them to be distinguished from Salmonella and Shigella.

A good medium for the selective and distinct isolation of Salmonella and Shigella species from clinical and environmental samples is SS agar, in conclusion. For the identification of bacterial species, the growth patterns of the bacteria on this medium can offer crucial information.

KFS Agar:

The Enterobacteriaceae family of bacteria, specifically Klebsiella, Enterobacter, and Serratia, can be isolated and distinguished using KFS (Klebsiella-Enterobacter-Serratia) agar, a selective and differentiating culture medium. These are a few of its characteristics:

Selective properties:

Because it contains crystal violet and bile salts, which prevent the growth of gram-positive and some gram-negative bacteria, KFS agar is specifically designed for Enterobacteriaceae.

In addition, lactose serves as the only carbon source in the medium. The media favors lactose-fermenting bacteria since not all Enterobacteriaceae can ferment lactose.

Differential characteristics. Lactose, peptone, and bromothymol blue are all ingredients in KFS agar. The lactose-fermenting bacteria will create acid, lowering the medium's pH and turning the medium's green color to yellow. When lactose is fermented, several bacteria of Serratia marcescens, Klebsiella pneumoniae, and Enterobacter aerogenes can create gas that can be observed as bubbles or fissures in the agar.

Bacteria growth on KFS agar:

The most typical bacteria identified on KFS agar are Klebsiella, Enterobacter, and Serratia.

Lactose-fermenting bacteria form yellow colonies with a definite boundary as they develop.

Bacteria that do not digest lactose are colorless. The development of bubbles or fissures on the agar surface near the colonies can indicate that certain bacteria are producing gas. In conclusion, Klebsiella, Enterobacter, and Serratia are isolated and distinguished from other

Enterobacteriaceae bacteria using KFS agar, a selective and differentiating medium, based on their capacity to digest lactose and release gas.

MSA

Staphylococcus aureus can be isolated and identified using the selective and differentiating agar known as MSA (Mannitol Salt Agar). Following are some characteristics of MSA agar and how bacteria develop on it:

Selective: MSA agar is selective because most bacteria find it difficult to grow on it due to its high salt content. On MSA agar, Staphylococcus aureus can thrive and can survive high salt concentrations.

Differential: Mannitol, a sugar alcohol that some bacteria may ferment, is a component of MSA agar, making it differential. Mannitol fermentation by Staphylococcus aureus results in the production of acid, which turns the pH indicator (phenol red) in the agar from red to yellow.

Apperance: MSA agar has a smooth surface and is a pinkish-red tint.

Growth: Staphylococcus aureus generates tiny, rounded, yellow colonies and thrives nicely on MSA agar. On MSA agar, other staphylococci may also proliferate, however they do not ferment mannitol or form yellow colonies.

Limitation: Staphylococcus aureus cannot be accurately identified with MSA agar. The presence of other tests, such as coagulase and catalase tests, is necessary to identify the bacterium.

In conclusion, Staphylococcus aureus is often isolated and identified using the selective and differential agar known as MSA agar. Staphylococci are chosen for by the high salt content of MSA agar, and Staphylococcus aureus can be distinguished from other staphylococci thanks to the presence of mannitol.

EMB Agar:

Gram-negative bacteria are isolated and distinguished using EMB (Eosin Methylene Blue) agar, a differential and selective medium. Eosin and methylene blue, together with a combination of nutrients that encourage bacterial development, are two of the dyes present.

The following characteristics apply to EMB agar:

Selectivity: Because the colors stop gram-positive bacteria from growing, the medium is selective for gram-negative bacteria.

Differential: EMB agar has the ability to distinguish between bacteria that ferment lactose and those that do not. Non-lactose fermenting bacteria generate colorless colonies, but lactose-fermenting bacteria produce colonies with a dark purple-black center and a green metallic sheen on the periphery.

Nutrient-rich: EMB agar, a medium that promotes the growth of a wide variety of gram-negative bacteria, is nutrient-rich.

When bacteria are injected on EMB agar, they proliferate when nutrients are present and are exposed to the selective and differential features of the medium. Eosin and methylene blue precipitate out of the media as a result of lactose-fermenting bacteria producing acid from the lactose, which lowers the pH of the medium. The colony's dark purple-black center and its outermost region's metallic green gloss are the result of this. Colonies with no color will develop from non-lactose fermenting bacteria since they do not create acid or cause the colors to precipitate.

Overall, the capacity of gram-negative bacteria to ferment lactose allows for the identification and separation of these organisms using EMB agar.

TCBS Agar:

A selective and differentiating medium called thiosulfate citrate bile sucrose (TCBS) agar is used to isolate and identify Vibrio species, including Vibrio cholerae, from clinical and environmental samples.

The following characteristics of TCBS agar make it ideal for this use:

Selective: Because of its high salt content (2% NaCl) and the presence of bile salts, TCBS agar is selective for Vibrio species. These elements prevent the development of numerous other bacterial species, enabling the selective proliferation of Vibrio species.

Differential: The presence of sucrose and the pH indicator bromothymol blue in TCBS agar makes it also different. Acid is produced by Vibrio species that are able to ferment sucrose, and this acid causes the pH indicator to change from green to yellow.

Alkaline pH: The growth of Vibrio species is best supported by the alkaline pH of 8.5 that characterizes TCBS agar.

Due to their capacity to ferment sucrose, Vibrio species will develop as yellow or green colonies when bacteria are inoculated on TCBS agar. Other bacteria won't grow or form white colonies if they can't grow on the media. Because some Vibrio species create chromopyrrolic acid, a pigment, they can also produce dark green colonies.

The identification and isolation of Vibrio species from clinical and environmental samples can be accomplished with the help of TCBS agar.

Luria Broth

A typical bacterial growth media used in molecular biology and microbiology is luria broth (LB). These are a few of its characteristics:

Nutrient-rich: LB is a nutrient-rich medium that includes a number of components that encourage bacterial growth, such as sodium chloride, yeast extract, and peptone.

pH level: The neutral pH of LB is excellent for the growth of the majority of bacteria.

Sterilization: To ensure that the medium is free of any impurities, LB is normally autoclaved.

Use: Both gram-positive and gram-negative bacteria, as well as a large variety of others, can thrive in LB.

Liquid or solid form: LB can be created in either a liquid or a solid form, depending on the particular needs of the experiment.

Agar concentration: Depending on the use, the LB agar's agar concentration can be changed to produce softer or firmer agar.

Growth rate: LB encourages rapid bacterial growth, making it helpful for investigations that call for plenty of bacterial cells.

Antibiotic compatibility: LB is suitable for assessing antibiotic susceptibility as it is compatible with a variety of antibiotics.

Luria broth is an all-purpose bacterial growth medium that is often used and appropriate for a wide range of molecular biology and microbiology applications.

Utilizing selective media, hichrome agar media, and nutrient agar media, we were able to isolate 23 bacteria from the 4 samples we obtained from three different pharmaceutical industries.

2.2 Choice of Site and Sample Collection:

Collection of samples from different sites was an initial step of our research objective alongside media selection and preparation. As the ultimate goal was to sketch a significant difference of growth and survivability in between conventional laboratory bacteria and ROS induced bacteria, we simply targeted sites which were the source of highly chemically stressed microbes. This is due to bacteria illicit responses to different types of stressors differently through their defense line mechanisms, hence influencing us to choose such adverse and harsh environment for sample collection. When a particular amount of stress is introduced to the bacteria, they can typically recognize the environmental shift and simultaneously initiate stress responses to survive through the hostile metabolic pressure. In chemically exposed water, stress factors such as ROS-reactive oxygen species, RCS-reactive chlorine species, superoxide anions, and free hydroxyl radicals are dominantly active basing upon the adversity due to imbalance in pH, temperature, and oxidation. These stress factors primarily target the bacterial cell wall to operate cellular disruption, followed by protein denaturation and interaction with amino acids/lipids. However, bacteria in return produce their defense line mechanisms as a reflex by chaperon protein activation and transcriptional regulations. Genes such as katG and sodA are responsible for the catalytic activation that suffice the stress responses.

Our first targeted sites while keeping all of the conditions in check were pharmaceutical and leather industries. The discharge water of these industries are heavily submerged in a variety of chemical exposures, detergent substances, and other toxic metabolites which enhance an imbalance of pH and temperature, creating an adverse effect by generating large amounts of reactive oxygen species and free radicals. Note that the drainage water supply from such factories can be both treated and untreated. As per how our research requisites, we chose to

collect the untreated water. However the microbial stage of the untreated water should be latent enough to elicit survivability through such stressful conditions from the chemically exposed water. Ensuring this was a crucial part of our sample collection because not only the stress factors of untreated drainage water should be submerged enough to initiate disruption of conventional cellular metabolism and protein production of the microbes that are present there, but also the microbes need to reach a certain stage of latency to fight through the disruptive stage which in return can prevent chances of cellular apoptosis. To ensure such measures, we have collected the water exactly from the middle area of the drainage pipeline-from where it is not far enough from the direct exposure of chemical treatment discharge, rather not close enough from the end of the pipeline where almost the entire untreated water is assumed to consume the adaptability of microbes leading to apoptosis.

Hence, we have gathered samples from 4 different sites in total, all with the similar system of manufacturing, releasing the ideal discharged water from which we have found our desirable bacteria with adequate measure of latency.

2.3 Isolating bacteria:

To carry out the bacterial species received from 4 sites, we have initially diluted the samples (from 10-1 to 10-9), and we took 100ul from the 10-9 dilution before pouring it into the selective medium plates and spreading it evenly using a spreader (Spread technique).

For our research on isolating bacteria, we chose 5 distinct selective media: SS agar, KFS agar, TCBS agar, EMB agar, and MSA agar. The plates were then placed in an incubator set at 37 degrees Celsius. The following day, we received microorganisms in their appropriate environmental plates.

Bacteria, we got the selective plates are down below:

- SS agar: Salmonella
- KFS agar: Streptococcus
- TCBS: Vibrio 1 & 2
- EMB: Klebsiella & *E.coli*
- MSA: Staplylococcus

Hence, Number of isolated bacterial species: **7 (Vibrio with 2 subtypes).** We have further streaked the microbes whose identity we weren't certain on HiChrome plates after receiving all the findings from all sites. Using HiChrome agar, we obtained distinct findings. Once we were certain we had all the bacteria, we streaked them on NA plates and stocked the 23 bacteria in soft agar. After effective stocking and recovering of isolated bacteria, we have proceeded to operate a microbial analytical assay or **Drop spread** method. This assay was used throughout our thesis for further comparative and statistical analysis of growth and survivability in between.

2.4 Drop Spreading Assay:

As previously mentioned, we have gathered 7 different bacterial species from our site sampling. For our comparative analysis, we have taken 7 laboratory strains which are correspondent to each of the factory strain. Thus, the assay includes activities of a total of 14 strains of bacteria. (7 from sites, and 7 laboratory strains opposite to them individually)

Day:01

1) Prepare 350ml of fresh NA media (autoclaved) to perform bacterial streak plating. Take 14 medium sized plates and pour about 25 ml of NA to each plate.

2) Use a total of 14 strains of bacteria for streaking in each plate: 7 factory strains of each bacterial type received from all the sites, and 7 correspondent laboratory strains of them. Note that all of the stocks should be actively functioning in order to produce single colonies.

3) After the NA solidifies, start streaking all of the strains separately into each NA plate inside the laminar air flow cabinet. Use 70% ethanol to disinfect the surface of laminar flow prior to streaking.

4) Place the plates into the incubator at 37˚C for overnight incubation.

Day:02

1) Take the streak plates out of the incubator the next day (approximately after 18-24 hours). Make 42 ml of fresh LB to prepare bacterial liquid culture. Take 14 glass vials and proceed to autoclave them alongside LB.

2) After autoclaving, pour 3ml of fresh LB into each sterile glass vial inside the laminar flow. Point out proper single colonies from the previously performed streak plates of factory and laboratory bacterial strains in use.

3) Use a sterile needle and inoculate those single colonies of each strain into individual glass vials. (Note: make sure to burn the needle in between switching to the next strain to avoid contamination)

4) After inoculation, place the glass vials into shaker incubator at 37˚overnight.

Day:03

1) Prepare 1400 ml of fresh NA media, around 28 ml of fresh LB and around 300ml distilled saline water for drop spreading. Take 14 glass vials and around 280 eppendorf tubes in total and autoclave them with NA, LB, and saline water

2) After sterilizing all the components, take out the liquid culture of 1st incubation from the previous day from shaker incubator and proceed to 2nd set of incubation. Inside laminar flow, take 14 glass vials and pipette 1.9ml of fresh autoclaved LB into each vial. Label these glass vials as per to each of 14 strains of bacteria in use (factory and laboratory samples). After this, add 100ul of liquid culture of each strain from 1st set of incubation individually into their fresh LB containing labeled vial. Vortex each vial for 1-2 seconds or shake by hand afterwards.

3) Place the glass vials into shaker incubator at 37˚C for 1 hour (2nd incubation).

4) 4 different concentrations of the oxidants: 3%, 5%, 7%, and 9% (exceptions in sodium hypochlorite) are considered for this experiment to visualize the contrast in survivability and specific reaction to each concentration in between factory and laboratory strains of each bacteria. As a total of 14 bacterial strains are in use, prepare 4 NA plates (for 4 individual oxidant concentration) for each strain of bacteria inside laminar flow cabinet. Hence the total of 56 NA plates are to be prepared in order to drop spread using one particular oxidant. Plate size may vary from semi medium to large (25ml each).

5) Pipette 900ul saline water into each of the eppeondorf tubes required for serial dilation during drop spreading.

6) After an hour, take out glass vials of 2nd incubation set to start drop spreading. Carry out mathematical calculations prior to creating an oxidant+ culture mixture of a particular concentration.

7) Using glass vials, make oxidant+ culture mixtures of four different concentrations as per the calculations. Note that one vial stands for a specific concentration of the mixture. Hence 4 vials containing mixtures of individual concentrations mark up for one particular bacterial strain.

8) After constructing all the oxidant+ culture mixture of 4 concentrations for each and every strain, proceed to serial dilation. Serially dilute each mixture of concentration from 10^{-1} to 10^{-5} using the eppendorf tubes. Make sure to shake the tubes by hand before each dilation.

9) After operating serial dilutions, proceed to pipette two 10ul drops of the raw mixture of a certain concentration and each of its dilations on a NA plate. Continue the procedure in the exact manner for each and every bacterial strain.

10) Keep all the plates opened inside the laminar cabinet and wait till the drops completely dry down.

10) After the drops have properly dried down, close the plates and place all the plates into the incubator at 37˚C for 24 hours. The results of the drop spread will be visible the next day after the incubation period.

2.5 Calculations of Oxidant+ Culture Concentrations:

During drop spreading with 4 different oxidants, one of the crucial steps was to determine a certain measure of bacterial culture aliquot and ensure to maintain that exact measurement for every distinct bacterial species we were working with.

We determined 100ul as the measure of the liquid bacterial culture aliquot from the 2nd set of incubation. Hence this measurement has been retained for all the bacterial species distinctly for each oxidant calculations throughout the experiment.

1)H_2O_2 (Hydrogen peroxide):

The stock concentration of hydrogen peroxide that we used was at 30%. We have selected and hence optimized 4 individual concentration levels: 3%, 5%, 7%, and 9% from the preliminary concentration. In order to carry out the measurements for each concentration, we

have simply followed the formula of dilution of titration, **S1V1=S2V2.**

Here, **S1**=Concentration of initial solution

S2=Concentration of final solution

V1=Volume of initial solution

V2=Volume of final solution

We need to figure out the initial volume of the mixture solution in order to effectuate the amount of both oxidant and Liquid broth (100ul fixed culture aliquot + fresh LB). We have determined our final volume of mixture solution 500ul for all the concentrations.

2) NaOCl (Sodium Hypochlorite):

Sodium hypochlorite can readily act as a bleaching agent to completely disinfect microbial agents at 0.5% percentage. However, the primary stock concentration of this oxidant that we used was at 8%. Under this condition, we have chosen to create the four concentration levels: 0.0625%, 0.125%, 0.25%, and 0.5% and calculated the measurements by S1V1=S2V2 (formula of dilution), likewise H_2O_2 calculations. Final volume, V2 is set as 500ul in terms of this oxidant as well. The calculations are hereby:

3) $K_2Cr_2O_7$ (Potassium Dichromate):

Potassium dichromate comes in a powdered form unlike hydrogen peroxide and sodium hypochlorite. However, the complete saturating level for this oxidant is 13.6 grams of oxidant powder per 100 ml of water at 25˚celcius. The concentration levels remain similar to those of potassium permanganate, 0.3%, 0.5%, 0.7%, & 0.9%; certainly because of the same issue. The calculations of this oxidant also remain similar as every other oxidant types. The obtained measures are hereby:

Chapter: 03

Results

We have sufficiently operated the drop spread assay with the correct oxidant and culture mixture for individual concentrations for around 11/12 repetitive cycles. The results that are received of growing and survivable microbial colonies from plates where further calculated and statistically described through graphical representation. An important thing to note here is that, we could not carry out appropriate results for all the microbes, and in some cases no result at all for some bacteria because of laboratory mismanagement, repetitive contaminations, technical and hands on errors in the assay, and lack of adequate time and resources. These problems will be demonstrated thoroughly in the errors and limitations segment of this report. The results of the strains that we have failed to collect as well as the ones that are appropriately collected are listed down below:

Name of Oxidant	Names of strains (No results)	Names of strains (With results)
H_2O_2	*E.coli* (fac), *E.coli* (lab), Vibrio2 (lab), Streptococcus (fac), Streptococcus (lab), Staphylococcus (lab), Klebsiella (fac), Salmonella (fac), Salmonella (lab) .	Vibrio1 (fac), Vibrio1 (lab), Staphylococcus (fac), Klebsiella (lab), Vibrio2 (fac).
NaOCl	Streptococcus (lab), Staphylococcus (fac)	*E.coli* (fac), *E.coli* (lab), Vibrio1 (fac), Vibrio1 (lab), Vibrio2 (fac), Vibrio2 (lab), Streptococcus (fac), Syaphylococcus (lab), Klebsiella (fac), Klebsiella (lab), Salmonella (fac), Salmonella (lab).
$K_2Cr_2O_7$	Vibrio1 (fac), Vibrio1 (lab), Staphylococcus (lab), Streptococcus (fac), Streptococcus (lab), Salmonella (fac), Salmonella (lab), Klebsiella (fac), Klebsiella (lab).	*E.coli* (fac), *E.coli* (lab), Staphylococcus (fac), Vibrio2 (fac). Vibrio2 (lab).

Table: The result of the strains that has collected and failed to collect.

To statistically portray the visible growth and survivability differences, we have individually calculated the mean value of each bacterial strain separately for the chosen oxidant

concentration level for of the particular oxidants. After accumulating the mean values, we have made bar graphs using Microsoft Excel which has successfully visualized an approximate difference in colony counts on different concentration levels respectively.

H_2O_2 Graphs:

●Vibrio1 (fac)/Vibrio1 (lab):

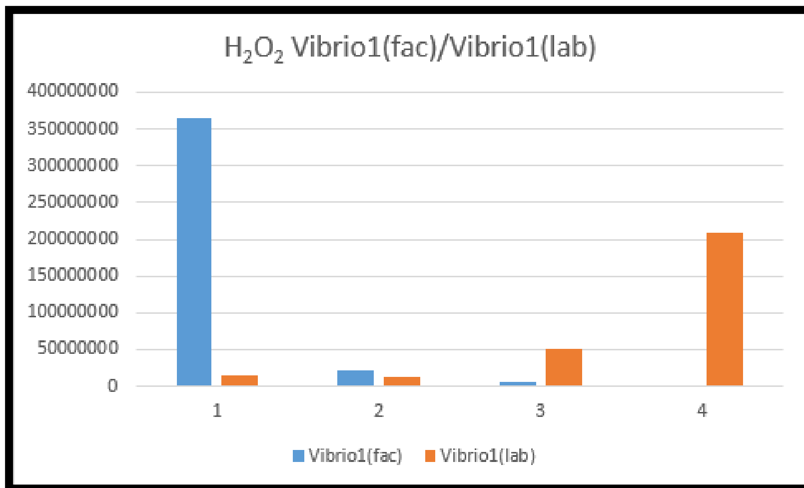

Fig: Graph of the growth and survivability of H_2O_2 on Vibrio 1 (fac) & Vibrio 1 (lab)

Interpretation:

H_2O_2 (%)	Vibrio1(fac) CFU/ml	Vibrio1(lab) CFU/ml
3%	364368916.7	15382833.33
5%	21183600	11531754.17
7%	5178525	50126587.5
9%	1954450	209881787.5

Fig: Interpretation of the graph of **H_2O_2** on Vibrio 1 (fac) & Vibrio 1 (lab)

- **Staphylococcus (fac):**

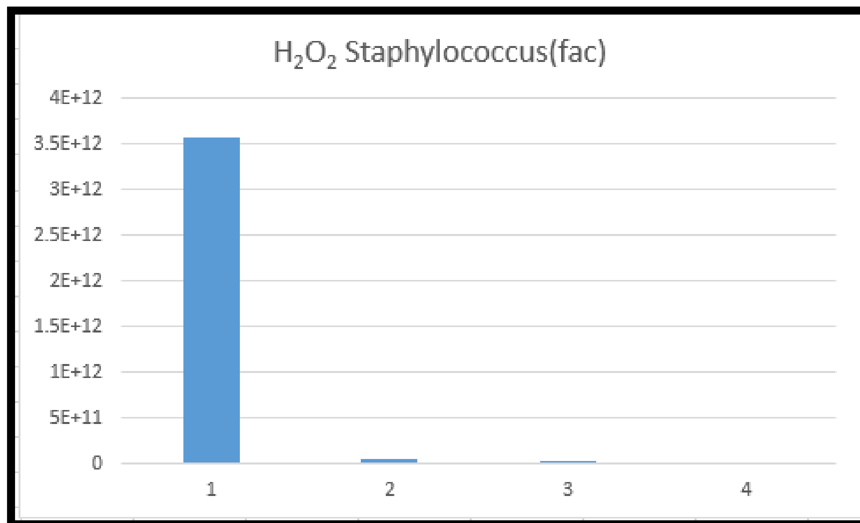

Fig: Graph of the growth and survivability of **H_2O_2** on Staphylococcus (fac)

Interpretation:

H_2O_2 (%)	Staphylococcus(fac) CFU/ml
3%	3.5837E+12
5%	42887185764
7%	30036470000
9%	17878495293

Fig: Interpretation of the graph of **H_2O_2** on Staphylococcus (fac)

- **Klebsiella (lab):**

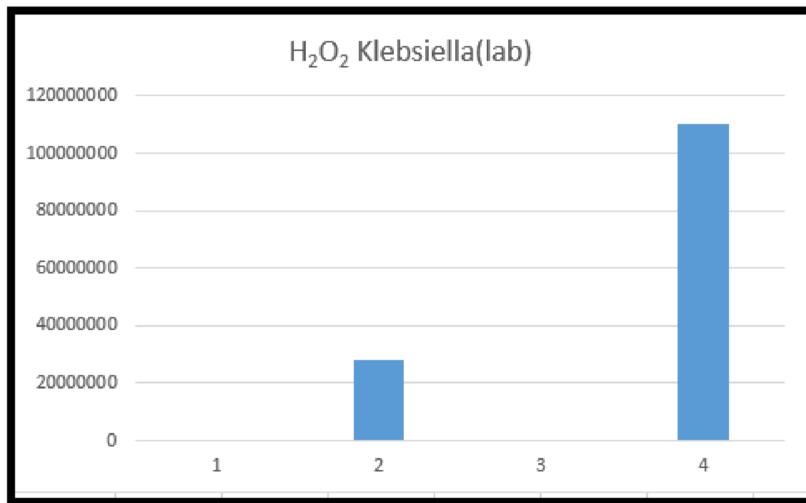

Fig: Graph of the growth and survivability of **H_2O_2** on Klebsiella (lab)

Interpretation:

H_2O_2 (%)	Klebsiella(lab) CFU/ml
3%	
5%	28200625
7%	
9%	110010000

Fig: Interpretation of the graph of **H_2O_2** on Klebsiella (lab)

- **Vibrio2 (fac):**

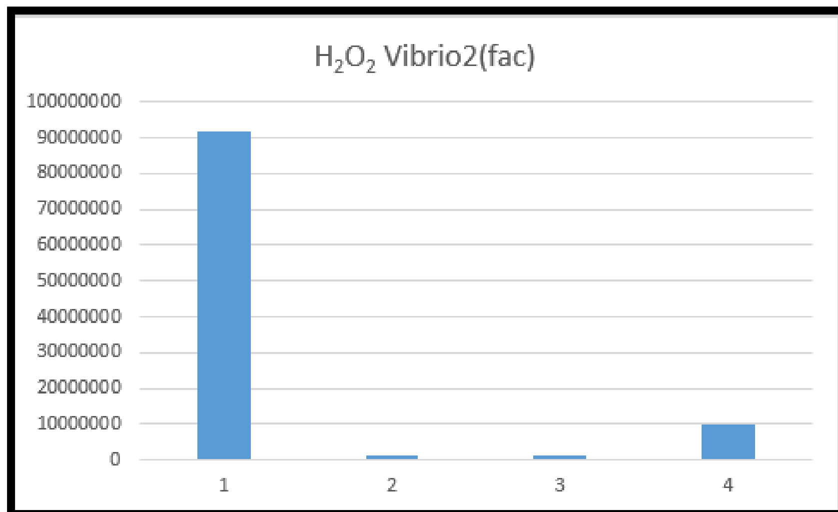

Fig: Graph of the growth and survivability of **H_2O_2** on Vibrio 2 (fac)

Interpretation:

H_2O_2 (%)	Vibrio2(fac) CFU/ml
3%	91681666.67
5%	1275908.333
7%	1223740
9%	10000000

Fig: Interpretation of the graph of **H_2O_2** on Vibrio 2(fac)

NaOCl Graphs:

● *E.coli* **(fac)/***E.coli* **(lab):**

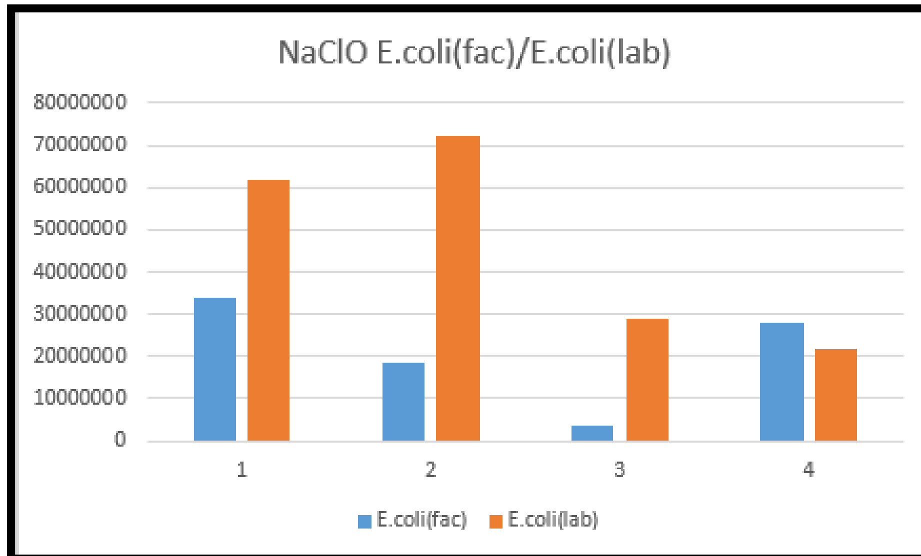

Fig: Graph of the growth and survivability of **NaOCl** on *E.coli* (fac) & *E.coli* (lab)

Interpretation:

NaClO	E.coli(fac) CFU/ml	E.coli(lab) CFU/ml
0.625%NaClO	33983600	61742987.5
0.125%NaClO	18680241.67	72385410
0.25%NaClO	3436280	29005912.5
0.5%NaClO	28045783.33	21515683.33

Fig: Interpretation of the graph of **NaOCl** on *E.coli* (fac) & *E.coli* (lab)

- **Vibrio1 (fac)/Vibrio1 (lab):**

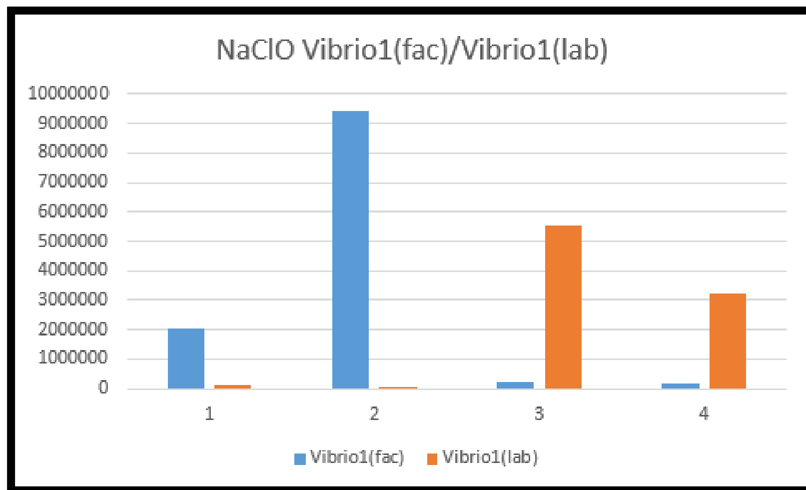

Fig: Graph of the growth and survivability of **NaOCl** on Vibrio1 (fac) &Vibrio1 (lab)

Interpretation:

NaClO	Vibrio1(fac) CFU/ml	Vibrio1(lab) CFU/ml
0.625%NaClO	2017100	90425
0.125%NaClO	9432500	13208.33333
0.25%NaClO	256050	5529400
0.5%NaClO	166866.6667	3199270.833

Fig: Interpretation of the graph of **NaOCl** on Vibrio1 (fac) &Vibrio1 (lab)

- **Vibrio2 (fac)/Vibrio2 (lab):**

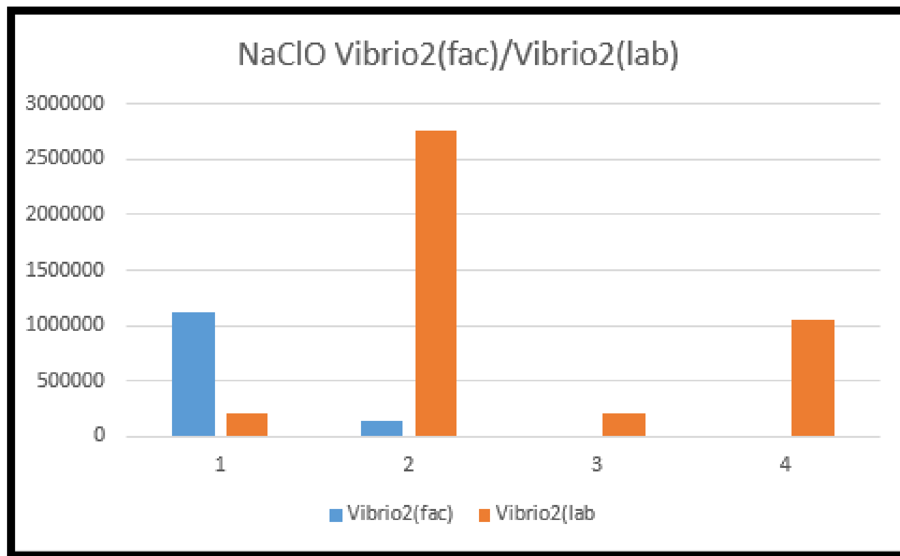

Fig: Graph of the growth and survivability of **NaOCl** on Vibrio2 (fac) &Vibrio2 (lab)

Interpretation:

NaClO	Vibrio2(fac) CFU/ml	Vibrio2(lab) CFU/ml
0.625%NaClO	1116808.333	201600
0.125%NaClO	137830	2758980
0.25%NaClO		212000
0.5%NaClO		1043550

Fig: Interpretation of the graph of **NaOCl** on Vibrio2 (fac) &Vibrio2 (lab)

● **Streptococcus (fac):**

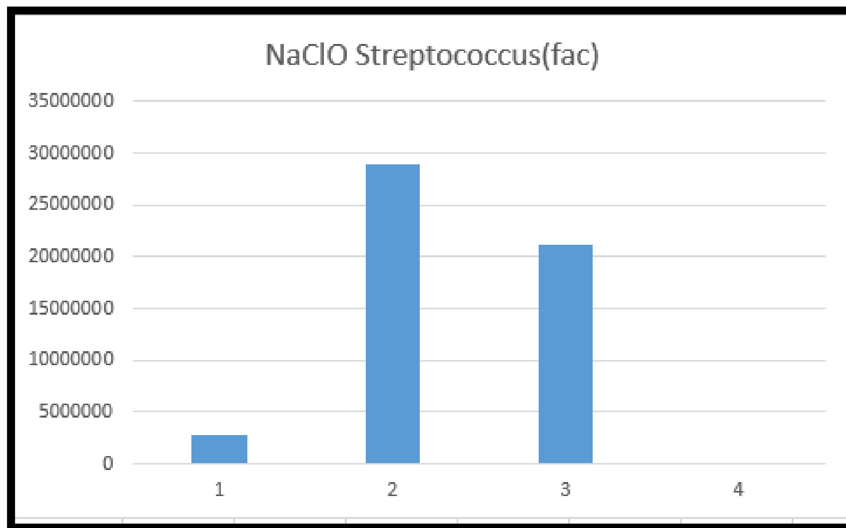

Fig: Graph of the growth and survivability of **NaOCl** on Staphylococcus (fac)

Interpretation:

NaClO	Streptococcus(fac)CFU/ml
0.625%NaClO	2835875
0.125%NaClO	28956025
0.25%NaClO	21138125
0.5%NaClO	

Fig: Interpretation of the graph of **NaOCl** on Staphylococcus (fac)

● **Staphylococcus (lab):**

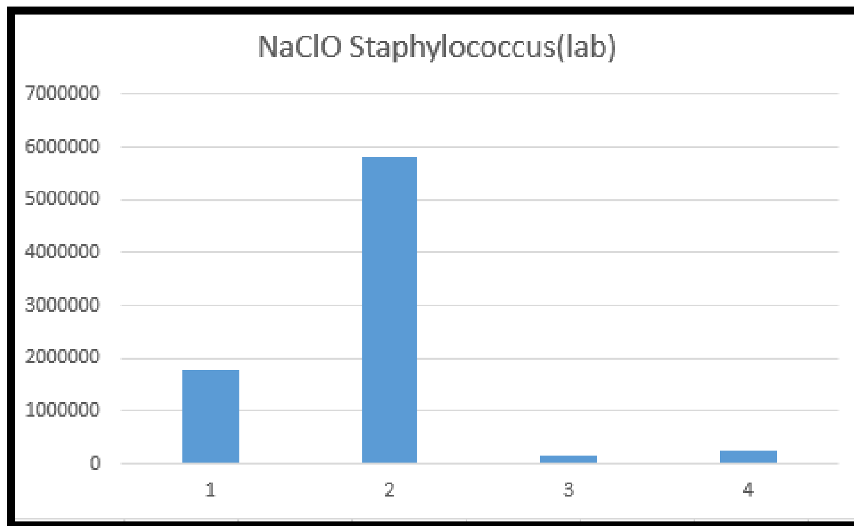

Fig: Graph of the growth and survivability of **NaOCl** on Staphylococcus (lab)

Interpretation:

NaClO	Staphylococcus(lab) CFU/ml
0.625%NaClO	1760583.333
0.125%NaClO	5811310
0.25%NaClO	155370
0.5%NaClO	267437.5

Fig: Interpretation of the graph of **NaOCl** on Staphylococcus (lab)

- **Klebsiella (fac)/Klebsiella (lab):**

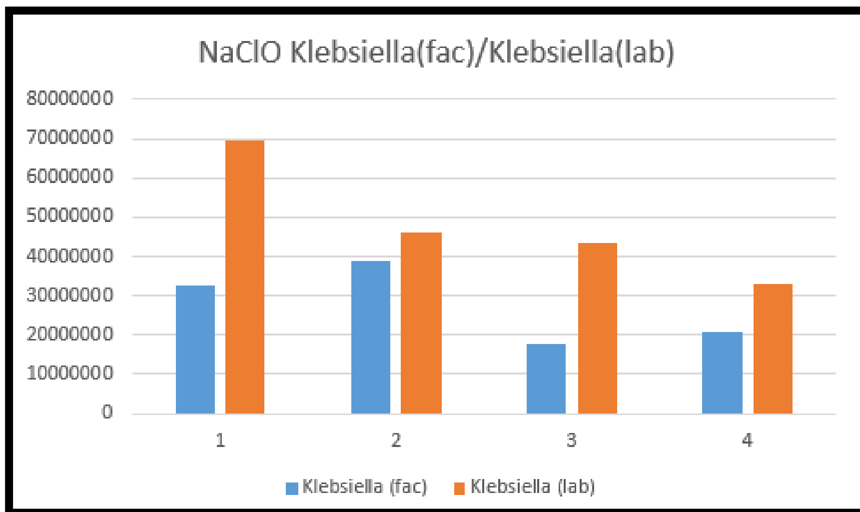

Fig: Graph of the growth and survivability of **NaOCl** on Klebsiella (fac) & Klebsiella (lab)

Interpretation:

NaClO	Klebsiella (fac) CFU/ml	Klebsiella (lab) CFU/ml
0.625%NaClO	32642900	69559375
0.125%NaClO	38844591.67	46214345
0.25%NaClO	17733883.33	43577360
0.5%NaClO	20596883.33	32931254.17

Fig: Interpretation of the graph of **NaOCl** on Klebsiella (fac) & Klebsiella (lab)

● **Salmonella (fac)/Salmonella (lab):**

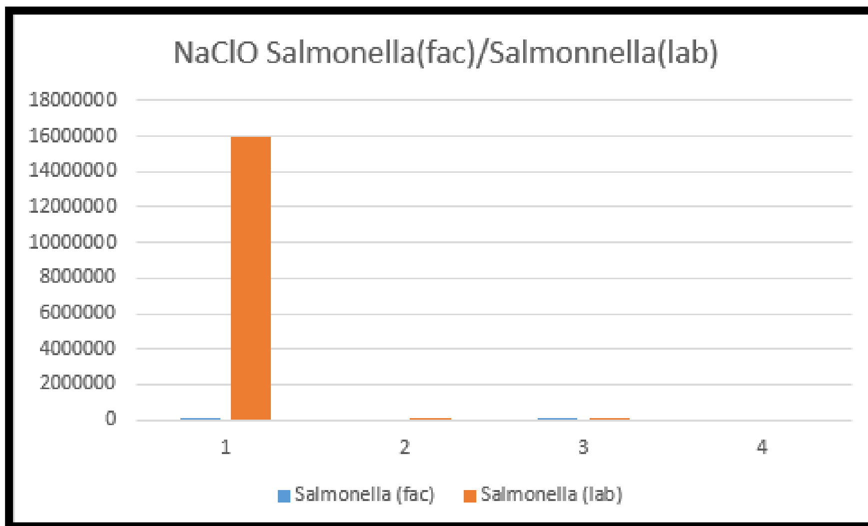

Fig: Graph of the growth and survivability of **NaOCl** on Salmonella (fac) & Salmonella (lab)

Interpretation:

NaClO	Salmonella (fac) CFU/ml	Salmonella (lab) CFU/ml
0.625%NaClO	23833.33333	15964166.67
0.125%NaClO		113712.5
0.25%NaClO	29216.66667	102500
0.5%NaClO		

Fig: Interpretation of the graph of **NaOCl** on Salmonella (fac) & Salmonella (lab)

$K_2Cr_2O_7$ Graphs:

●*E.coli* **(fac)**/*E.coli* **(lab):**

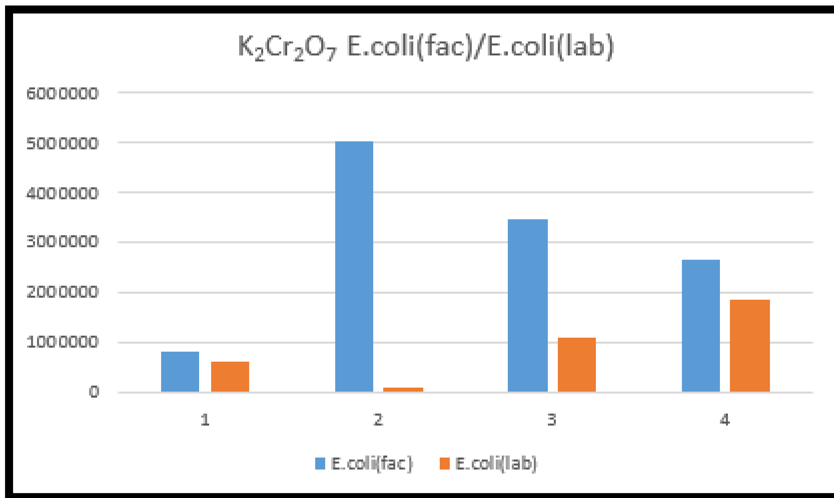

Fig: Graph of the growth and survivability of $K_2Cr_2O_7$ on *E.coli* (fac) & *E.coli* (lab)

Interpretation:

$K_2Cr_2O_7$ Concentration (%)	E.coli(fac) CFU/ml	E.coli(lab) CFU/ml
0.3%K2Cr2O7	809608.3333	593095.8333
0.5%K2Cr2O7	5032880	97816.66667
0.7%K2Cr2O7	3451675	1096475
0.9%K2Cr2O7	2660400	1854058.333

Fig: Interpretation of the graph of $K_2Cr_2O_7$ on *E.coli* (fac) & *E.coli* (lab)

•Staphylococcus (fac):

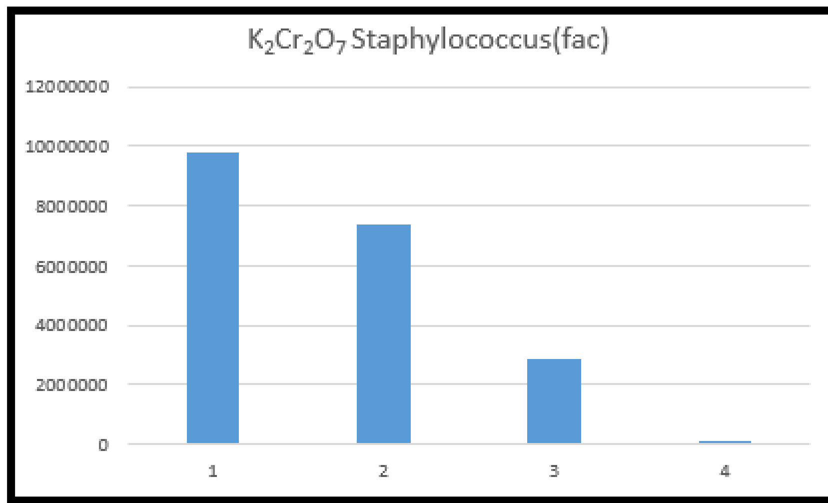

Fig: Graph of the growth and survivability of $K_2Cr_2O_7$ on Staphylococcus (fac)

Interpretation:

$K_2Cr_2O_7$Concentration (%)	Staphylococcus(fac) CFU/ml
0.3%K2Cr2O7	9783750
0.5%K2Cr2O7	7363406.239
0.7%K2Cr2O7	2890632.852
0.9%K2Cr2O7	145829.9364

Fig: Interpretation of the graph of $K_2Cr_2O_7$ on Staphylococcus (fac)

●Vibrio2 (fac)/Vibrio2 (lab):

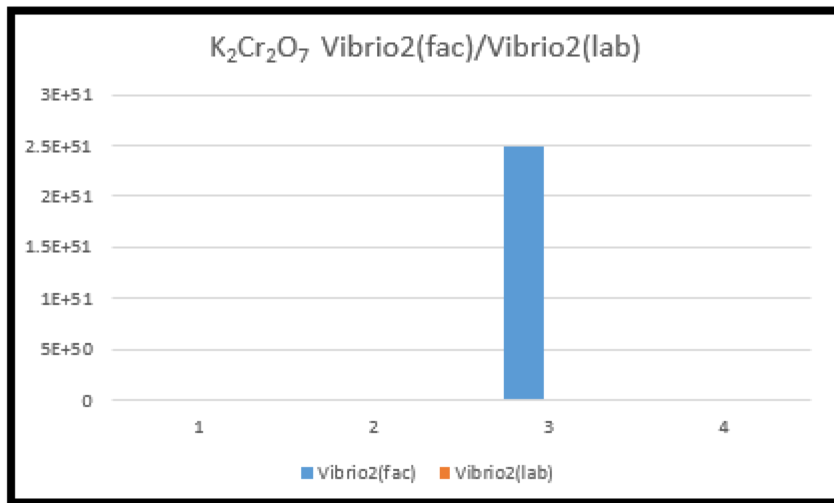

Fig: Graph of the growth and survivability of $K_2Cr_2O_7$ on Vibrio2 (fac) & Vibrio2 (lab)

Interpretation:

$K_2Cr_2O_7$Concentration (%)	Vibrio2(fac) CFU/ml	Vibrio2(lab) CFU/ml
0.3%K2Cr2O7		148750
0.5%K2Cr2O7		3925666.667
0.7%K2Cr2O7	2.5E+51	3925666.667
0.9%K2Cr2O7	1.125E+32	154965

Fig: Interpretation of the graph of $K_2Cr_2O_7$ on Vibrio2 (fac) & Vibrio2 (lab)

Chapter: 04

Discussion

In this study, the ultimate motive of analysis was to observe how different microorganisms react to different stress factors by keeping them under similar environmental conditions. The research was based on two groups of microorganisms, one of which are chemically stressed microbes collected and retrieved from industrial wastewater and the other group is the corresponding conventional laboratory microbial strain (not naturally stressed) of those industrial strains, to be used as our control group. By formation of the assay, both the groups were tested simultaneously at the same time with our chosen oxidants of interest. Ideally, it was expected that the conventional laboratory strains will show less growth and survivability due to lack of genetic modifications to tolerate stressors, and also the lack of correct antioxidant functions to survive through this extreme oxidative conditions. On the other hand, the industrial wastewater microbes were expected to show grater survivability and growth because of its naturally stressed metabolic conditions and hostile environment. The industrial wastewater can be basically of two forms: treated, and untreated. The samples we chose for our study was from an intermediate level of untreated wastewater where chemical substances are efficiently present but not at a stage where the wastewater is completely washed out using chemical treatment prior to ETP. Hence the sample was considered to be ideal as chemically induced stressors in the sample water could profoundly generate ROS, with sufficient amount of microorganisms present in the sample water that were constantly battling to stay alive despite the harsh external conditions.

However, some of the results did meet the desired goal and has shown discretely specific results. Despite controlling all the measures and techniques as neatly as possible to eradicate contacts, there are some results which have shown the complete opposite results of what was ideally expected. The drop spread results for each oxidant category is discussed hereby:

1) H_2O_2: Only results for Vibrio type 1 both laboratory and factory strain, Staphylococcus and Vibrio type 2 factory strain, and Klebsiella Laboratory strain could be appropriately collected and graphically represented.

In VIB1S1/VIB1S1L graph, it is seen that the factory vibrio type strain had its highest growth in 3% of the oxidant concentration having slightly more than 3.5 billion/ml cells, lesser than 4 billion. However under the same concentration, the control strain of Vibrio type 1 has grown into much lesser than 50 million/ml cells, supposedly 5-10 million cells of an approximate value. Now, in the next concentration levels: 5%, 7%, and 9%, we observe a massive fall in colony numbers of factory strain vibrio type 1, indicating an intense suppression in growth and

survivability of this strain. The colony numbers have drastically decreased from 3.5 billion cells per ml in 3% oxidant level to merely 2 million cells per ml in 9% oxidant level. To the contrary of the factory strain, the laboratory vibrio type 1 strain has responded unusually to the assay by actually increasing in colony numbers from 7% to 9% oxidant concentrations. Where there were initially around 5-10 million cells/ml in the lowest level of oxidant concentration, the colony numbers of the laboratory strain hiked up to around 2.1 billion cells/ml. This is a massive drawback and a contradiction to our research theme. It is important to note that these results are free of contaminations, and with the highest strength of correctness of the assay protocol. Further thorough research is nevertheless required to substantially demonstrate the cause of such phenomenon.

There are two distinct results for the factory strains of Staphylococcus and Vibrio type 2. Results for their control strains are hence not obtained due to technical limitations and shortcoming of our research procedure. One great similarity in both of these factory strains is that both of the microorganisms have survived and grown the most in the lowest oxidant concentration at 3%. The approximate colony numbers for Staphylococcus and Vibrio type 2 are 3.8 billion/ml and 9.1 million/ml respectively. Moreover, for staphylococcus strain the colony numbers have drastically decreased with inversely increasing concentration levels. The numbers have barged down towards an approximate of 1.8billion/ml cells in the highest concentration levels. The sufficient decline in factory staphylococcus growth reportedly demonstrates the active metabolic suppression of the bacteria, making it weakly capable of surviving in highly concentrated levels. Apart from the factory strain, as there is no control result for Staphylococcus under this oxidant we are unable to sketch a contrasting image for survivability in this case. In terms of factory Vibrio type 2 strain, the scenario is almost similar with factory staphylococcus strain with a slightly unusual picture for the highest concentration. For 5% and 7% of concentration, the growth numbers for Vibrio type 2 factory strains have remarkably lowered into an approximate of 1.2million/ml of cells, contrarily increasing up to 10million/ml in 9% concentration level.

The last result under this oxidant category is the laboratory strain of Klebsiella. An exception in this case is we have no results of 3% and 7% concentrated plates due to severe contamination in the plates, sufficiently making it incapable for counting colonies. The results of 5% and 9% concentrated plates have abnormally shown increasing number in colonies such as: 2.8million/ml cells in 5% concentration, and more than 1.1billion/ml cells in 9% concentration.

2) NaOCl: We have sufficiently collected and calculated the most amount of graphical results demonstrating effective comparison between both groups of microbes. The assay for this batch has the least amount of contaminations and technical errors. The only results that were unable of collection were: Streptococcus laboratory strain and Staphylococcus factory strain.

The comparison study of microbes under this oxidant were true to be less contaminated, but highly unusual mostly for majority of bacteria. Let us firstly consider the case for Vibrio type 1. This factory strain and its control strain have shown an unusual increase and decrease, opposing to our study expectations. For the factory strain, the colony growth unusually hiked up in 0.125% concentration level at an approximate 9.4million/ml cell numbers. Whereas the colony count was significantly lower in rest of the concentration ranging from 2million/ml (0.625%) to lesser than 270 thousand to 170 thousand cells per ml. The control strain in this case have also unusually grown in higher level of concentrations, 0.25% and 0.5%, marking a range of approximately 5.6million/ml cells at 0.25% and 3.2million/ml cells at 0.5%. In lower concentrated levels of the oxidants, the survivability of the control microbe is extremely low. This however sketches a contradictory image of the expected outcomes for both the groups. We can observe similar results in opposite directions for Vibrio type 2 strain. There are no results obtained for factory strain Vibrio type 2 at 0.25% and 0.5% concentration. However in between the rest two concentrations, surprisingly the factory strain has more countable colonies and at the lowest concentration (0.0625%). The CFU/ml has substantially decreased for the consecutive concentration. Results for the laboratory strain is properly portrayed for all the concentration. The growth for the laboratory strain Vibrio type pitches the highest at 0.125% oxidant concentration with an approximate of 2.8million cells/ml whilst the CFU/ml remains quite similar for both 0.625% and 0.25% at approximately 200-220 thousand cells/ml. This is an abnormality to a point as the CFU/ml has unexpectedly peaked at an intermediate concentration level. By the last concentration at 0.5%, the CFU/ml for Vibrio type 2 has increased up around 10 million cells/ml.

For *E.coli* (fac) and *E.Coli* (lab), again an unusual occurrence in the results is observed where the control microbe *E.Coli* (lab) is observed to surprisingly survive more than factory *E.coli* (fac) strain. Needless to mention that *E.coli* (fac) itself is a facultative anaerobe bacterium, meaning it can naturally survive in the presence of free oxygen radical formations. Under hydrogen peroxide, *E.coli* (fac) has preliminarily shown colonial growth of approximately 3.4million/ml cells at 0.625%, slowly reducing to less than 2 million cells for both 0.125% and 0.25% concentrated levels. On the other hand for *E.Coli* (lab), the growth rate was much higher

than *E.coli* (fac) at 0.625%, 0.125%, and 0.25% concentrated levels with the highest cell count of approximately 7.1million cells per ml at 0.125% of oxidant expression. An exception for both of the microbes is observed in 0.5% concentrated level where the *E.Coli* (lab) colony count was slightly lower in number than that of *E.coli* (fac) colony count. Being a facultative anaerobe, both the microbes have shown sufficient survivability and growth at every concentration levels. However the comparison study was not satisfying as the CFU/ml for both the microbes were at undesirable levels at any of the given concentrated levels.

A similar result to *E.coli* (fac)/ *E.Coli* (lab) can be noticed in terms of factory strain of Klebsiella and its control strain. The control Klebsiella strain had a higher CFU/ml level than its factory strain throughout the four concentration levels. Where CFU/ML for control klebsiella strain ranged from approximately 7million/ml at 0.625% to 3.2million/ml at 0.5%, CFU/ml for the factory strain remained less than 4million cells/ml for all the four concentrations. Despite the growth and survivability for both of the grouped microbes have for sure simultaneously decreased with the increasing number of concentration levels, it was still expected that the CFU/ml count for the factory klebsiella strain to be grater in number than its control strain.

For Streptococcus and Staphylococcus, there is only one group of results gathered from both which are: factory strain for Streptococcus, and laboratory strain for Staphylococcus. There are no comparative study proceeded for these bacterial types. Only the received results are hereby explained. For Staphylococcus laboratory strain, the growth bar has peaked the highest at 0.125%, highly resembling with the results of Vibrio type 2 laboratory strain. Where the CFU/ml for the rest of the three concentration remains under approximately 2 million cells/ml, the colony growth has survived for up to around 6 million cells/ml for the 0.125% concentration of oxidant. We sketch a similar scenario again for the same concentration in terms of Streptococcus factory strain. Whilst the survivability rate marks under 20million cells/ml for 0.625% and 0.25%, the growth is above approximately 30 million cells/ml only for 0.125% of concentration. There are no results obtained for 0.5% of concentration.

For the last bacterial type under this oxidant is Salmonella which probably has the most unusual results out of all the bacterial types. We could not attain results for 0.5% concentration level for any of the strains of Salmonella, and 0.125% concentration result for factory Salmonella strain. Regardless of the unachievable results, the most unusual growth rate is observed in laboratory salmonella strain. For laboratory Salmonella, CFU/ml is marked up to around

10.5million whilst all the other results of this bacterial type remain under 2 million CFU/ml. This is a gigantic shift of unusualness as there are so many questions that arise with this atypical comparative difference. Factory strain of Salmonella remained under 2 million cells/ml for all the three concentrations with results. Laboratory strain of this bacterial type also remained remarkably low opposite to the factory strain at both 0.125% and 0.25% of oxidant concentration. The astonishing factor of such significant difference in only 0.625% of concentration only for the laboratory strain of Salmonella is what has made the comparative analysis more difficult to sketch and explain.

3) $K_2Cr_2O_7$: Being the only oxidant of our list which comes as a powdered format unlike the other two oxidants, it was quite difficult to articulate contamination and error free results for most of the bacterial types under Potassium Dichromate. It took us quite a lot of time to firstly figuring out the proper procedure of making different concentration rates for this oxidant let alone proceeding the strains through drop spread assay. Results for only *E.coli* (fac) */ E.Coli* (lab), Vibrio type 2 factory and laboratory strain, and Staphylococcus factory strain are achieved and presented under this concentration.

Starting with *E.coli* (fac) */ E.Coli* (lab), there are two different factors that are to be noticed in this comparative analysis. Firstly, we observe that the growth and survivability rate for *E.Coli* (lab) remains lower than *E.coli* (fac) for all the concentration, hence serving the main motive of our study. Secondly however, the growth bars have not pitched at an expected value for any of the concentrations. For *E.coli* (fac), the highest growth rate marks at 0.5% with approximately 5 million CFU/ml whereas the number is marginally low at 0.3% of concentration with under 1 million cells/ml. The rate for 0.7% and 0.9% remain in between 2.5 million-3.6 million CFU/ml which are both lower than that of 0.5% of concentration. In terms of *E.Coli* (lab), this strain has survived lesser than its factory strain, however it has grater survivability in higher concentrations than lower concentration. For the lower concentrations at 0.3% and 0.5%, the growth rate of *E.Coli* (lab), remains under 1 million CFU/ml whereas the numbers for 0.7% and 0.9% of concentration remain in between approximately 1 milion-2 million cells/ml. Despite showing low levels of survivability than the factory strain, the laboratory strain still has unusual peaks at different concentration levels separately.

Vibrio type 2 results are hence devoid of the research expectations as well. There are no results for the two lower concentrations of the factory strain of this bacterial type. However the CFU/ml has astonishingly marked up to trillions of cells for the factory strain at the two higher concentrations. The highest growth rate of Vibrio type 2 factory strain stands at around more than 2.6 trillion CFU/ml at 0.7% of the concentration which has on the other hand decreased to less than 1.4 trillion cells/ml at 0.9% of concentration. As we can see that the growth bar has visibly decreased with higher level of concentration, one idea could be an assumption of back calculation that there can be possibly higher growth and survivability rate in the lower oxidant levels for the factory strain of Vibrio type 2. Despite the assumption, we cannot overlook and ignore the unusualness and abnormality of results for the other bacterial types. Hence, the back calculation only avails as an assuming factor for the study. For the laboratory strain of the bacteria, there are insanely low levels of growth observed with enormously lower levels of survivability rates for all the concentrations. The CFU/ml range only up to 3.9 million cells to even lower than only 100 thousand cells. Highest growth rates are at the 0.5% and 0.7% concentration levels by similarly ranging up to 3.9 million CFU/ml.Whereas for 0.3% and 0.9%, the rates are notably lowered to about 150 thousand cells/ml. Although the growth rates are not in an expected order by their concentration points, the survivability of the laboratory strains remain extremely lower comparative to the factory strain of Vibrio type 2. This part of the analysis does partially validate our study expectations.

The last bacteria under this oxidant type is Staphylococcus factory strain. This strain has appropriately met our study objective by discretely showing desired results for each of the concentration levels. At first we see that the growth rate has pitched its highest mark at the lowest concentration level 0.3% with a CFU/ml of an approximate of 9.8 million cells. The rate is shortly decreased to 7.3 million CFU/ml for 0.5% of concentration. The growth bar again drops rationally at around 2.8 million CFU/ml at 0.7% concentration. Finally, the lowest level of growth is seen to be in the highest concentration level, 0.9%, with an approximate of only about 145 thousand CFU/ml, much lesser than a million. As we can clearly see, this bacterial strain has shown difficulty surviving with each increasing value of the concentration. Thus only for this strain it has given us a desired outcome. However as there are no results for the laboratory strain opposite to this strain, no comparative analysis can be sketched for this bacterial type.

By adequately scrutinizing the received results, it is observed that a large portion of the comparative analysis has abnormally shown unfamiliar outcomes. However there are still study

gaps to confirm the unusualness of the results and how both the groups of the microbes are supposed to react to stress factors. From our perspectives, there are a bunch of different errors and limitations of our study that have been expected to take pace throughout or laboratory operations. These errors and limitations can possibly explain the unexpected results apart from the study gaps. Hence these factors are described hereby.

4.1 Errors:

- **SODIUM HYPOCHLORITE:**

The necessity of keeping sodium hypochlorite in closed, opaque containers. Due to the breakdown effects of ultraviolet rays and heat, sodium hypochlorite is best maintained for the longest storage life at temperatures around or below 60°F (15°C), when filtered and free of contaminants, at dilute concentrations that preserve pH above 10, and without direct sun exposure. The range of 2.5%–5% is the most widely utilized NaOCl concentration. It is necessary to improve the storage conditions for NaOCl and the activation techniques used. Additionally, the concentrations, length of irrigation, storage of NaOCl, and usage of irrigation adjuncts varied amongst specialized practices and GDPs. The sodium hypochlorite in bleach starts to deteriorate at a rate of roughly 20% each year after the six-month expiration date. The solubility and bactericidal effects of sodium hypochlorite (NaOCl) are improved when the temperature is raised. However, despite the solution's warmth, multi-species biofilms' strong resistance could be able to limit its effects, allowing the surviving bacteria to recover over time. Undiluted household bleach has a shelf life of six to twelve months after the date of manufacturing, after which it deteriorates at a rate of 20% each year until it is completely converted to salt and water, while a 1:10 bleach solution has a shelf life of twenty-four hours, according to Clorox.

Hypochlorite is quite difficult to store in anything other than the original box, a plastic bucket with a screw-on cover. ALL other attempts fail. All metal containers, plastic straws with flame seals on both ends, screw-on plastic containers, plastic bags, rubber seals, wax seals, and glues were destroyed. Evaporated from all containers, including ordinary food-grade buckets with snap-on lids and glass beakers with glass tops. When trying to hold little amounts, vapors ate through the fabric of the Go Bag.

We essentially had no idea how to maintain hypochlorite, so we put it in a conical flask that was typically wrapped in aluminum foil. We were unable to get the required outcome since we were unaware of the proper preservation methods for hypochlorite.

This material is toxic and unsafe to handle or breathe in. However, for producing bleach or placing a few grains in a water bottle to disinfect water, nothing beats hypochlorite (70% or more without additives, do your homework). For long-term storage to create disinfection solutions, keep a few one-pound bags in the original bucket.

● HYDROGEN PEROXIDE:

To preserve hydrogen peroxide and extend its shelf life, you can follow these guidelines:

Storage container: Transfer hydrogen peroxide to a container that is either dark in color or opaque. It may degrade more quickly when exposed to light. Pick a glass or plastic container if possible, as these materials offer better light protection.

Seal tightly: After each use, make sure the container is well sealed. This helps keep the hydrogen peroxide's efficacy by preventing air from getting inside.

Temperature control: Keep hydrogen peroxide away from heat sources and direct sunlight in a cold, dark location. The deterioration process may be accelerated by higher temperatures. If at all possible, store it in a refrigerator.

Avoid contaminants: Maintaining a clean and contaminant-free container is important. This applies to any foreign materials, such as dirt or dust, that might come into contact with the hydrogen peroxide.

Original container: Hydrogen peroxide can be kept in its original container if you'd prefer because those are usually made to shield it from light. Make sure the original cap is tightened all the way.

Check expiration date: When properly stored, hydrogen peroxide typically has a shelf life of one to three years. Always check the expiration date before using hydrogen peroxide, and throw away any that has.

But we didn’t know about it as we didn’t imagine of it also the lab assistants didn’t tell us anything about it.

2 The incubator at BRAC Lab is highly contaminated. We repeatedly requested to our lab officer clean it, but it was never completed. As a result, fungus, pseudomonas, and other live things used to infect our plates. As a result, we had to deal with a lot of issues. Because of that, the majority of the experiment has become polluted.
3 In the beginning of the drop spread assay, we didn't know how to autoclave saline, vials, or eppendoufs due to a lack of knowledge. And as a result, our results were tainted.
4 The lab's refrigerators are also infected. Many culture plates used to be placed on the fresh media fridge because first-year students don't know much about laboratory work, which caused our plates to become contaminated.
5 A lot of students had to work in a tiny space. For the autoclave, each group had a lot of beakers filled with eppendous, vials, test tubes, etc. as well as pipette tips, media, etc. Due to overloaded conditions, some of the foil paper used to tear during re-autoclave, and as autoclave time increased, our drop assay time decreased. As a result, we were unable to finish our drop assay in a timely manner, and the next day when we returned to work, the results were messy due to bacterial regrowth.
6 Students have been known to embezzle pipette tips, autoclaved vials, and eppendoufs from one another, which has caused our experiment to be delayed and caused bacterial solution contamination or bacterial self-regrowth.

4.2 Limitations:

There are several reasons why we didn't get the desired result in our scientific experiment. Here are some possible factors to consider:

Experimental Design: We were unable to properly plan our experiment because we are undergraduate students. Examining the experimental design to make sure it was well thought out and carried out. We started out making a lot of blunders.

Methodology and Techniques: Since few people have conducted this topic-related experiment, we were unable to read numerous articles in order to build our protocol. We had to experiment with a variety of our supervisor's ways.

Equipment and Materials: The majority of the tools and supplies were seriously harmed. Due to broken pipettes and our inability to do accurate measurements, we obtained inaccurate data.

Environmental Factors: Our plates used to become contaminated because we had to share an incubator and a refrigerator with other groups, and there was nothing we could do about it in this topic but accept the difficulties.

Human Error: Take into account the likelihood of human error when gathering, analyzing, or interpreting data. To ensure accuracy, double-check the computations, measurements, and observations. Collaboration and peer review can reduce the possibility of human error.

Biological Variability: For this investigation, we combined laboratory samples with factory samples that we had obtained. As living things are a part of our experiment, biological systems can have inherent variability. Our findings could be impacted by genetic variations, individual variance, or unforeseen biological reactions. Replicates or a larger sample size can also contribute to accounting for this variability.

Unforeseen Factors: Unexpected or unpredictable events can occasionally have an impact on the experiment. It might be an unidentified confounding factor, an outside occurrence, or an unaccounted-for interaction. Our laboratory strains were more potent than industrial strains for some strange reason.

Chapter: 05

Conclusion

This research has widely focused on sketching a contrasting image of the tolerability of reactive Oxygen species and other stress factors between two groups of bacteria: chemically stressed microbes and their correspondent laboratory microbes as control groups. Two indicators of this study to accurately portray stress tolerance was to check the growth and survivability rate of the microbes simultaneously. A microbial assay was established where primarily three oxidants o interest were chosen for ROS formation in both groups of bacteria. Later on a drop spread protocol was built and operated throughout the research to identify growth rates of both group of the microbes. After 11/12 repetitive cycles of the assay, sufficiently possible outcomes are achieved of both the microbe groups avoiding all the contaminated erroneous results. Visible single colonies and scattered colonies are counted as accurately as possible, later on the CFU/ml was calculated for every single microbe separately for their dilutions and oxidant concentrations using Microsoft Excel operations. After this calculation, a mean value of the CFU/ml for every microbe of both groups (with results) were statistically calculated and represented through bar graphs on Excel. Hence a proper comparative analysis was pictured through the graphs. The graph results have shown both desirable and unusually unexpected results apart from the research objective. An event of errors and limitations of our study is hence described that could possibly describe the abnormal and unfamiliar results of the comparisons. The study requires more resources, time, and in fact further optimizations to substantially reach our initial research objective, or even reach different aspects of visualizing the comparative analysis.

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Appendix

Appendix 1

Hydrogen Peroxide’s mastersheet, calculation and statistics:

EXCEL NEW .XLSX
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A1 Exp No:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	Exp No:	0	H2O2																	
2	Organism	3%	5%	7%	9%	Control	3%	5%	7%	9%										
3	VIB1S1	107	92	33	44	VIB1L	20	25.5	65.5	14.5		5350	4600	1650	2200	#VALUE!	1000	1275	3275	725
4	E.COLI3	0	0	0	0	DH5a	50.5		1	6.5		0	0	0	0	#VALUE!	2525	0	50	325
5	SPY3	m	87	conta	71	SPYL						#VALUE!	4350	#VALUE!	3550	#VALUE!	0	0	0	0
6	SAL4	n/d	n/d	n/d	n/d	SALL						#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0	0	0	0
7	STR2	28	32	5	n/g	STRL						1400	1600	250	#VALUE!	#VALUE!	0	0	0	0
8	VIB2	60	59	64	33	VIB2L						3000	2950	3200	1650	#VALUE!	0	0	0	0
9	KLB2		122	110	96	KLBL						0	6100	5500	4800	#VALUE!	0	0	0	0
10																				
11	Exp No:	1	H2O2																	
12	Organism	3%	5%	7%	9%	Control	3%	5%	7%	9%										
13	VIB1S1	92	84	29	29	VIB1L	17	13.5	27.5	9		46000	42000	14500	14500	#VALUE!	8500	6750	13750	45000
14	E.COLI3	0	0	0	0	DH5a	7		27	2		0	0	0	0	#VALUE!	3500	0	13500	10000
15	SPY3	70	52	conta	47	SPYL						35000	26000	#VALUE!	23500	#VALUE!	0	0	0	0
16	SAL4	n/d	n/d	n/d	n/d	SALL						#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0	0	0	0
17	STR2	23	25	1	n/g	STRL						11500	12500	500	#VALUE!	#VALUE!	0	0	0	0
18	VIB2	32	45	41	34	VIB2L						16000	22500	20500	17000	#VALUE!	0	0	0	0
19	KLB2		96			KLBL		55	43.5	13		0	48000	0	0	#VALUE!	0	27500	21750	65000
20																				
21	Exp No:	2	H2O2																	
22	Organism	3%	5%	7%	9%	Control	3%	5%	7%	9%										
23	VIB1S1	84	51	21	32	VIB1L	27.5	36.5	48.5	9.9		420000	255000	105000	160000	#VALUE!	137500	182500	242500	495000
24	E.COLI3	0	0	0	0	DH5a	1.5		2			0	0	0	0	#VALUE!	7500	0	10000	0
25	SPY3	68	44	50	38	SPYL						340000	220000	250000	190000	#VALUE!	0	0	0	0
26	SAL4	n/d	n/d	n/d	n/d	SALL						#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0	0	0	0
27	STR2	11	12	n/g	n/g	STRL						55000	60000	#VALUE!	#VALUE!	#VALUE!	0	0	0	0
28	VIB2	38	46	39	26	VIB2L						190000	230000	195000	130000	#VALUE!	0	0	0	0
29	KLB2					KLBL	31	180		34.5		0	0	0	0	#VALUE!	155000	900000	0	1725000
30																				
31																				
32	Exp No:	3	H2O2																	
33	Organism	3%	5%	7%	9%	Control	3%	5%	7%	9%										

ALL STATS | H2O2 MASTER | H2O2 CALCULATIONS | H2O2 STATS | Hypo | HYPO CALCULATIONS | HYPO STATS | K2Cr2O7 master | K2Cr | Explore

EXCEL NEW .XLSX
File Edit View Insert Format Data Tools Help
A1 Exp No:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
32	Exp No:	3	H2O2																	
33	Organism	3%	5%	7%	9%	Control	3%	5%	7%	9%										
34	VIB1S1	73	46	19	31	VIB1L	13	15	20	2.5		3650000	2300000	950000	1550000	#VALUE!	650000	750000	1000000	1250000
35	E.COLI3	0	0	0	0	DH5a						0	0	0	0	#VALUE!	0	0	0	0
36	SPY3	56	31	42	45	SPYL						2800000	1550000	2100000	2250000	#VALUE!	0	0	0	0
37	SAL4	n/d	n/d	n/d	n/d	SALL						#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0	0	0	0
38	STR2	n/g	n/g	n/g	n/g	STRL						#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0	0	0	0
39	VIB2	36	8	18	22	VIB2L						1800000	400000	900000	1100000	#VALUE!	0	0	0	0
40	KLB2					KLBL		117.5		51.5		0	0	0	0	#VALUE!	0	5875000	0	25750000
41																				
42	Exp No:	4	H2O2																	
43	Organism	3%	5%	7%	9%	Control	3%	5%	7%	9%										
44	VIB1S1	69	39	10	10	VIB1L	18	11	24	6.5		34500000	19500000	5000000	5000000	#VALUE!	9000000	5500000	12000000	32500000
45	E.COLI3	0	0	0	0	DH5a	1					0	0	0	0	#VALUE!	500000	0	0	0
46	SPY3	45	37	40	34	SPYL						22500000	18500000	20000000	17000000	#VALUE!	0	0	0	0
47	SAL4	10	4	n/g	n/g	SALL						5000000	2000000	#VALUE!	#VALUE!	#VALUE!	0	0	0	0
48	STR2	n /g	n/g	n/g	n/g	STRL						#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0	0	0	0
49	VIB2	16	4	conta	11	VIB2L						8000000	2000000	#VALUE!	5500000	#VALUE!	0	0	0	0
50	KLB2					KLBL	37.5	212		82.5		0	0	0	0	#VALUE!	18750000	106000000	0	412500000
51																				
52	Exp No:	5	H2O2																	
53	Organism	3%	5%	7%	9%	Control	3%	5%	7%	9%										
54	VIB1S1	36	21	5	1	VIB1L	16.5	12.55	57.5	24.5		180000000	105000000	25000000	5000000	#VALUE!	82500000	62750000	287500000	1225000000
55	E.COLI3	0	0	0	0	DH5a						0	0	0	0	#VALUE!	0	0	0	0
56	SPY3	39	38	32	26	SPYL						195000000	190000000	160000000	130000000	#VALUE!	0	0	0	0
57	SAL4	3	1	n/g	n/g	SALL						15000000	5000000	#VALUE!	#VALUE!	#VALUE!	0	0	0	0
58	STR2	n/g	n/g	n/g	n/g	STRL						#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0	0	0	0
59	VIB2	9	1	1	2	VIB2L						45000000	5000000	5000000	10000000	#VALUE!	0	0	0	0
60	KLB2		85			KLBL	80.5	101												
61																				
62	Exp No: Old	9	H2O2																	
63	Organism	3%	5%	7%	9%	Control	3%	5%	7%	9%										

ALL STATS | H2O2 MASTER | H2O2 CALCULATIONS | H2O2 STATS | Hypo | HYPO CALCULATIONS | HYPO STATS | K2Cr2O7 master | K2Cr | Explore

	A	B	C	D	E	F	G	H	I	J	L	M	N	O	P	Q	R	S	T
62	Exp No: Old	9				H2O2													
63	Organism	3%	5%	7%	9%	Control	3%	5%	7%	9%									
64	VIB1S1					VIB1L					0	0	0	0	#VALUE!	0	0	0	0
65	E.COLI3					DH5a	20	19	1	19	0	0	0	0	#VALUE!	1000000000000	950000000000	50000000000	950000000000
66	SPY3	43	6	3	2.5	SPYL	5	108	58.5	1	2150000000000	300000000000	150000000000	125000000000	#VALUE!	250000000000	5400000000000	2925000000000	50000000000
67	SAL4					SALL					0	0	0	0	#VALUE!	0	0	0	0
68	STR2					STRL					0	0	0	0	#VALUE!	0	0	0	0
69	VIB2					VIB2L					0	0	0	0	#VALUE!	0	0	0	0
70	KLB2		85			KLBL					0	425000000000C	0	0	#VALUE!	0	0	0	0

Fig: Mastersheet of Hydrogen Peroxide

Calculation:

	A	B	C	D	E	F	G	H	I	J
1					0					
3	VIB1S1	5350	4600	1650	2200	VIB1L	1000	1275	3275	725
4	E.COLI3	0	0	0	0	DH5a	2525	0	50	325
5	SPY3	#VALUE!	4350	#VALUE!	3550	SPYL	0	0	0	0
6	SAL4	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SALL	0	0	0	0
7	STR2	1400	1600	250	#VALUE!	STRL	0	0	0	0
8	VIB2	3000	2950	3200	1650	VIB2L	0	0	0	0
9	KLB2	0	6100	5500	4800	KLBL	0	0	0	0
11					1					
13	VIB1S1	46000	42000	14500	14500	VIB1L	8500	6750	13750	45000
14	E.COLI3	0	0	0	0	DH5a	3500	0	13500	10000
15	SPY3	35000	26000	#VALUE!	23500	SPYL	0	0	0	0
16	SAL4	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SALL	0	0	0	0
17	STR2	11500	12500	500	#VALUE!	STRL	0	0	0	0
18	VIB2	16000	22500	20500	17000	VIB2L	0	0	0	0
19	KLB2	0	48000	0	0	KLBL	0	27500	21750	65000
21					2					
23	VIB1S1	420000	255000	105000	160000	VIB1L	137500	182500	242500	495000
24	E.COLI3	0	0	0	0	DH5a	7500	0	10000	0
25	SPY3	340000	220000	250000	190000	SPYL	0	0	0	0
26	SAL4	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SALL	0	0	0	0
27	STR2	55000	60000	#VALUE!	#VALUE!	STRL	0	0	0	0
28	VIB2	190000	230000	195000	130000	VIB2L	0	0	0	0
29	KLB2	0	0	0	0	KLBL	155000	900000	0	1725000
32					3					

	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
2								3% H2O2						
3	VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
4	53500		350000		14000	30000		1000	2525	2500000C				155000
5	460000		3400000		115000	160000		8500	3500					1875000C
6	4200000		2800000C		550000	1900000		137500	7500					
7	3650000C		2250000C			1800000C		650000	500000					
8	3450000C		1950000C			8000000C		9000000	1000000C					
9	1800000C		2150000C			4500000C		8250000C						
11								5% H2O2						
12	VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
13	4600		4350	2000000	1600	2950	6100	1275	9500000C					27500
14	42000		26000	5000000	12500	22500	48000	6750						900000
15	255000		220000		60000	230000	4250000C	182500						5875000
16	2300000		1550000			400000		750000						1060000C
17	1950000C		1850000C			2000000		5500000						
18	1050000C		1900000C			5000000		6275000C						
19			3000000C											
21								7% H2O2						
22	VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
23	1650		250000		250	3200	5500	3275	50	5000000C				21750
24	14500		2100000		500	20500		13750	13500	10000				
25	105000		2000000C			195000		242500	10000					
26	950000		1600000C			900000		1000000	5000000C					
27	5000000		1500000C			5000000		1200000C						
28	2500000C							2875000C						
32								9% H2O2						
33	VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL

3

VIB1S1	3650000	2300000	950000	1550000	VIB1L	650000	750000	1000000	1250000
E.COLI3	0	0	0	0	DH5a	0	0	0	0
SPY3	2800000	1550000	2100000	2250000	SPYL	0	0	0	0
SAL4	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SALL	0	0	0	0
STR2	#VALUE!	#VALUE!	#VALUE!	#VALUE!	STRL	0	0	0	0
VIB2	1800000	400000	900000	1100000	VIB2L	0	0	0	0
KLB2	0	0	0	0	KLBL	0	5875000	0	2575000C

9% H202

VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
2200		3550			1650	4800	725	325	5000000C				65000
14500		23500			17000		45000	9500000C					1725000
160000		190000			130000		495000	10000					2575000C
1550000		2250000			1100000		1250000						4125000C
5000000		1700000C			5500000		3250000C						
5000000		1300000C			1000000C		1225000C						
		1250000C											

4

VIB1S1	3450000C	1950000C	5000000	5000000	VIB1L	9000000	5500000	1200000C	3250000C
E.COLI3	0	0	0	0	DH5a	500000	0	0	0
SPY3	2250000C	1850000C	2000000C	1700000C	SPYL	0	0	0	0
SAL4	5000000	2000000	#VALUE!	#VALUE!	SALL	0	0	0	0
STR2	#VALUE!	#VALUE!	#VALUE!	#VALUE!	STRL	0	0	0	0
VIB2	8000000	2000000	#VALUE!	5500000	VIB2L	0	0	0	0
KLB2	0	0	0	0	KLBL	1875000C	1060000C	0	4125000C

5

VIB1S1	1800000C	1050000C	2500000C	5000000	VIB1L	8250000C	6275000C	2875000C	1225000C
E.COLI3	0	0	0	0	DH5a	0	0	0	0
SPY3	1950000C	1900000C	1600000C	1300000C	SPYL	0	0	0	0
SAL4	1500000C	5000000	#VALUE!	#VALUE!	SALL	0	0	0	0
STR2	#VALUE!	#VALUE!	#VALUE!	#VALUE!	STRL	0	0	0	0
VIB2	4500000C	5000000	5000000	1000000C	VIB2L	0	0	0	0
KLB2					KLBL				

9 OLD

VIB1S1	0	0	0	0	VIB1L	0	0	0	0
E.COLI3	0	0	0	0	DH5a	1000000C	9500000C	5000000C	9500000C
SPY3	2150000C	3000000C	1500000C	1250000C	SPYL	2500000C	5400000C	5000000C	5000000C
SAL4	0	0	0	0	SALL	0	0	0	0
STR2	0	0	0	0	STRL	0	0	0	0
VIB2	0	0	0	0	VIB2L	0	0	0	0
KLB2	0	4250000C	0	0	KLBL	0	0	0	0

Fig: Calculation of Hydrogen Peroxide

Statistics:

FAC VIB1 3%		FAC VIB1 5%		FAC VIB1 7%		FAC VIB1 9%	
Mean	364368916.7	Mean	21183600	Mean	5178525	Mean	1954450
Standard Error	292334674.6	Standard Error	17047128.16	Standard Error	4041497.292	Standard Error	991784.0201
Median	20350000	Median	1277500	Median	527500	Median	855000
Mode	#N/A	Mode	#N/A	Mode	#N/A	Mode	5000000
Standard Deviation	716070787	Standard Deviation	41756765.57	Standard Deviation	9899606.163	Standard Deviation	2429364.784
Sample Variance	5.12757E+17	Sample Variance	1.74363E+15	Sample Variance	98002202183750	Sample Variance	5901813255000
Kurtosis	5.228720191	Kurtosis	5.279131443	Kurtosis	5.176229004	Kurtosis	-2.006564299
Skewness	2.271724206	Skewness	2.282590125	Skewness	2.258856954	Skewness	0.7514086246
Range	1799946500	Range	104995400	Range	24998350	Range	4997800
Minimum	53500	Minimum	4600	Minimum	1650	Minimum	2200
Maximum	1800000000	Maximum	105000000	Maximum	25000000	Maximum	5000000
Sum	2186213500	Sum	127101600	Sum	31071150	Sum	11726700
Count	6	Count	6	Count	6	Count	6
Confidence Level(95.(	751470204.6	Confidence Level(95.(	43821038	Confidence Level(95.(	10388999.53	Confidence Level(95.(	2549461.987

LAB VIB1 3%		LAB VIB1 5%		LAB VIB1 7%		LAB VIB1 9%	
Mean	15382833.33	Mean	11531754.17	Mean	50126587.5	Mean	209881787.5
Standard Error	13500496.42	Standard Error	10280272.74	Standard Error	47513247.4	Standard Error	203091184.1
Median	393750	Median	466250	Median	621250	Median	872500
Mode	#N/A	Mode	#N/A	Mode	#N/A	Mode	#N/A
Standard Deviation	33069327.51	Standard Deviation	25181422.63	Standard Deviation	116383212.2	Standard Deviation	497469772.2
Sample Variance	1.09358E+15	Sample Variance	634104045695104	Sample Variance	1.35451E+16	Sample Variance	2.47476E+17
Kurtosis	5.753300134	Kurtosis	5.846723451	Kurtosis	5.965424849	Kurtosis	5.98589684
Skewness	2.389690998	Skewness	2.411680212	Skewness	2.440696366	Skewness	2.44586384
Range	82499000	Range	62748725	Range	287496725	Range	1224999275
Minimum	1000	Minimum	1275	Minimum	3275	Minimum	725
Maximum	82500000	Maximum	62750000	Maximum	287500000	Maximum	1225000000
Sum	92297000	Sum	69190525	Sum	300759525	Sum	1259290725
Count	6	Count	6	Count	6	Count	6
Confidence Level(95.(	34704130.88	Confidence Level(95.(	26426282.37	Confidence Level(95.(	122136690.7	Confidence Level(95.(	522062508.7

FAC SPY3 3%		FAC SPY3 5%		FAC SPY3 7%		FAC SPY3 9%	
Mean	3583701125000	Mean	42887185764	Mean	30036470000	Mean	17878495293
Standard Error	3583259788395	Standard Error	42852143783	Standard Error	29990897247	Standard Error	17853593100
Median	126500000	Median	1550000	Median	20000000	Median	2250000
Mode	#N/A	Mode	#N/A	Mode	#N/A	Mode	#N/A
Standard Deviation	8777158097402	Standard Deviation	113376115596	Standard Deviation	67061684952	Standard Deviation	47236167352
Sample Variance	7.70385E+25	Sample Variance	1.28541E+22	Sample Variance	4.49727E+21	Sample Variance	2.23126E+21
Kurtosis	5.999999843	Kurtosis	6.999991554	Kurtosis	4.999980321	Kurtosis	6.999977453
Skewness	2.449489702	Skewness	2.645749217	Skewness	2.236062482	Skewness	2.645745723
Range	21499999650000	Range	299999995650	Range	149999750000	Range	124999996450
Minimum	350000	Minimum	4350	Minimum	250000	Minimum	3550
Maximum	21500000000000	Maximum	300000000000	Maximum	150000000000	Maximum	125000000000
Sum	21502206750000	Sum	300210300350	Sum	150182350000	Sum	125149467050
Count	6	Count	7	Count	5	Count	7
Confidence Level(95.(	9211062524415	Confidence Level(95.(	104855418469	Confidence Level(95.(	83268079863	Confidence Level(95.(	43686168542

FAC VIB2 3%		FAC VIB2 5%		FAC VIB2 7%		FAC VIB2 9%	
Mean	91681666.67	Mean	1275908.333	Mean	1223740	Mean	10000000
Standard Error	72754030.47	Standard Error	805103.7718	Standard Error	958142.9317	Standard Error	0
Median	9950000	Median	315000	Median	195000	Median	10000000
Mode	#N/A	Mode	#N/A	Mode	#N/A	Mode	#N/A
Standard Deviation	178210251.4	Standard Deviation	1972093.431	Standard Deviation	2142472.727	Standard Deviation	#DIV/0!
Sample Variance	3.17589E+16	Sample Variance	3889152500417	Sample Variance	4590189388000	Sample Variance	#DIV/0!
Kurtosis	5.353682959	Kurtosis	2.982717972	Kurtosis	4.387252115	Kurtosis	#DIV/0!
Skewness	2.297451522	Skewness	1.806332081	Skewness	2.083132923	Skewness	#DIV/0!
Range	449970000	Range	4997050	Range	4996800	Range	0
Minimum	30000	Minimum	2950	Minimum	3200	Minimum	10000000
Maximum	450000000	Maximum	5000000	Maximum	5000000	Maximum	10000000
Sum	550090000	Sum	7655450	Sum	6118700	Sum	10000000
Count	6	Count	6	Count	5	Count	1

A	B	D	E	G	H	J	K
Mean	91681666.67	Mean	1275908.333	Mean	1223740	Mean	10000000
Standard Error	72754030.47	Standard Error	805103.7718	Standard Error	958142.9317	Standard Error	0
Median	9950000	Median	315000	Median	195000	Median	10000000
Mode	#N/A	Mode	#N/A	Mode	#N/A	Mode	#N/A
Standard Deviation	178210251.4	Standard Deviation	1972093.431	Standard Deviation	2142472.727	Standard Deviation	#DIV/0!
Sample Variance	3.17589E+16	Sample Variance	3889152500417	Sample Variance	4590189388000	Sample Variance	#DIV/0!
Kurtosis	5.353682959	Kurtosis	2.982717972	Kurtosis	4.387252115	Kurtosis	#DIV/0!
Skewness	2.297451522	Skewness	1.806332081	Skewness	2.083132923	Skewness	#DIV/0!
Range	449970000	Range	4997050	Range	4996800	Range	0
Minimum	30000	Minimum	2950	Minimum	3200	Minimum	10000000
Maximum	450000000	Maximum	5000000	Maximum	5000000	Maximum	10000000
Sum	550090000	Sum	7655450	Sum	6118700	Sum	10000000
Count	6	Count	6	Count	5	Count	1
Confidence Level(95.(	187020189.2	Confidence Level(95.(	2069585.132	Confidence Level(95.(	2660231.253	Confidence Level(95.(	#NUM!

LAB KLB 5%		*LAB KLB 9%*	
Mean	28200625	Mean	110010000
Standard Error	25965081.33	Standard Error	101000615.5
Median	3387500	Median	13737500
Mode	#N/A	Mode	#N/A
Standard Deviation	51930162.65	Standard Deviation	202001231
Sample Variance	2.69674E+15	Sample Variance	4.08045E+16
Kurtosis	3.949330275	Kurtosis	3.929958932
Skewness	1.985411619	Skewness	1.980044965
Range	105972500	Range	412435000
Minimum	27500	Minimum	65000
Maximum	106000000	Maximum	412500000
Sum	112802500	Sum	440040000
Count	4	Count	4
Confidence Level(95.(	82632477.13	Confidence Level(95.(	321429035.6

3.00%	Standard deviation	Mean	5.00%	Standard deviation	Mean	7.00%	Standard deviation	Mean	9.00%	Standard deviation	Mean
VIB1S1	716070787	36436891	VIB1S1	41756765.57	2118360C	VIB1S1	9899606.163	5178525	VIB1S1	2429364.784	1954450
E.COLI3			E.COLI3			E.COLI3			E.COLI3		
SPY3	8777158097402	35837011	SPY3	113376115596	4288718!	SPY3	67061684952	3003647C	SPY3	47236167352	1787849!
SAL4			SAL4			SAL4			SAL4		
STR2			STR2			STR2			STR2		
VIB2	178210251.4	9168166€	VIB2	1972093.431	1275908.	VIB2	2142472.727	1223740	VIB2		1000000C
KLB2			KLB2			KLB2			KLB2		
VIB1L	33069327.51	15382833	VIB1L	25181422.63	11531754	VIB1L	116383212.2	50126587	VIB1L	497469772.2	2098817€
DH5a			DH5a			DH5a			DH5a		
SPYL			SPYL			SPYL			SPYL		
SALL			SALL			SALL			SALL		
STRL			STRL			STRL			STRL		
VIB2L			VIB2L			VIB2L			VIB2L		
KLBL			KLBL	51930162.65	28200625	KLBL			KLBL	202001231	1100100C

Fig: Statistics of Hydrogen Peroxide

Hypochlorite mastersheet, calculation and statistics

Row	A	B	C	D	E	F	G	H	I	J	N	O	P	Q	R	S	T	U	V
1	Exp No:	RAW	Hypo																
2	Organism	0.0625%	0.125%	0.25%	0.50%	Control	0.0625%	0.125%	0.25%	0.50%									
3	VIB1S1	52	Conta	24	2	VIB1L	34	22.5	8	7.5	2600	#VALUE!	1200	100	#VALUE!	1700	1125	400	375
4	E.COLI3	n/g	69	68	84	DH5a	98.5	86	79.5	52	#VALUE!	3450	3400	4200	#VALUE!	4925	4300	3975	2600
5	SPY3	M+C	M+C	M+C	M+C	SPYL	50	101	97	45	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	2500	5050	4850	2250
6	SAL4	140	127	63	48	SALL	70	47	30	55	7000	6350	3150	2400	#VALUE!	3500	2350	1500	2750
7	STR2	75	63	95	Matte	STRL	43	49	40	n/g	3750	3150	4750	#VALUE!	#VALUE!	2150	2450	2000	#VALUE!
8	VIB2	37	33	2	n/g	VIB2L	15	83	70	81	1850	1650	100	#VALUE!	#VALUE!	750	4150	3500	4050
9	KLB2	n/g	51	56	66	KLBL	N/G	84.5	41	30.5	#VALUE!	2550	2800	3300	#VALUE!	#VALUE!	4225	2050	1525
10																			
11	Exp No:	-1	Hypo																
12	Organism	0.0625%	0.125%	0.25%	0.50%	Control	0.0625%	0.125%	0.25%	0.50%									
13	VIB1S1	30	Conta	16	1	VIB1L	10	7	7	5.5	15000	#VALUE!	8000	500	#VALUE!	5000	3500	3500	2750
14	E.COLI3	46	36	56	81	DH5a	151	25.5	68	28	23000	18000	28000	40500	#VALUE!	75500	12750	34000	14000
15	SPY3	M+C	M+C	M+C	M+C	SPYL	22	33	14	15	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	11000	16500	7000	7500
16	SAL4	39	50	29	9	SALL	63	35	17	44	19500	25000	14500	4500	#VALUE!	31500	17500	8500	22000
17	STR2	53	56	68	s.m	STRL	30	45	n/g	n/g	26500	28000	34000	#VALUE!	#VALUE!	15000	22500	#VALUE!	#VALUE!
18	VIB2	58	35	n/g	n/g	VIB2L	19.5	71.5	43	64.5	29000	17500	#VALUE!	#VALUE!	#VALUE!	9750	35750	21500	32250
19	KLB2	79	50	31	56	KLBL	25	35	49.5	72	39500	25000	15500	28000	#VALUE!	12500	17500	24750	36000
20																			
21	Exp No:	-2	Hypo																
22	Organism	0.0625%	0.125%	0.25%	0.50%	Control	0.0625%	0.125%	0.25%	0.50%									
23	VIB1S1	27	36	3	conta	VIB1L	11	7	4.5	3.5	135000	180000	15000	#VALUE!	#VALUE!	55000	35000	22500	17500
24	E.COLI3	39	32	50	46	DH5a	35.5	32	74.5	35.5	195000	160000	250000	230000	#VALUE!	177500	160000	372500	177500
25	SPY3	M+C	M+C	M+C	M+C	SPYL	10	7	43		#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	50000	35000	215000	0
26	SAL4	9	18	14	conta	SALL	50	17	10	19	45000	90000	70000	#VALUE!	#VALUE!	250000	85000	50000	95000
27	STR2	37	61	58	s.m	STRL	19	12	n/g	n/g	185000	305000	290000	#VALUE!	#VALUE!	95000	60000	#VALUE!	#VALUE!
28	VIB2	24	24	n/g	n/g	VIB2L	24.5	51	17	14.5	120000	120000	#VALUE!	#VALUE!	#VALUE!	122500	255000	85000	72500
29	KLB2	35	48	27	30	KLBL	30	34.5	22	55	175000	240000	135000	150000	#VALUE!	150000	172500	110000	275000
30											0	0	0	0	0	0	0	0	0
31	Exp No:	-3	Hypo																
32	Organism	0.0625%	0.125%	0.25%	0.50%	Control	0.0625%	0.125%	0.25%	0.50%									
33	VIB1S1	9	61	conta	conta	VIB1L	6	N/G	8	3.5	450000	3050000	#VALUE!	#VALUE!	#VALUE!	300000	#VALUE!	400000	175000

Row	A	B	C	D	E	F	G	H	I	J	N	O	P	Q	R	S	T	U	V
30											0	0	0	0	0	0	0	0	0
31	Exp No:	-3	Hypo																
32	Organism	0.0625%	0.125%	0.25%	0.50%	Control	0.0625%	0.125%	0.25%	0.50%									
33	VIB1S1	9	61	conta	conta	VIB1L	6	N/G	8	3.5	450000	3050000	#VALUE!	#VALUE!	#VALUE!	300000	#VALUE!	400000	175000
34	E.COLI3	44	28	38	40	DH5a	34	42	67.5	68	2200000	1400000	1900000	2000000	#VALUE!	1700000	2100000	3375000	3400000
35	SPY3	M+C	M+C	M+C	M+C	SPYL			1		#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0	0	50000	0
36	SAL4	conta	conta	conta	conta	SALL	40	7	7	19	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	2000000	350000	350000	950000
37	STR2	26	58	60	68	STRL	4	3	n/g	n/g	1300000	2900000	3000000	3400000	#VALUE!	200000	150000	#VALUE!	#VALUE!
38	VIB2	1	1	n/g	n/g	VIB2L	7.5	16.5	9	4	50000	50000	#VALUE!	#VALUE!	#VALUE!	375000	825000	450000	200000
39	KLB2	40	36	25	48	KLBL	29.5	26	CONT	25.5	2000000	1800000	1250000	2400000	#VALUE!	1475000	1300000	#VALUE!	1275000
40																			
41	Exp No:	-4	Hypo																
42	Organism	0.0625%	0.125%	0.25%	0.50%	Control	0.0625%	0.125%	0.25%	0.50%									
43	VIB1S1	3	9	2	1	VIB1L	N/G	N/G	5.5	8	1500000	4500000	1000000	500000	#VALUE!	#VALUE!	#VALUE!	2750000	4000000
44	E.COLI3	35	21	30	32	DH5a	37	33.5	30.5	26	1750000C	1050000C	1500000C	1600000C	#VALUE!	1850000C	1675000C	1525000C	1300000C
45	SPY3	M+C	M+C	M+C	M+C	SPYL	1	48	1		#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	500000	2400000C	500000	0
46	SAL4	conta	conta	conta	conta	SALL	37				#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	1850000C	0	0	0
47	STR2	21	3	37	matte	STRL	1	2	n/g	n/g	1050000C	1500000	1850000C	#VALUE!	#VALUE!	500000	1000000	#VALUE!	#VALUE!
48	VIB2	3	1	n/g	n/g	VIB2L	1	7	1	1.5	1500000	500000	#VALUE!	#VALUE!	#VALUE!	500000	3500000	500000	750000
49	KLB2	32	32	20	32	KLBL	78.5	59.5	45.5	37	1600000C	1600000C	1000000C	1600000C	#VALUE!	3925000C	2975000C	2275000C	1850000C
50																			
51	Exp No:	-5	Hypo																
52	Organism	0.0625%	0.125%	0.25%	0.50%	Control	0.0625%	0.125%	0.25%	0.50%									
53	VIB1S1	2	6	n/g	n/g	VIB1L	N/G	N/G	6	3	1000000C	3000000C	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	3000000C	1500000C
54	E.COLI3	30	20	21	30	DH5a	70	69	31	22.5	1500000C	1000000C	1050000C	1500000C	#VALUE!	3500000C	3450000C	1550000C	1125000C
55	SPY3	M+C	M+C	M+C	M+C	SPYL	2	1			#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	1000000C	5000000	0	0
56	SAL4	conta	conta	conta	conta	SALL	15				#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	7500000C	0	0	0
57	STR2	1	34	21	n/g	STRL	n/g	2	n/g	n/g	5000000	1700000C	1050000C	#VALUE!	#VALUE!	#VALUE!	1000000C	#VALUE!	#VALUE!
58	VIB2	1	n/g	n/g	n/g	VIB2L	N/G	2	N/G	1	5000000	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	1000000C	#VALUE!	5000000
59	KLB2	29	43	19	21	KLBL	47.5	40	39	35.5	1450000C	2150000C	9500000C	1050000C	#VALUE!	2375000C	2000000C	1950000C	1775000C

Fig: Mastersheet of Sodium Hypochlorite

Calculation:

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	A	B	C	D	E	F	G	H	I	J
1						0				
2	VIB1S1	2600	#VALUE!	1200	100	VIB1L	1700	1125	400	375
3	E.COLI3	#VALUE!	3450	3400	4200	DH5a	4925	4300	3975	2600
4	SPY3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SPYL	2500	5050	4850	2250
5	SAL4	7000	6350	3150	2400	SALL	3500	2350	1500	2750
6	STR2	3750	3150	4750	#VALUE!	STRL	0	0	0	0
7	VIB2	1850	1650	100	#VALUE!	VIB2L	750	4150	3500	4050
8	KLB2	#VALUE!	2550	2800	3300	KLBL	#VALUE!	4225	2050	1525
9										
10										
11						1				
12	VIB1S1	15000	#VALUE!	8000	500	VIB1L	5000	3500	3500	2750
13	E.COLI3	23000	18000	28000	40500	DH5a	75500	12750	34000	14000
14	SPY3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SPYL	11000	16500	7000	7500
15	SAL4	19500	25000	14500	4500	SALL	31500	17500	8500	22000
16	STR2	26500	28000	34000	#VALUE!	STRL	0	0	0	0
17	VIB2	29000	17500	#VALUE!	#VALUE!	VIB2L	9750	35750	21500	32250
18	KLB2	39500	25000	15500	28000	KLBL	12500	17500	24750	36000
19										
20										
21						2				
22	VIB1S1	135000	180000	15000	#VALUE!	VIB1L	55000	35000	22500	17500
23	E.COLI3	195000	160000	250000	230000	DH5a	177500	160000	372500	177500
24	SPY3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SPYL	50000	35000	215000	0
25	SAL4	45000	90000	70000	#VALUE!	SALL	250000	85000	50000	95000
26	STR2	185000	305000	290000	#VALUE!	STRL	0	0	0	0
27	VIB2	120000	120000	#VALUE!	#VALUE!	VIB2L	122500	255000	85000	72500
28	KLB2	175000	240000	135000	150000	KLBL	150000	172500	110000	275000
29		0	0	0	0	0	0	0	0	0
30										
31						3				
32	VIB1S1	450000	3050000	#VALUE!	#VALUE!	VIB1L	300000	#VALUE!	400000	175000
33	E.COLI3	2200000	1400000	1900000	2000000	DH5a	1700000	2100000	3375000	3400000

	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
1							0.06%							
2	VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
3				7000	3750	1850	39500				3500		750	12500
4				19500	26500	29000	175000				31500		9750	1475000
5				45000	185000	120000	2000000				250000		122500	3925000C
6					1300000	50000	1600000C				2000000		375000	2375000C
7					1050000C	1500000	1450000C				1850000C		500000	
8					5000000	5000000					7500000C			
11							0.13%							
12	VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
13				6350	3150	1650	2550				2350		4150	4225
14				90000	28000	17500	25000				17500		35750	17500
15					305000	120000	240000				85000		255000	1300000
16					2900000	50000	1800000				350000		3500000	2975000C
17					500000	500000	1600000C						1000000C	2000000C
18					1700000C		2150000C							
21							0.25%							
22	VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
23				3150	4750	100	2800				1500		3500	2050
24				14500	34000		15500				8500		21500	24750
25				70000	290000		135000				50000		85000	110000
26					3000000		1250000				350000		450000	2275000C
27					1850000C		1000000C						500000	1950000C
28					1050000C		9500000C							
31							0.50%							
32	VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
33				2400	3400000		3300				2750		4050	1525

ALL STATS | H2O2 MASTER | H2O2 CALCULATIONS | H2O2 STATS | Hypo | HYPO CALCULATIONS | HYPO STATS | K2Cr2O7 master | K2Cr

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	A	B	C	D	E	F	G	H	I	J
30										
31						3				
32	VIB1S1	450000	3050000	#VALUE!	#VALUE!	VIB1L	300000	#VALUE!	400000	175000
33	E.COLI3	2200000	1400000	1900000	2000000	DH5a	1700000	2100000	3375000	3400000
34	SPY3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SPYL	0	0	50000	0
35	SAL4	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SALL	2000000	350000	350000	950000
36	STR2	1300000	2900000	3000000	3400000	STRL	0	0	0	0
37	VIB2	50000	50000	#VALUE!	#VALUE!	VIB2L	375000	825000	450000	200000
38	KLB2	2000000	1800000	1250000	2400000	KLBL	1475000	1300000	#VALUE!	1275000
39										
40										
41						4				
42	VIB1S1	1500000	4500000	1000000	500000	VIB1L	#VALUE!	#VALUE!	2750000	4000000
43	E.COLI3	1750000C	1050000C	1500000C	1600000C	DH5a	1850000C	1675000C	1525000C	1300000C
44	SPY3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SPYL	500000	2400000C	500000	0
45	SAL4	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SALL	1850000C	0	0	0
46	STR2	1050000C	1500000	1850000C	#VALUE!	STRL	0	0	0	0
47	VIB2	1500000	500000	#VALUE!	#VALUE!	VIB2L	500000	3500000	500000	750000
48	KLB2	1600000C	1600000C	1000000C	1600000C	KLBL	3925000C	2975000C	2275000C	1850000C
49										
50										
51						5				
52	VIB1S1	1000000C	3000000C	#VALUE!	#VALUE!	VIB1L	#VALUE!	#VALUE!	3000000C	1500000C
53	E.COLI3	1500000C	1000000C	1050000C	1500000C	DH5a	3500000C	3450000C	1550000C	1125000C
54	SPY3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SPYL	1000000C	5000000	0	0
55	SAL4	#VALUE!	#VALUE!	#VALUE!	#VALUE!	SALL	7500000C	0	0	0
56	STR2	5000000	1700000C	1050000C	#VALUE!	STRL	0	0	0	0
57	VIB2	5000000	#VALUE!	#VALUE!	#VALUE!	VIB2L	#VALUE!	1000000C	#VALUE!	5000000
58	KLB2	1450000C	2150000C	9500000C	1050000C	KLBL	2375000C	2000000C	1950000C	1775000C
59										
60										
61										

	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
31							0.50%							
32	VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
33				2400	3400000		3300				2750		4050	1525
34				4500			28000				22000		32250	36000
35							150000				95000		275000	275000
36							2400000				950000		200000	1275000
37							1600000C						750000	1850000C
38							1050000C						5000000	1775000C

ALL STATS | H2O2 MASTER | H2O2 CALCULATIONS | H2O2 STATS | Hypo | HYPO CALCULATIONS | HYPO STATS | K2Cr2O7 master | K2Cr

Fig: Calculation of Sodium Hypochlorite

Statistics:

	FAC VIB1S1 0.0625%	FAC VIBS1 0.125%	FAC VIBS1 0.25%	FAC VIBS1 0.5%
Mean	2017100	9432500	256050	
Standard Error	1613059.229	6914337.007	247999.333	
Median	292500	3775000	11500	
Mode	#N/A	#N/A	#N/A	#N/A
Standard Deviation	3951172.036	13828674.01	495998.666	
Sample Variance	15611760460000	191232225000000	246014676667	8
Kurtosis	5.560730354	3.684314142	3.997419257	#DIV/(
Skewness	2.343543214	1.897886176	1.999225935	
Range	9997400	29820000	998800	
Minimum	2600	180000	1200	
Maximum	10000000	30000000	1000000	
Sum	12102600	37730000	1024200	
Count	6	4	4	
Confidence Level(95.0%)	4146500.754	22004506.26	789244.561	

	LAB VIBS1 0.0625 %	LAB VIBS1 0.125%	LAB VIB1S1 0.25%	LAB VIB1S1 0.5%
Mean	90425	13208.33333	5529400	
Standard Error	70914.3659	10917.38229	4913510.057	
Median	30000	3500	211250	
Mode	#N/A	#N/A	#N/A	#N/A
Standard Deviation	141828.7318	18909.46082	12035592.49	
Sample Variance	20115389167	357567708.3	144855486500000	3592
Kurtosis	3.354223955	#DIV/0!	5.830123536	
Skewness	1.831699363	1.701362906	2.40768921	
Range	298300	33875	29999600	
Minimum	1700	1125	400	
Maximum	300000	35000	30000000	
Sum	361700	39625	33176400	
Count	4	3	6	
Confidence Level(95.0%)	225681.1618	46973.70472	12630579.7	

	FAC E.COLI 0.0625%	FAC E.COLI 0.125%	FAC E.COLI 0.25%	FAC E.COLI 0.5%
Mean	33983600	18680241.67	3436280	
Standard Error	29186215.25	16348830.09	2912328.917	
Median	2200000	780000	250000	
Mode	#N/A	#N/A	#N/A	#N/A
Standard Deviation	65262361.3	40046291.62	6512165.43	
Sample Variance	4.25918E+15	1.60371E+15	42408298592000	
Kurtosis	4.74037638	5.776002001	4.693747678	
Skewness	2.169389778	2.394887993	2.157903366	
Range	149977000	99996550	14996600	
Minimum	23000	3450	3400	
Maximum	150000000	100000000	15000000	
Sum	169918000	112081450	17181400	
Count	5	6	5	
Confidence Level(95.0%)	81033924.46	42026005.67	8085921.365	

	Dh5a 0.0625%	Dh5a 0.125%	Dh5a 0.25%	Dh5a 5%
Mean	61742987.5	72385410	29005912.5	
Standard Error	57726942.93	68230253.69	25312075.7	
Median	938750	160000	1873750	
Mode	#N/A	#N/A	#N/A	#N/A
Standard Deviation	141401554.6	152567485.4	62001669.81	
Sample Variance	1.99944E+16	2.32768E+16	3.84421E+15	
Kurtosis	5.944087892	4.954112249	5.807587576	
Skewness	2.435383115	2.223736612	2.402123994	
Range	349995075	344995700	154996025	
Minimum	4925	4300	3975	
Maximum	350000000	345000000	155000000	
Sum	370457925	361927050	174035475	
Count	6	5	6	

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A1:B1 | FAC VIB1S1 0.0625%

LAB SPY 0.0625%

Statistic	Value
Mean	1760583.333
Standard Error	1649794.989
Median	30500
Mode	#N/A
Standard Deviation	4041155.904
Sample Variance	16330941041667
Kurtosis	5.95053423
Skewness	2.436980332
Range	10000000
Minimum	0
Maximum	10000000
Sum	10563500
Count	6
Confidence Level(95.0%)	4240933.032

LAB SPY 0.125%

Statistic	Value
Mean	5811310
Standard Error	4648359.405
Median	35000
Mode	#N/A
Standard Deviation	10394047.61
Sample Variance	108036225793000
Kurtosis	4.083082923
Skewness	2.016198736
Range	23994950
Minimum	5050
Maximum	24000000
Sum	29056550
Count	5
Confidence Level(95.0%)	12905914.72

LAB SPY 0.25%

Statistic	Value
Mean	155370
Standard Error	94365.86724
Median	50000
Mode	#N/A
Standard Deviation	211008.4939
Sample Variance	44524584500
Kurtosis	1.609438784
Skewness	1.476723047
Range	495150
Minimum	4850
Maximum	500000
Sum	776850
Count	5
Confidence Level(95.0%)	262001.6502

FAC SAL 0.0625%

Statistic	Value
Mean	23833.33333
Standard Error	11181.58208
Median	19500
Mode	#N/A
Standard Deviation	19367.06827
Sample Variance	375083333.3
Kurtosis	#DIV/0!
Skewness	0.9564572313
Range	38000
Minimum	7000
Maximum	45000
Sum	71500
Count	3

FAC SAL 0.25%

Statistic	Value
Mean	29216.66667
Standard Error	20653.21471
Median	14500
Mode	#N/A
Standard Deviation	35772.41721
Sample Variance	1279665833
Kurtosis	#DIV/0!
Skewness	1.537960999
Range	66850
Minimum	3150
Maximum	70000
Sum	87650
Count	3

ALL STATS | H2O2 MASTER | H2O2 CALCULATIONS | H2O2 STATS | Hypo | HYPO CALCULATIONS | HYPO STATS | K2Cr2O7 master | K2Cr2O7 calc

EXCEL NEW .XLSX

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A1:B1 | FAC VIB1S1 0.0625%

LAB SAL 0.0625%

Statistic	Value
Mean	15964166.67
Standard Error	12168519.22
Median	1125000
Mode	#N/A
Standard Deviation	29806663.01
Sample Variance	888437160066667
Kurtosis	4.72480705
Skewness	2.164421705
Range	74996500
Minimum	3500
Maximum	75000000
Sum	95785000
Count	6
Confidence Level(95.0%)	31280174.47

LAB SAL 0.125%

Statistic	Value
Mean	113712.5
Standard Error	80785.02433
Median	51250
Mode	#N/A
Standard Deviation	161570.0487
Sample Variance	26104880625
Kurtosis	2.922255234
Skewness	1.722393842
Range	347650
Minimum	2350
Maximum	350000
Sum	454850
Count	4
Confidence Level(95.0%)	257094.0022

LAB SAL 0.25%

Statistic	Value
Mean	102500
Standard Error	83191.29562
Median	29250
Mode	#N/A
Standard Deviation	166382.5912
Sample Variance	27683166667
Kurtosis	3.646302522
Skewness	1.904087756
Range	348500
Minimum	1500
Maximum	350000
Sum	410000
Count	4
Confidence Level(95.0%)	264751.8314

LAB SAL 0.5%

Statistic	Value
Mean	
Standard Error	
Median	
Mode	#N/A
Standard Deviation	
Sample Variance	2(
Kurtosis	
Skewness	
Range	
Minimum	
Maximum	
Sum	
Count	
Confidence Level(95.0%)	

FAC STR 0.0625%

Statistic	Value
Mean	2835875
Standard Error	1719707.526
Median	742500
Mode	#N/A
Standard Deviation	4212405.945
Sample Variance	17744363843750
Kurtosis	1.873004983
Skewness	1.578040782
Range	10496250
Minimum	3750
Maximum	10500000
Sum	17015250
Count	6

FAC STR 0.125%

Statistic	Value
Mean	28956025
Standard Error	28212317.35
Median	402500
Mode	#N/A
Standard Deviation	69105781.98
Sample Variance	4.77561E+15
Kurtosis	5.994726296
Skewness	2.448122004
Range	169996850
Minimum	3150
Maximum	170000000
Sum	173736150
Count	6

FAC STR 0.25%

Statistic	Value
Mean	21138125
Standard Error	17024896.78
Median	1645000
Mode	#N/A
Standard Deviation	41702310.03
Sample Variance	1.73908E+15
Kurtosis	5.358503471
Skewness	2.299521229
Range	104995250
Minimum	4750
Maximum	105000000
Sum	126828750
Count	6

ALL STATS | H2O2 MASTER | H2O2 CALCULATIONS | H2O2 STATS | Hypo | HYPO CALCULATIONS | HYPO STATS | K2Cr2O7 master | K2Cr2O7 calc

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A1:B1 | FAC VIB1S1 0.0625%

	A	B	C	D	E	F	G	H	I	J	K
137	FAC VIB2 0.0625%			FAC VIB2 0.125%							
138											
139	Mean	1116808.333		Mean	137830						
140	Standard Error	812079.2101		Standard Error	92796.39756						
141	Median	85000		Median	50000						
142	Mode	#N/A		Mode	#N/A						
143	Standard Deviation	1989179.695		Standard Deviation	207499.053						
144	Sample Variance	3956835860417		Sample Variance	43055857000						
145	Kurtosis	4.15088148		Kurtosis	4.014457168						
146	Skewness	2.048077743		Skewness	1.984628246						
147	Range	4998150		Range	498350						
148	Minimum	1850		Minimum	1650						
149	Maximum	5000000		Maximum	500000						
150	Sum	6700850		Sum	689150						
151	Count	6		Count	5						
152	Confidence Level(95.0%)	2087516.066		Confidence Level(95.0%)	257644.1038						
153											
154	LAB VIB2 0.0625%			LAB VIB2 0.125%			LAB VIB2 0.25%			LAB VIB2 0.5%	
155											
156	Mean	201600		Mean	2758980		Mean	212000		Mean	
157	Standard Error	100626.5559		Standard Error	1926868.795		Standard Error	108507.949		Standard Error	
158	Median	122500		Median	255000		Median	85000		Median	
159	Mode	#N/A		Mode	#N/A		Mode	#N/A		Mode	#N/A
160	Standard Deviation	225007.8193		Standard Deviation	4308609.61		Standard Deviation	242631.1501		Standard Deviation	
161	Sample Variance	50628518750		Sample Variance	18564116770750		Sample Variance	58869875000		Sample Variance	382
162	Kurtosis	-2.219309352		Kurtosis	2.523561306		Kurtosis	-3.110886259		Kurtosis	
163	Skewness	0.5898005961		Skewness	1.6839969		Skewness	0.5642664658		Skewness	
164	Range	499250		Range	9995850		Range	496500		Range	
165	Minimum	750		Minimum	4150		Minimum	3500		Minimum	
166	Maximum	500000		Maximum	10000000		Maximum	500000		Maximum	
167	Sum	1008000		Sum	13794900		Sum	1060000		Sum	
168	Count	5		Count	5		Count	5		Count	

ALL STATS | H2O2 MASTER | H2O2 CALCULATIONS | H2O2 STATS | Hypo | HYPO CALCULATIONS | HYPO STATS | K2Cr2O7 master | K2Cr2O7 calc

EXCEL NEW .XLSX

A1:B1 | FAC VIB1S1 0.0625%

	A	B	C	D	E	F	G	H	I	J	K
171	FAC KLB 0.0625%			FAC KLB 0.125%			FAC KLB 0.25%			FAC KLB 0.5%	
172											
173	Mean	32642900		Mean	38844591.67		Mean	17733883.33		Mean	
174	Standard Error	28246448.97		Standard Error	35322742.64		Standard Error	15534517.76		Standard Error	
175	Median	2000000		Median	1020000		Median	692500		Median	
176	Mode	#N/A		Mode	#N/A		Mode	#N/A		Mode	#N/A
177	Standard Deviation	63160980.01		Standard Deviation	86522695.78		Standard Deviation	38051641.92		Standard Deviation	
178	Sample Variance	3.98931E+15		Sample Variance	7.48618E+15		Sample Variance	1.44793E+15		Sample Variance	
179	Kurtosis	4.768784042		Kurtosis	5.888585483		Kurtosis	5.774164004		Kurtosis	
180	Skewness	2.176432453		Skewness	2.421789264		Skewness	2.394475095		Skewness	
181	Range	144960500		Range	214997450		Range	94997200		Range	
182	Minimum	39500		Minimum	2550		Minimum	2800		Minimum	
183	Maximum	145000000		Maximum	215000000		Maximum	95000000		Maximum	
184	Sum	163214500		Sum	233067550		Sum	106403300		Sum	
185	Count	5		Count	6		Count	6		Count	
186	Confidence Level(95.0%)	78424714.97		Confidence Level(95.0%)	90800000.61		Confidence Level(95.0%)	39932749.19		Confidence Level(95.0%)	
187											
188	LAB KLB 0.0625%			LAB KLB 0.125%			LAB KLB 0.25%			LAB KLB 0.5%	
189											
190	Mean	69559375		Mean	46214345		Mean	43577360		Mean	
191	Standard Error	56711963.52		Standard Error	38863816.34		Standard Error	38110132.31		Standard Error	
192	Median	20362500		Median	1300000		Median	110000		Median	
193	Mode	#N/A		Mode	#N/A		Mode	#N/A		Mode	#N/A
194	Standard Deviation	113423927		Standard Deviation	86902135.19		Standard Deviation	85216846.48		Standard Deviation	
195	Sample Variance	1.2865E+16		Sample Variance	7.55198E+15		Sample Variance	7.26191E+15		Sample Variance	
196	Kurtosis	3.441680814		Kurtosis	4.549467811		Kurtosis	4.721354593		Kurtosis	
197	Skewness	1.853428241		Skewness	2.123510986		Skewness	2.164965525		Skewness	
198	Range	237487500		Range	199995775		Range	194997950		Range	
199	Minimum	12500		Minimum	4225		Minimum	2050		Minimum	
200	Maximum	237500000		Maximum	200000000		Maximum	195000000		Maximum	
201	Sum	278237500		Sum	231071725		Sum	217886800		Sum	
202	Count	4		Count	5		Count	5		Count	

ALL STATS | H2O2 MASTER | H2O2 CALCULATIONS | H2O2 STATS | Hypo | HYPO CALCULATIONS | HYPO STATS | K2Cr2O7 master | K2Cr2O7 calc

	A	B	C	D	E	F	G	H	I	J	K
205											
206	0.06%	Standard deviation	Mean	0.13%	Standard deviation	Mean	0.25%	Standard deviation	Mean	0.50%	Standard deviatio
207	VIB1S1	3951172.036	2017100	VIB1S1	13828674.01	9432500	VIB1S1	495998.666	256050	VIB1S1	
208	E.COLI3	65262361.3	3398360	E.COLI3	40046291.62	18680241	E.COLI3	6512165.43	3436280	E.COLI3	
209	SPY3			SPY3			SPY3			SPY3	
210	SAL4	19367.06827	23833.33	SAL4			SAL4	35772.41721	29216.66	SAL4	
211	STR2	4212405.945	2835875	STR2	69105781.98	28956025	STR2	41702310.03	21138125	STR2	
212	VIB2	1989179.695	1116808.	VIB2	207499.053	137830	VIB2			VIB2	
213	KLB2	63160980.01	3264290	KLB2	86522695.78	38844591	KLB2	38051641.92	1773388	KLB2	
214	VIB1L	141828.7318	90425	VIB1L	18909.46082	13208.33	VIB1L	12035592.49	5529400	VIB1L	
215	DH5a	141401554.6	6174298	DH5a	152567485.4	7238541	DH5a	62001669.81	2900591	DH5a	
216	SPYL	4041155.904	1760583.	SPYL	10394047.61	5811310	SPYL	211008.4939	155370	SPYL	
217	SALL	29806663.01	1596416	SALL	161570.0487	113712.5	SALL	166382.5912	102500	SALL	
218	STRL			STRL			STRL			STRL	
219	VIB2L	225007.8193	201600	VIB2L	4308609.61	2758980	VIB2L	242631.1501	212000	VIB2L	
220	KLBL	113423927	6955937	KLBL	86902135.19	4621434	KLBL	85216846.48	4357736	KLBL	

Fig: Statistics of Sodium Hypochlorite

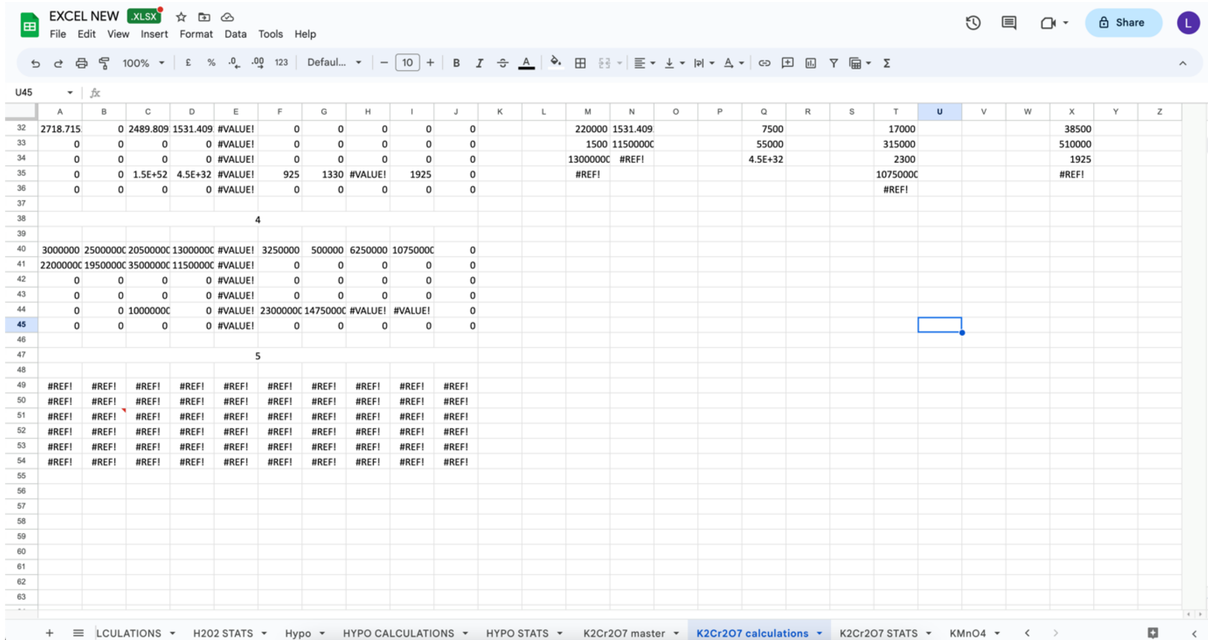
Potassium Dichromate Mastersheet, calculation and statistics:

Row	A	B	C	D	E	F	G	H	I	J	L	M	N	O	P	Q	R	S	T	U
62	Exp No:	0				K2Cr2O7									Dilution:0					
63	Organism	0.3%	0.5%	0.7%	0.9%	Control	0.3%	0.5%	0.7%	0.9%										
64	VIB1S1					VIB1L														
65	E.COLI3	103	48	52		DH5a	61.5	52	80.5	51	5150	2400	2600	0	#VALUE!	3075	2600	4025	2550	0
66	SPY3					SPYL					0	0	0	0	#VALUE!	0	0	0	0	0
67	SAL4					SALL					0	0	0	0	#VALUE!	0	0	0	0	0
68	STR2					STRL					0	0	0	0	#VALUE!	0	0	0	0	0
69	VIB2			79	18	VIB2L	21.5	81.5	OVER	63	0	0	3950	900	#VALUE!	1075	4075	#VALUE!	3150	0
70	KLB2					KLBL					0	0	0	0	#VALUE!	0	0	0	0	0
71																				
72																				
73	Exp No:	4				K2Cr2O7									Dilution:1					
74	Organism	0.3%	0.5%	0.7%	0.9%	Control	0.3%	0.5%	0.7%	0.9%										
75	VIB1S1	59	48	20	0	VIB1L	156	24	26	17										
76	E.COLI3	6	50	41	26	DH5a	6.5	1	12.5	21.5	3000000	25000000	2050000C	1300000C	#VALUE!	3250000	500000	6250000	1075000C	0
77	SPY3	44	39	70	23	SPYL					2200000C	19500000	3500000C	1150000C	#VALUE!	0	0	0	0	0
78	SAL4	16	62	1	11	SALL	148	81	63	97	8000000	31000000	500000	5500000	#VALUE!	7400000C	4050000C	3150000C	4850000C	0
79	STR2	0	10	1	0	STRL	0	4	91	0	0	5000000	500000	0	#VALUE!	0	2000000	4550000C	0	0
80	VIB2	132	20	64	20	VIB2L	46	29.5	CONT	CONT	6600000C	10000000	3200000C	1000000C	#VALUE!	2300000C	1475000C	#VALUE!	#VALUE!	0
81	KLB2	62	0	80	3	KLBL	0	0	0	0	3100000C	0	4000000C	1500000	#VALUE!	0	0	0	0	0
82																				
83																				
84	Exp No:	5				K2Cr2O7									Dilution:2					
85	Organism	0.3%	0.5%	0.7%	0.9%	Control	0.3%	0.5%	0.7%	0.9%										
86	VIB1S1	36	29	0	0	VIB1L	69	18	16	5										
87	E.COLI3	4	45	34	22	DH5a	2	15	2	15	2000000C	225000000	1700000C	1100000C	#VALUE!	1000000C	7500000C	1000000C	7500000C	0
88	SPY3	35	27	34	17	SPYL					1750000C	135000000	1700000C	8500000C	#VALUE!	0	0	0	0	0
89	SAL4	0	51	0	9	SALL	78	64	58	85	0	255000000	0	4500000C	#VALUE!	3900000C	3200000C	2900000C	4250000C	0
90	STR2					STRL					0	0	0	0	#VALUE!	0	0	0	0	0
91	VIB2	72	14	40	12	VIB2L	59.5	33.5	98	88.5	3600000C	70000000	2000000C	6000000C	#VALUE!	2975000C	1675000C	4900000C	4425000C	0
92	KLB2	34	0	42	1	KLBL	0	0	0	0	1700000C	0	2100000C	5000000	#VALUE!	0	0	0	0	0

Fig: Mastersheet of Potassium Dichromate

Calculation:

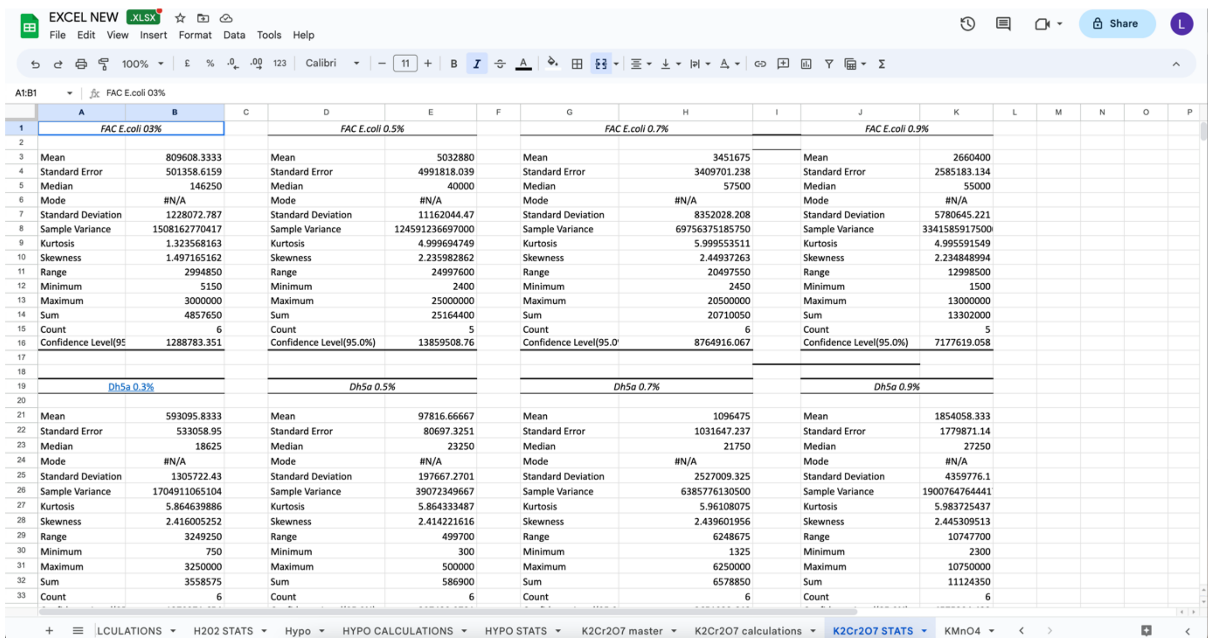
Row	A	B	C	D	E	F	G	H	I	J	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1																								
2					0													0.30%						
3											VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
4	5150	2400	2600	0	#VALUE!	3075	2600	4025	2550	0		5150	2718.715						3075				1075	
5	0	0	0	0	#VALUE!	0	0	0	0	0		27500	2200000C						32250				38250	
6	0	0	0	0	#VALUE!	0	0	0	0	0		265000	#REF!						267500				365000	
7	0	0	0	0	#VALUE!	0	0	0	0	0		1550000							750				925	
8	0	0	3950	900	#VALUE!	1075	4075	#VALUE!	3150	0		3000000							3250000				2300000C	
9	0	0	0	0	#VALUE!	0	0	0	0	0		#REF!							#REF!				#REF!	
10																								
11					1													0.50%						
12											VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
13	27500	9500	30000	25500	#VALUE!	32250	11500	38500	17000	0		2400	1950000C						2600				4075	
14	0	0	0	18500	#VALUE!	0	0	0	0	0		9500	#REF!						11500				31250	
15	0	0	0	0	#VALUE!	0	0	0	0	0		40000							35000				282500	
16	0	0	0	0	#VALUE!	0	0	0	0	0		2500000C							300				1330	
17	0	0	30500	7500	#VALUE!	38250	31250	14000	38500	0		#REF!							500000				1475000C	
18	0	0	0	0	#VALUE!	0	0	0	0	0									#REF!				#REF!	
19																								
20					2													0.70%						
21											VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
22	265000	40000	90000	220000	#VALUE!	267500	35000	280000	315000	0		2600	2489.809			3950			4025				14000	
23	0	0	0	0	#VALUE!	0	0	0	0	0		30000	350000			30500			38500				112500	
24	0	0	0	0	#VALUE!	0	0	0	0	0		90000	#REF!			230000			280000				#REF!	
25	0	0	0	0	#VALUE!	0	0	0	0	0		2450				1.5E+52			1325					
26	0	0	230000	55000	#VALUE!	365000	282500	112500	510000	0		2050000C				1000000C			6250000					
27	0	0	0	0	#VALUE!	0	0	0	0	0		#REF!				#REF!			#REF!					
28																								
29					3													0.90%						
30											VIB1S1	E.COLI3	SPY3	SAL4	STR2	VIB2	KLB2	VIB1L	DH5a	SPYL	SALL	STRL	VIB2L	KLBL
31	1550000	#VALUE!	2450	1500	#VALUE!	750	300	1325	2300	0		25500	18500			900			2550				3150	
32	2718.715	0	2489.809	1531.409	#VALUE!	0	0	0	0	0		220000	1531.409			7500			17000				38500	
33	0	0	0	0	#VALUE!	0	0	0	0	0		1500	11500000			55000			315000				510000	



	A	B	C	D	E	F	G	H	I	J	M	N	Q	T	X
32	2718.715	0	2489.809	1531.409	#VALUE!	0	0	0	0	0	220000	1531.409	7500	17000	38500
33	0	0	0	0	#VALUE!	0	0	0	0	0	1500	1150000C	55000	315000	510000
34	0	0	0	0	#VALUE!	0	0	0	0	0	1300000C	#REF!	4.5E+32	2300	1925
35	0	0	1.5E+52	4.5E+32	#VALUE!	925	1330	#VALUE!	1925	0	#REF!			1075000C	#REF!
36	0	0	0	0	#VALUE!	0	0	0	0	0				#REF!	
37															
38	4														
39															
40	3000000	2500000C	2050000C	1300000C	#VALUE!	3250000	500000	6250000	1075000C	0					
41	2200000C	1950000C	3500000C	1150000C	#VALUE!	0	0	0	0	0					
42	0	0	0	0	#VALUE!	0	0	0	0	0					
43	0	0	0	0	#VALUE!	0	0	0	0	0					
44	0	0	1000000C	0	#VALUE!	2300000C	1475000C	#VALUE!	#VALUE!	0					
45	0	0	0	0	#VALUE!	0	0	0	0	0					
46															
47	5														
48															
49	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!					
50	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!					
51	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!					
52	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!					
53	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!					
54	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!					

Fig: Calculation of Potassium Dichromate

Statistics:



	A	B	D	E	G	H	J	K
1	FAC E.coli 03%		FAC E.coli 0.5%		FAC E.coli 0.7%		FAC E.coli 0.9%	
2								
3	Mean	809608.3333	Mean	5032880	Mean	3451675	Mean	2660400
4	Standard Error	501358.6159	Standard Error	4991818.039	Standard Error	3409701.238	Standard Error	2585183.134
5	Median	146250	Median	40000	Median	57500	Median	55000
6	Mode	#N/A	Mode	#N/A	Mode	#N/A	Mode	#N/A
7	Standard Deviation	1228072.787	Standard Deviation	11162044.47	Standard Deviation	8352028.208	Standard Deviation	5780645.221
8	Sample Variance	1508162770417	Sample Variance	124591236697000	Sample Variance	69756375185750	Sample Variance	3341585917500
9	Kurtosis	1.323568163	Kurtosis	4.999694749	Kurtosis	5.999553511	Kurtosis	4.995591549
10	Skewness	1.497165162	Skewness	2.235982862	Skewness	2.44937263	Skewness	2.234848994
11	Range	2994850	Range	24997600	Range	20497550	Range	12998500
12	Minimum	5150	Minimum	2400	Minimum	2450	Minimum	1500
13	Maximum	3000000	Maximum	25000000	Maximum	20500000	Maximum	13000000
14	Sum	4857650	Sum	25164400	Sum	20710050	Sum	13302000
15	Count	6	Count	5	Count	6	Count	5
16	Confidence Level(95	1288783.351	Confidence Level(95.0%)	13859508.76	Confidence Level(95.0	8764916.067	Confidence Level(95.0%)	7177619.058
17								
18								
19	Dh5a 0.3%		Dh5a 0.5%		Dh5a 0.7%		Dh5a 0.9%	
20								
21	Mean	593095.8333	Mean	97816.66667	Mean	1096475	Mean	1854058.333
22	Standard Error	533058.95	Standard Error	80697.3251	Standard Error	1031647.237	Standard Error	1779871.14
23	Median	18625	Median	23250	Median	21750	Median	27250
24	Mode	#N/A	Mode	#N/A	Mode	#N/A	Mode	#N/A
25	Standard Deviation	1305722.43	Standard Deviation	197667.2701	Standard Deviation	2527009.325	Standard Deviation	4359776.1
26	Sample Variance	1704911065104	Sample Variance	39072349667	Sample Variance	6385776130500	Sample Variance	1900764764441
27	Kurtosis	5.864639886	Kurtosis	5.864333487	Kurtosis	5.96108075	Kurtosis	5.983725437
28	Skewness	2.416005252	Skewness	2.414221616	Skewness	2.439601956	Skewness	2.445309513
29	Range	3249250	Range	499700	Range	6248675	Range	10747700
30	Minimum	750	Minimum	300	Minimum	1325	Minimum	2300
31	Maximum	3250000	Maximum	500000	Maximum	6250000	Maximum	10750000
32	Sum	3558575	Sum	586900	Sum	6578850	Sum	11124350
33	Count	6	Count	6	Count	6	Count	6

FAC SPY3 0.3%		FAC SPY3 0.5%		FAC SPY3 0.7%		FAC SPY3 0.9%	
Mean	7363406.239	Mean	9783750	Mean	145829.9364	Mean	2890632.852
Standard Error	7318337.805	Standard Error	9716250	Standard Error	104826.9114	Standard Error	2869801.353
Median	87500	Median	9783750	Median	85000	Median	30500
Mode	#N/A	Mode	#N/A	Mode	#N/A	Mode	#N/A
Standard Deviation	12675732.9	Standard Deviation	13740852.53	Standard Deviation	181565.5365	Standard Deviation	5739602.707
Sample Variance	160674204670892	Sample Variance	188811028125000	Sample Variance	32966044035	Sample Variance	3294303923262
Kurtosis	#DIV/0!	Kurtosis	#DIV/0!	Kurtosis	#DIV/0!	Kurtosis	3.999828399
Skewness	1.731963638	Skewness	#DIV/0!	Skewness	1.338410929	Skewness	1.999948561
Range	21997281.28	Range	19432500	Range	347510.1907	Range	11498468.59
Minimum	2718.715506	Minimum	67500	Minimum	2489.809298	Minimum	1531.409226
Maximum	22000000	Maximum	19500000	Maximum	350000	Maximum	11500000
Sum	22090218.72	Sum	19567500	Sum	437489.8093	Sum	11562531.41
Count	3	Count	2	Count	3	Count	4
Confidence Level(95	31488266.13	Confidence Level(95.0%)	123456661.8	Confidence Level(95.0'	451033.7963	Confidence Level(95.0%)	9132988.714

FAC VIB2 0.7%		FAC VIB2 0.9%	
Mean	2.5E+51	Mean	1.125E+32
Standard Error	2.5E+51	Standard Error	1.125E+32
Median	130250	Median	31250
Mode	#N/A	Mode	#N/A
Standard Deviation	6.12372E+51	Standard Deviation	2.25E+32
Sample Variance	3.75E+103	Sample Variance	5.0625E+64
Kurtosis	6	Kurtosis	4
Skewness	2.449489743	Skewness	2
Range	1.5E+52	Range	4.5E+32
Minimum	3950	Minimum	900
Maximum	1.5E+52	Maximum	4.5E+32
Sum	1.5E+52	Sum	4.5E+32
Count	6	Count	4

LAB VIB2 0.3%		LAB VIB2 0.5%		LAB VIB2 0.7%		LAB VIB2 0.9%	
Mean	148750	Mean	3925666.667	Mean	3925666.667	Mean	154965
Standard Error	0	Standard Error	3815283.58	Standard Error	3815283.58	Standard Error	97590.13385
Median	148750	Median	93500	Median	93500	Median	38500
Mode	#N/A	Mode	#N/A	Mode	#N/A	Mode	#N/A
Standard Deviation	#DIV/0!	Standard Deviation	9345497.996	Standard Deviation	9345497.996	Standard Deviation	218218.1732
Sample Variance	#DIV/0!	Sample Variance	87338332793917	Sample Variance	87338332793917	Sample Variance	47619171125
Kurtosis	#DIV/0!	Kurtosis	5.99539271	Kurtosis	5.99539271	Kurtosis	1.465800739
Skewness	#DIV/0!	Skewness	2.448290338	Skewness	2.448290338	Skewness	1.458751364
Range	0	Range	22999075	Range	22999075	Range	508075
Minimum	148750	Minimum	925	Minimum	925	Minimum	1925
Maximum	148750	Maximum	23000000	Maximum	23000000	Maximum	510000
Sum	148750	Sum	23554000	Sum	23554000	Sum	774825
Count	1	Count	6	Count	6	Count	5
Confidence Level(95	#NUM!	Confidence Level(95.0%)	9807498.67	Confidence Level(95.0'	9807498.67	Confidence Level(95.0%)	270953.6494

0.30%	Standard deviation	Mean	0.50%	Standard deviation	Mean	0.70%	Standard deviation	Mean	0.90%	Standard deviation		Mean
VIB1S1			VIB1S1			VIB1S1			VIB1S1			
E.COLI3	1228072.787	809608.3	E.COLI3	11162044.47	5032880	E.COLI3	8352028.208	3451675	E.COLI3	5780645.221		2660400
SPY3	12675732.9	7363406.	SPY3	13740852.53	9783750	SPY3	181565.5365	145829.93	SPY3	5739602.707		2890632.
SAL4			SAL4			SAL4			SAL4			
STR2			STR2			STR2			STR2			
VIB2			VIB2			VIB2	6.12372E+51	2.5E+51	VIB2	2.25E+32		1.125E+3
KLB2			KLB2			KLB2			KLB2			
VIB1L			VIB1L			VIB1L			VIB1L			
DH5a	1305722.43	593095.8	DH5a	197667.2701	97816.66	DH5a	2527009.325	1096475	DH5a	4359776.1		1854058.
SPYL			SPYL			SPYL			SPYL			
SALL			SALL			SALL			SALL			
STRL			STRL			STRL			STRL			
VIB2L		148750	VIB2L	9345497.996	3925666.	VIB2L	9345497.996	3925666.6	VIB2L	218218.1732		154965

Fig: Statistics of Potassium Dichromate

Total Calculation:

3.00%	Standard d	Mean	5.00%	Standard d	Mean	7.00%	Standard d	Mean	9.00%	Standard d	Mean
VIB1S1	7.16E+08	3.64E+08	VIB1S1	41756766	21183600	VIB1S1	9899606	5178525	VIB1S1	2429365	1954450
E.COLI3			E.COLI3			E.COLI3			E.COLI3		
SPY3	8.78E+12	3.58E+12	SPY3	1.13E+11	4.29E+10	SPY3	6.71E+10	3E+10	SPY3	4.72E+10	1.79E+10
SAL4			SAL4			SAL4			SAL4		
STR2			STR2			STR2			STR2		
VIB2	1.78E+08	91681667	VIB2	1972093	1275908	VIB2	2142473	1223740	VIB2		10000000
KLB2			KLB2			KLB2			KLB2		
VIB1L	33069328	15382833	VIB1L	25181423	11531754	VIB1L	1.16E+08	50126588	VIB1L	4.97E+08	2.1E+08
DH5a			DH5a			DH5a			DH5a		
SPYL			SPYL			SPYL			SPYL		
SALL			SALL			SALL			SALL		
STRL			STRL			STRL			STRL		
VIB2L			VIB2L			VIB2L			VIB2L		
KLBL			KLBL	51930163	28200625	KLBL			KLBL	2.02E+08	1.1E+08

NaClo

0.06%	Standard d	Mean	0.13%	Standard d	Mean	0.25%	Standard d	Mean	0.50%	Standard deviation		Mean
VIB1S1	3951172	2017100	VIB1S1	13828674	9432500	VIB1S1	495998.7	256050	VIB1S1	288502		166866.7
E.COLI3	65262361	33983600	E.COLI3	40046292	18680242	E.COLI3	6512165	3436280	E.COLI3	60067726		28045783
SPY3			SPY3			SPY3			SPY3			
SAL4	19367.07	23833.33	SAL4			SAL4	35772.42	29216.67	SAL4			
STR2	4212406	2835875	STR2	69105782	28956025	STR2	41702310	21138125	STR2			
VIB2	1989180	1116808	VIB2	207499.1	137830	VIB2			VIB2			
KLB2	63160980	32642900	KLB2	86522696	38844592	KLB2	38051642	17733883	KLB2	41812423		20596883
VIB1L	141828.7	90425	VIB1L	18909.46	13208.33	VIB1L	12035592	5529400	VIB1L	5993646		3199271
DH5a	1.41E+08	61742988	DH5a	1.53E+08	72385410	DH5a	62001670	29005913	DH5a	44853749		21515683
SPYL	4041156	1760583	SPYL	10394048	5811310	SPYL	211008.5	155370	SPYL	456773.2		267437.5
SALL	29806663	15964167	SALL	161570	113712.5	SALL	166382.6	102500	SALL			
STRL			STRL			STRL			STRL			
VIB2L	225007.8	201600	VIB2L	4308610	2758980	VIB2L	242631.2	212000	VIB2L	1956793		1043550
KLBL	1.13E+08	69559375	KLBL	86902135	46214345	KLBL	85216846	43577360	KLBL	71194659		32931254

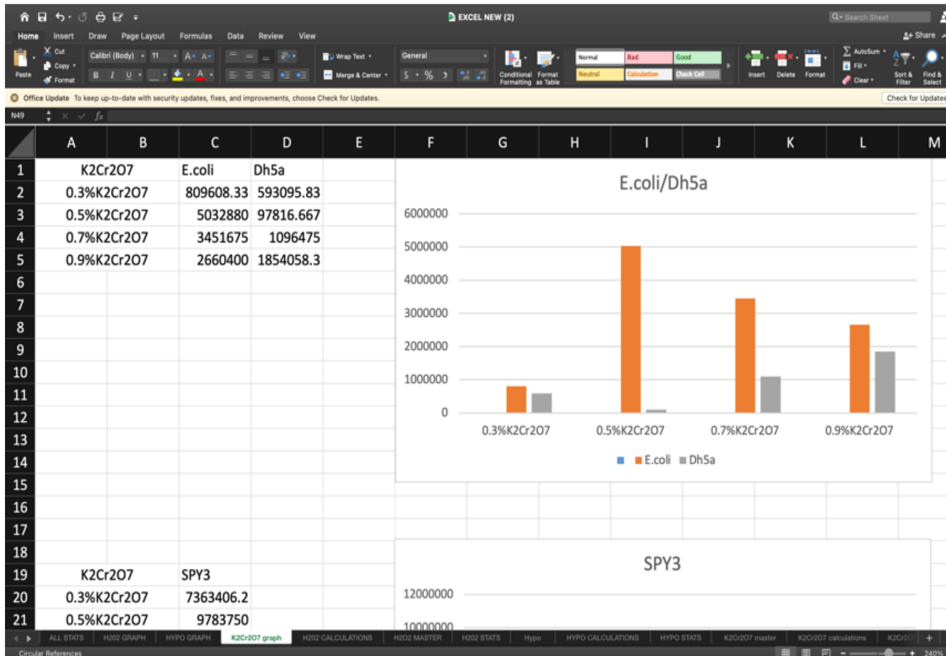
K2Cr2O7

0.30%	Standard d	Mean	0.50%	Standard d	Mean	0.70%	Standard d	Mean	0.90%	Standard deviation		Mean
VIB1S1			VIB1S1			VIB1S1			VIB1S1			
E.COLI3	1228073	809608.3	E.COLI3	11162044	5032880	E.COLI3	8352028	3451675	E.COLI3	5780645		2660400
SPY3	12675733	7363406	SPY3	13740853	9783750	SPY3	181565.5	145829.9	SPY3	5739603		2890633
SAL4			SAL4			SAL4			SAL4			
STR2			STR2			STR2			STR2			
VIB2			VIB2			VIB2	6.12E+51	2.5E+51	VIB2	2.25E+32		1.13E+32
KLB2			KLB2			KLB2			KLB2			
VIB1L			VIB1L			VIB1L			VIB1L			
DH5a	1305722	593095.8	DH5a	197667.3	97816.67	DH5a	2527009	1096475	DH5a	4359776		1854058
SPYL			SPYL			SPYL			SPYL			
SALL			SALL			SALL			SALL			
STRL			STRL			STRL			STRL			
VIB2L		148750	VIB2L	9345498	3925667	VIB2L	9345498	3925667	VIB2L	218218.2		154965
KLBL			KLBL			KLBL			KLBL			

Fig: Total Calculation of All Oxidant

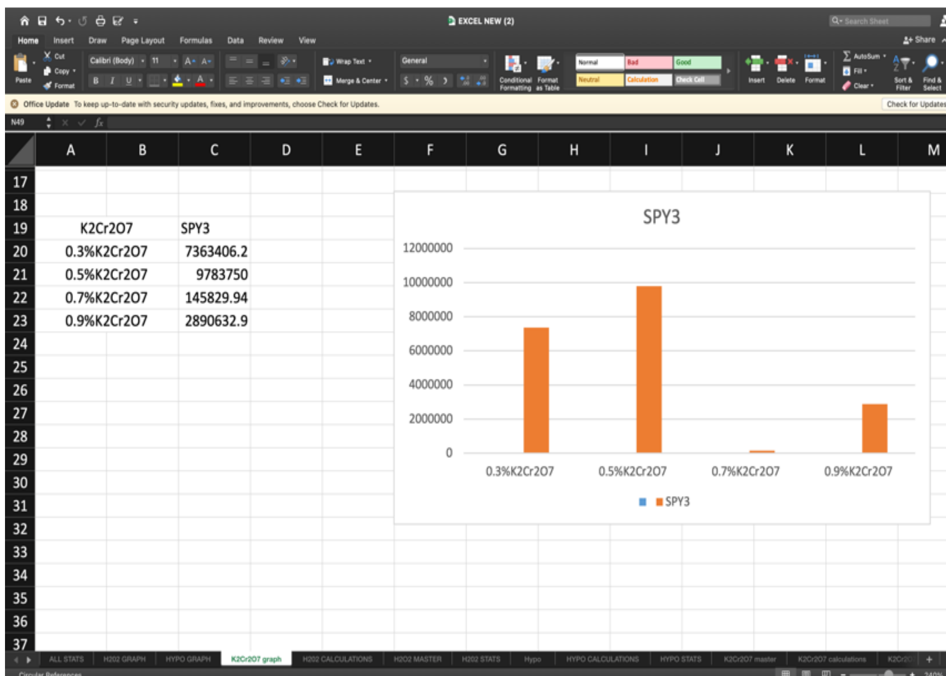
Graphs:

Potassium Dichromate

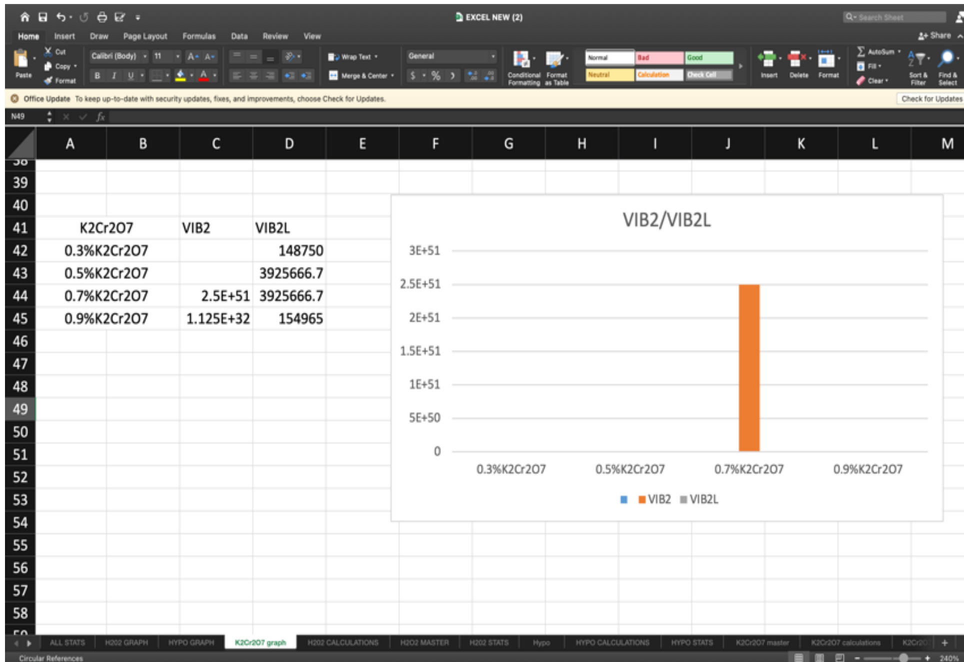


K2Cr2O7	E.coli	Dh5a
0.3%K2Cr2O7	809608.33	593095.83
0.5%K2Cr2O7	5032880	97816.667
0.7%K2Cr2O7	3451675	1096475
0.9%K2Cr2O7	2660400	1854058.3

K2Cr2O7	SPY3
0.3%K2Cr2O7	7363406.2
0.5%K2Cr2O7	9783750



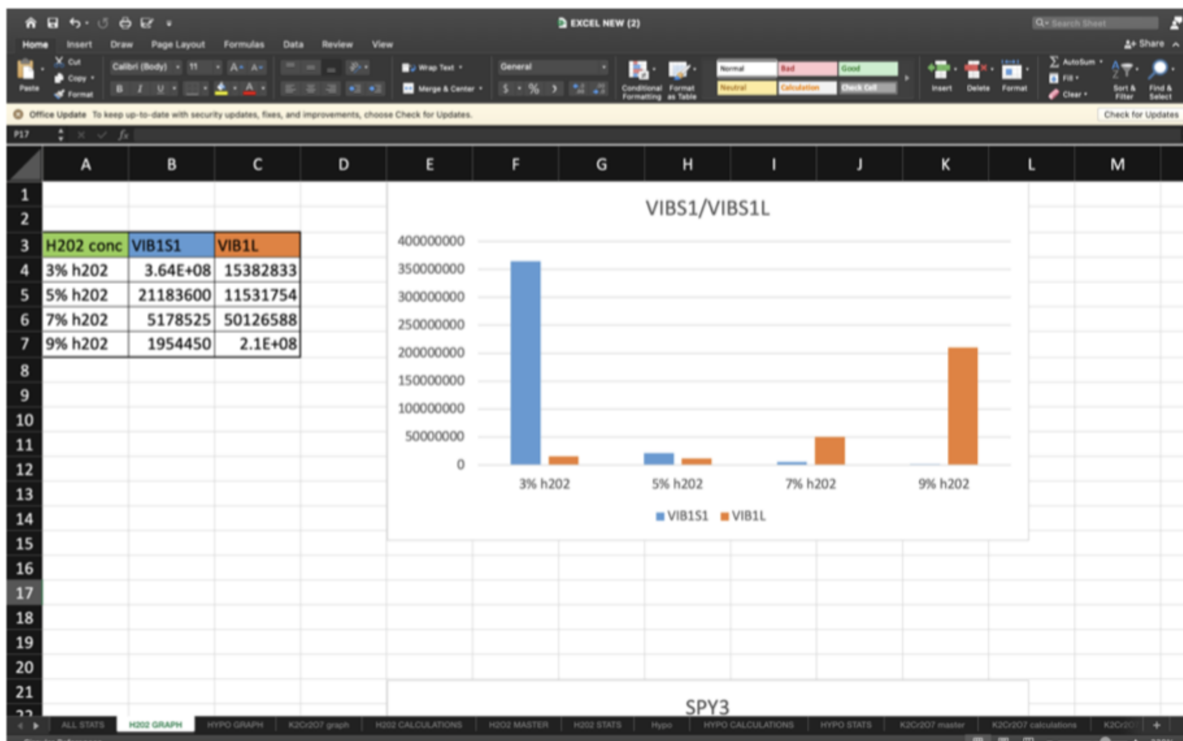
K2Cr2O7	SPY3
0.3%K2Cr2O7	7363406.2
0.5%K2Cr2O7	9783750
0.7%K2Cr2O7	145829.94
0.9%K2Cr2O7	2890632.9



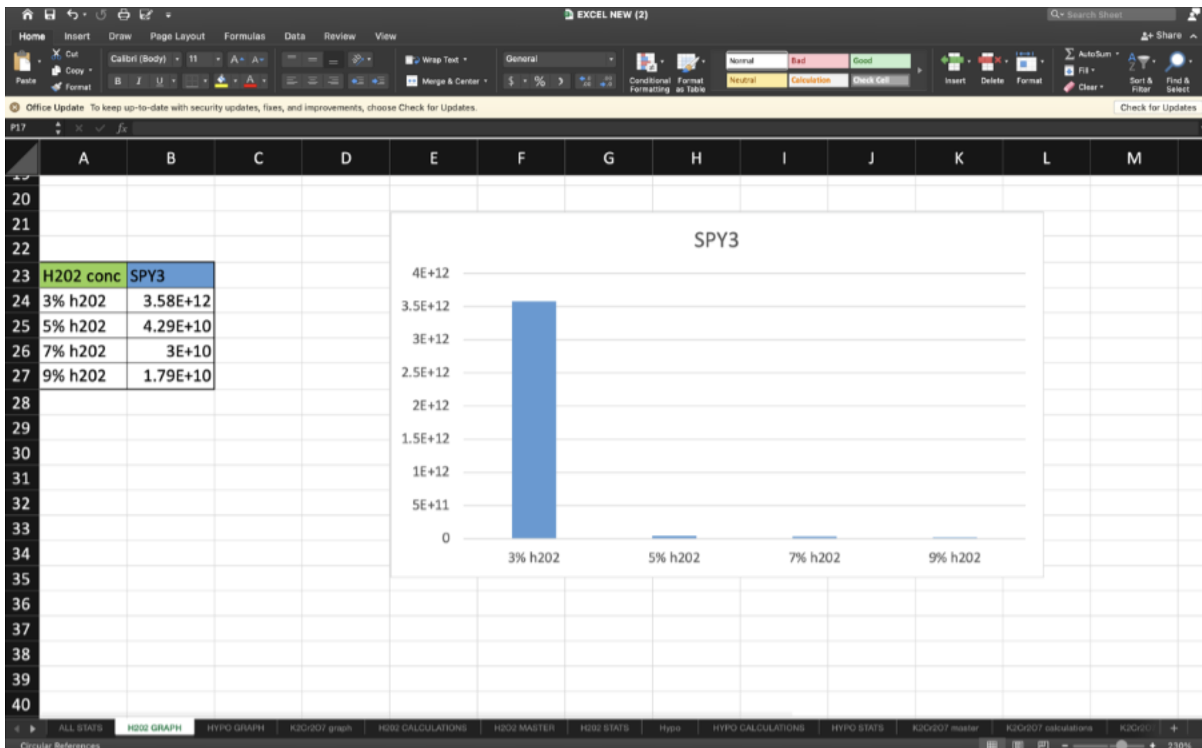
K2Cr2O7	VIB2	VIB2L
0.3%K2Cr2O7		148750
0.5%K2Cr2O7		3925666.7
0.7%K2Cr2O7	2.5E+51	3925666.7
0.9%K2Cr2O7	1.125E+32	154965

Fig: Graphs of Potassium Dichromate

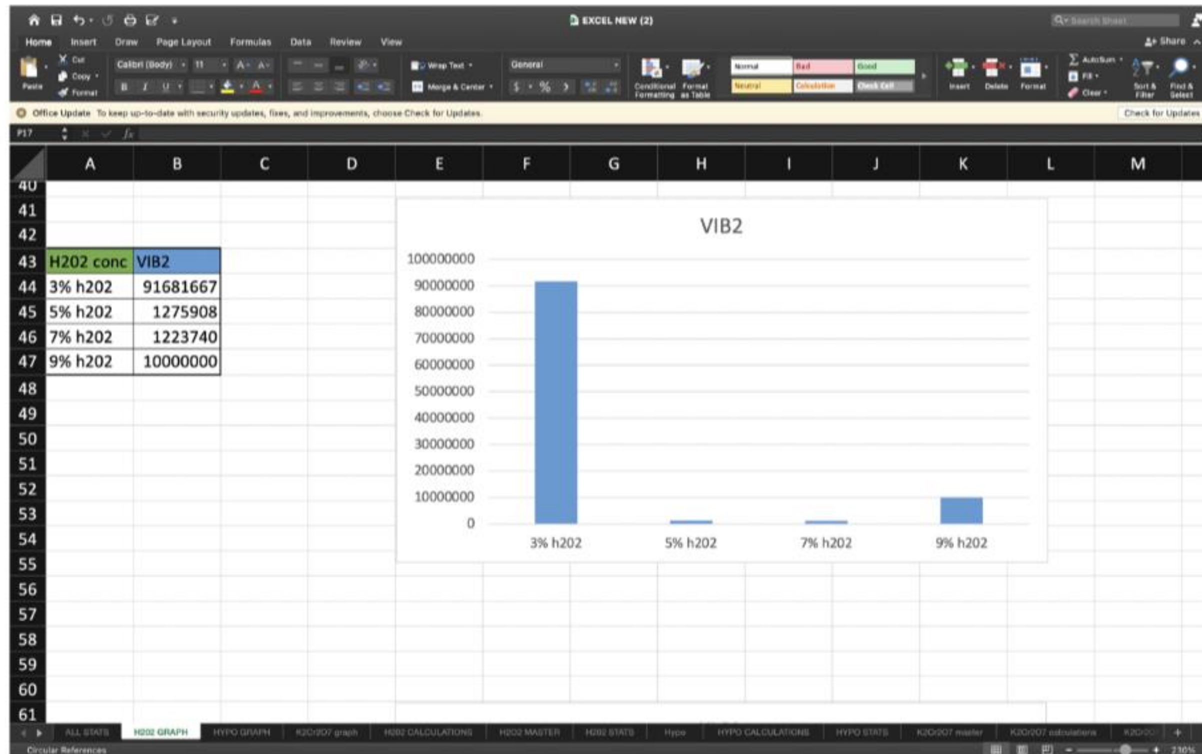
Hydrogen Peroxide:



H202 conc	VIB1S1	VIB1L
3% h202	3.64E+08	15382833
5% h202	21183600	11531754
7% h202	5178525	50126588
9% h202	1954450	2.1E+08



H202 conc | SPY3
3% h202 | 3.58E+12
5% h202 | 4.29E+10
7% h202 | 3E+10
9% h202 | 1.79E+10
SPY3



H202 conc | VIB2
3% h202 | 91681667
5% h202 | 1275908
7% h202 | 1223740
9% h202 | 10000000
VIB2

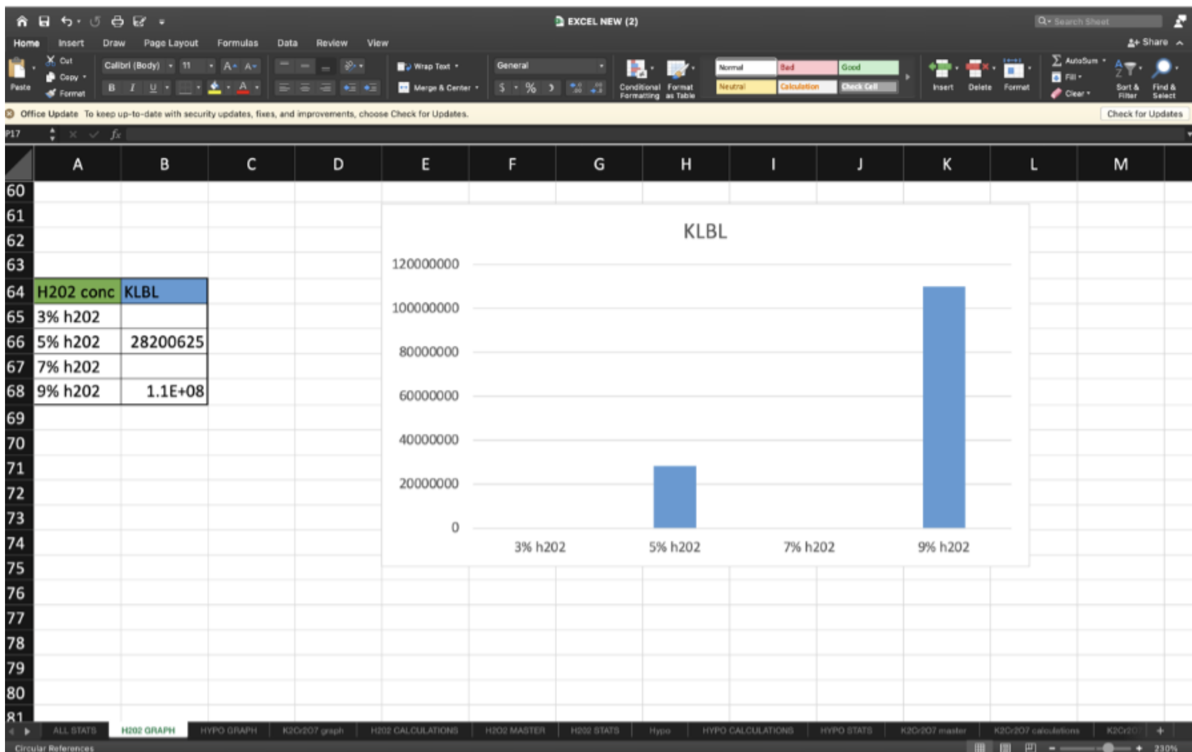
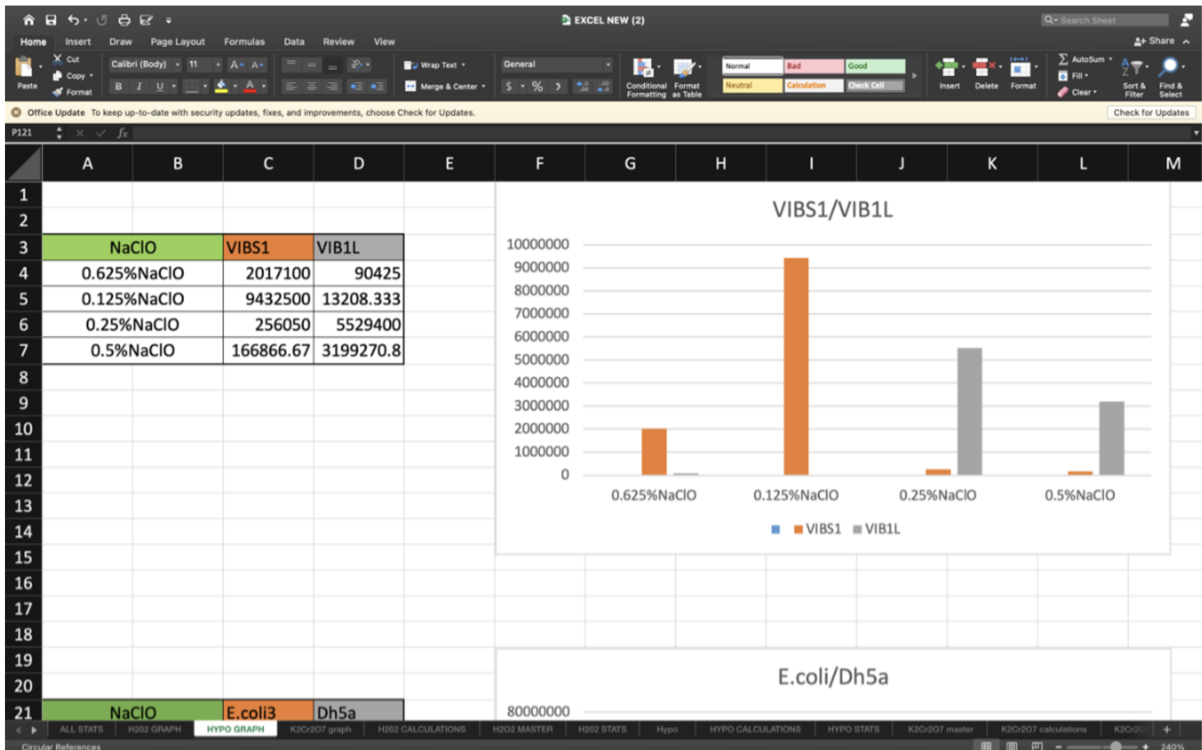
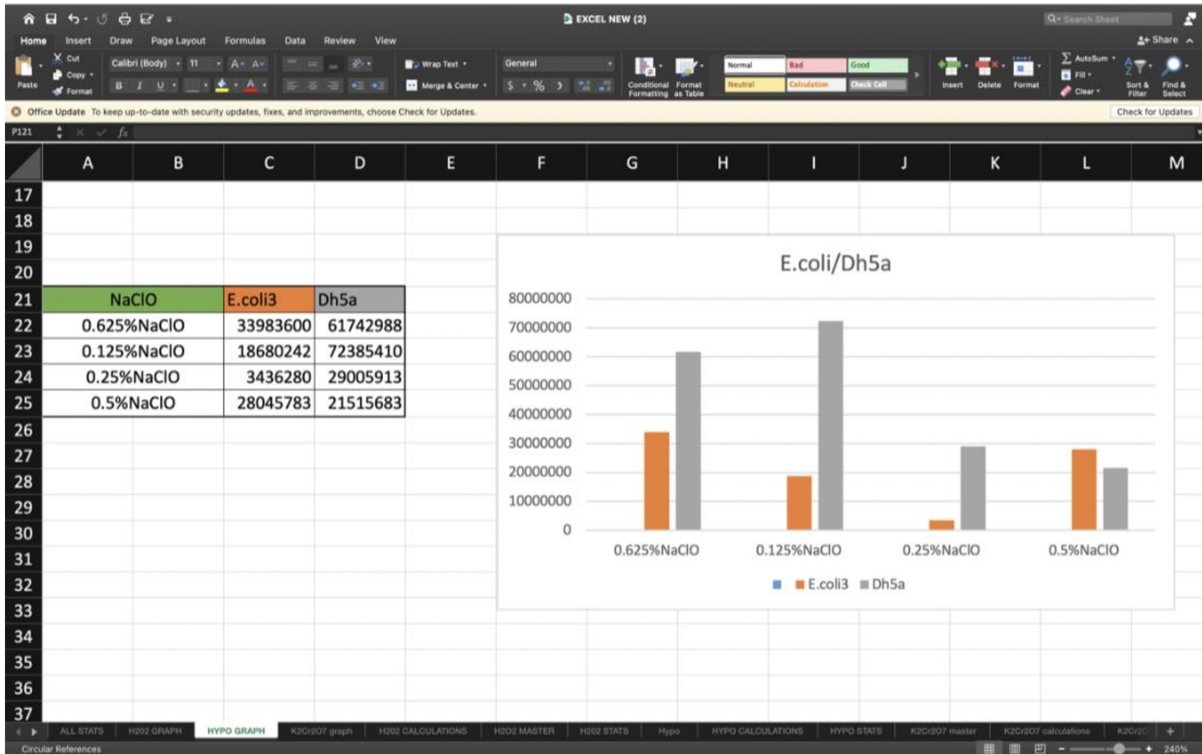


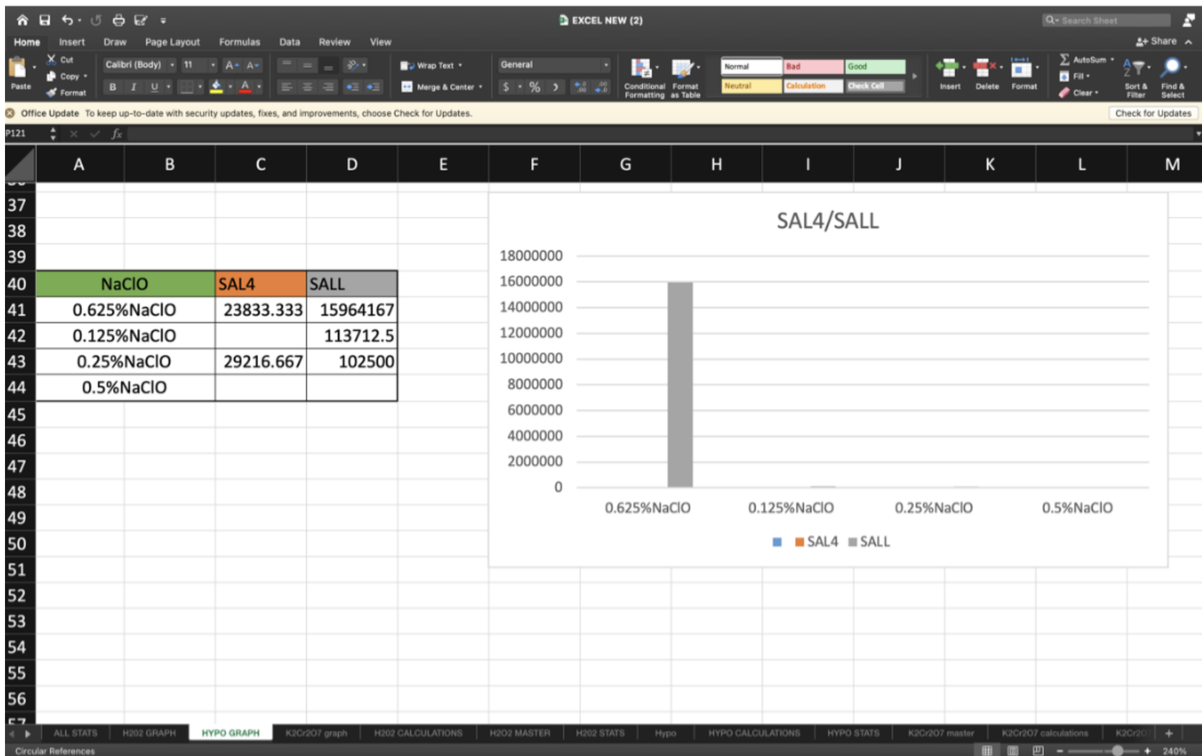
Fig: Graphs of Hydrogen Peroxide

Sodium Hypochlorite:

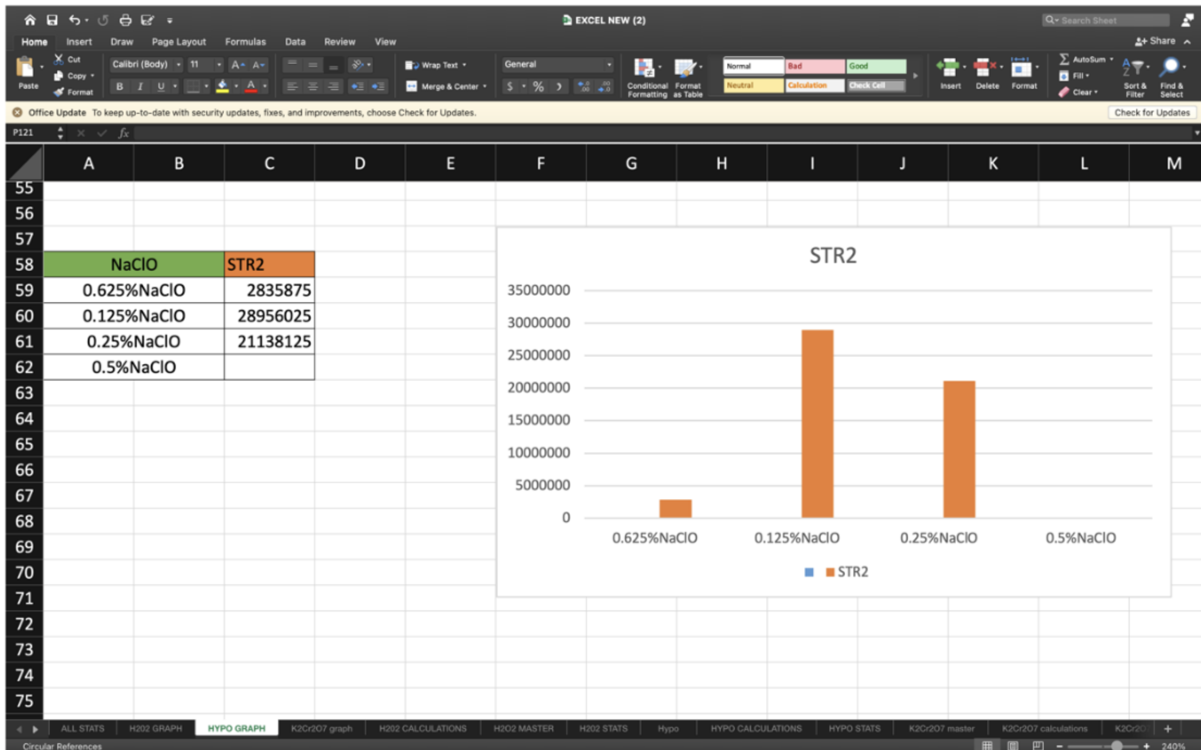




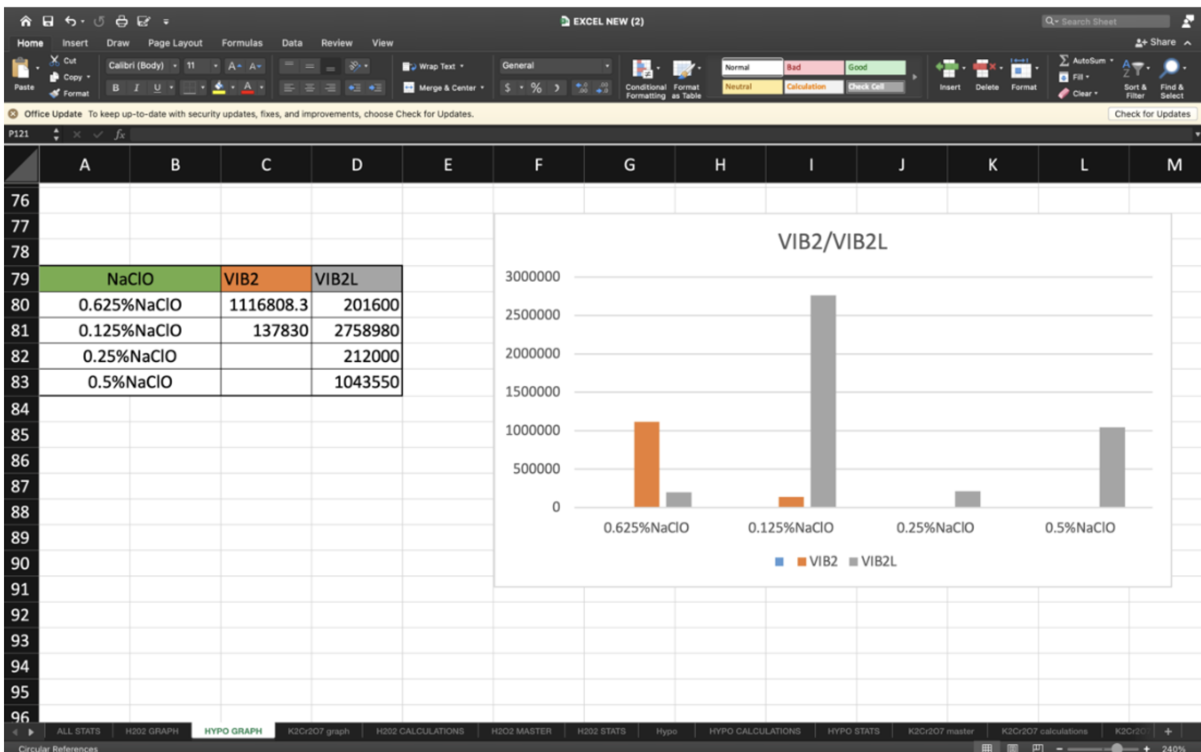
NaClO	E.coli3	Dh5a
0.625%NaClO	33983600	61742988
0.125%NaClO	18680242	72385410
0.25%NaClO	3436280	29005913
0.5%NaClO	28045783	21515683



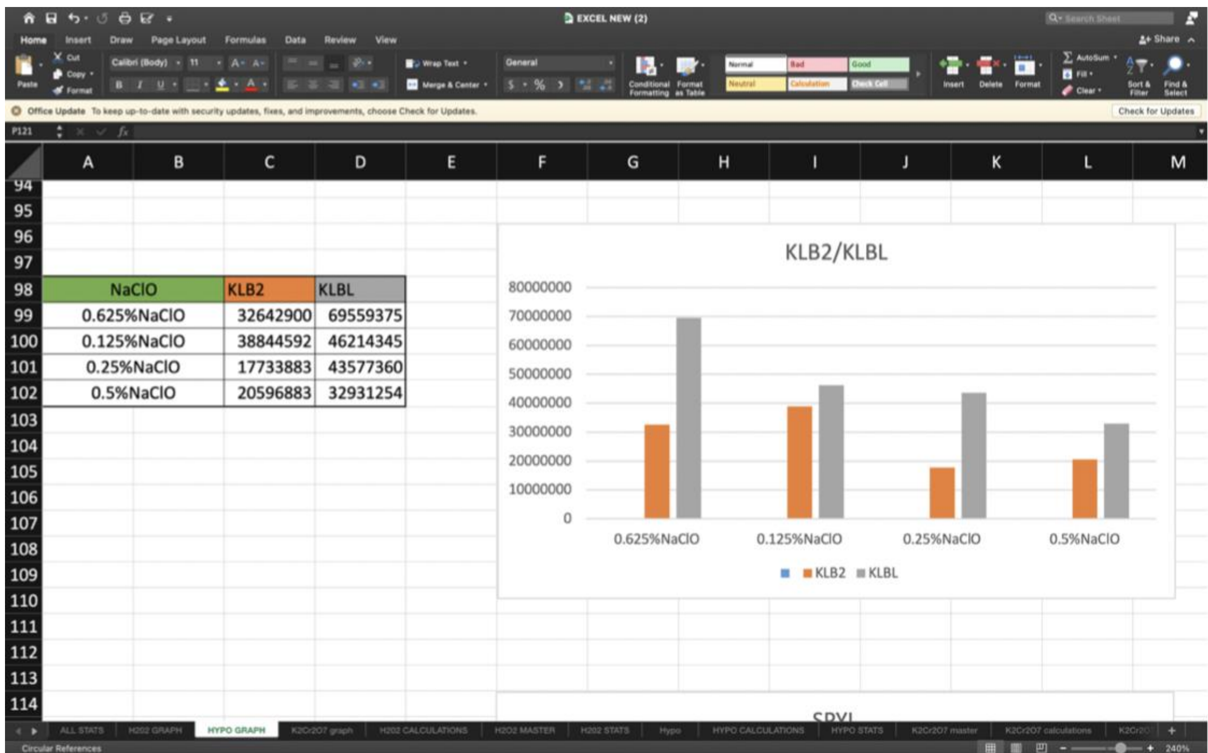
NaClO	SAL4	SALL
0.625%NaClO	23833.333	15964167
0.125%NaClO		113712.5
0.25%NaClO	29216.667	102500
0.5%NaClO		



NaClO	STR2
0.625%NaClO	2835875
0.125%NaClO	28956025
0.25%NaClO	21138125
0.5%NaClO	



NaClO	VIB2	VIB2L
0.625%NaClO	1116808.3	201600
0.125%NaClO	137830	2758980
0.25%NaClO		212000
0.5%NaClO		1043550



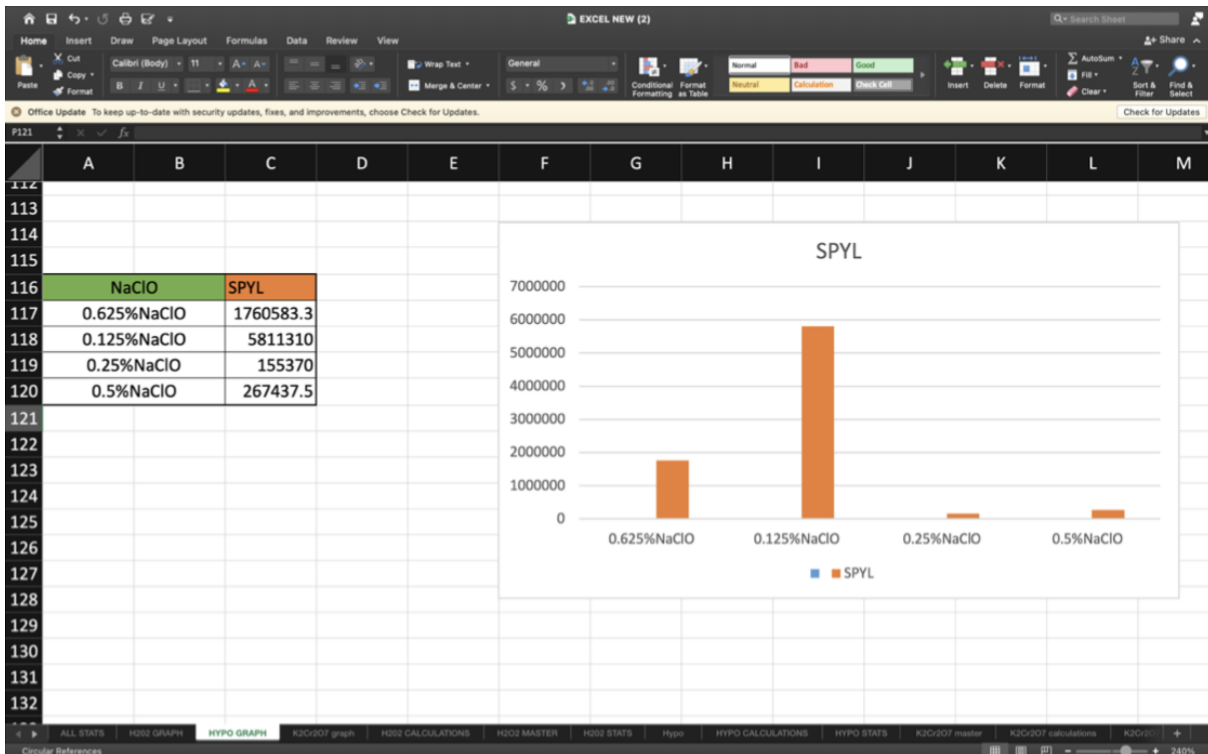
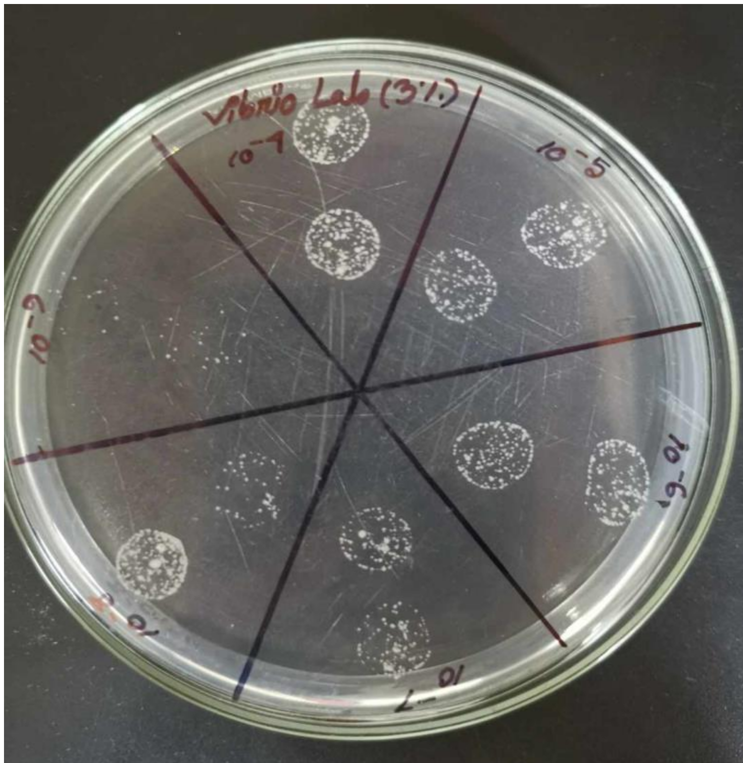
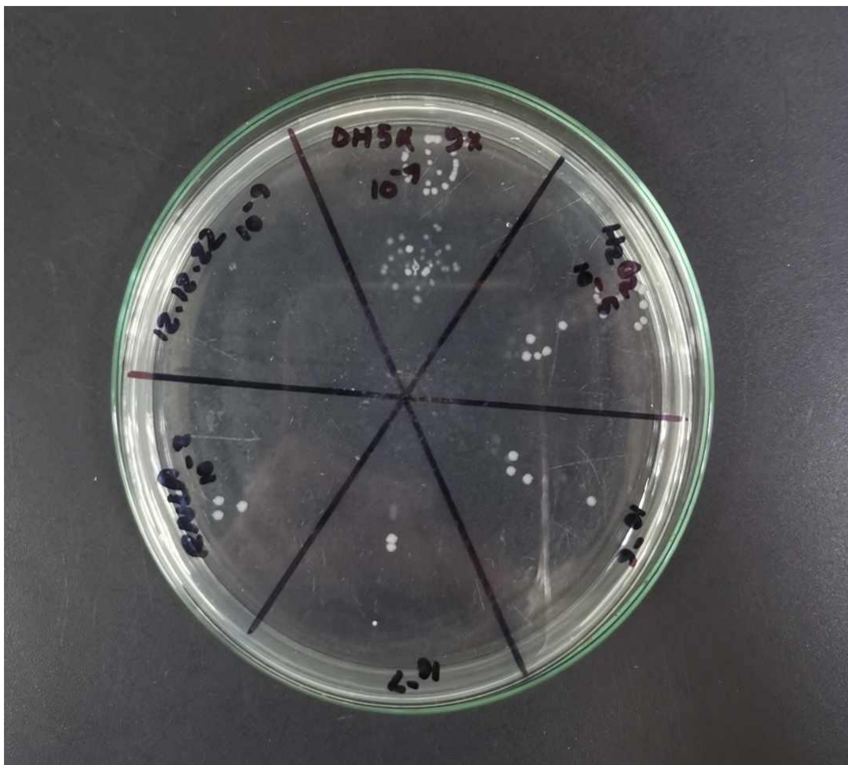


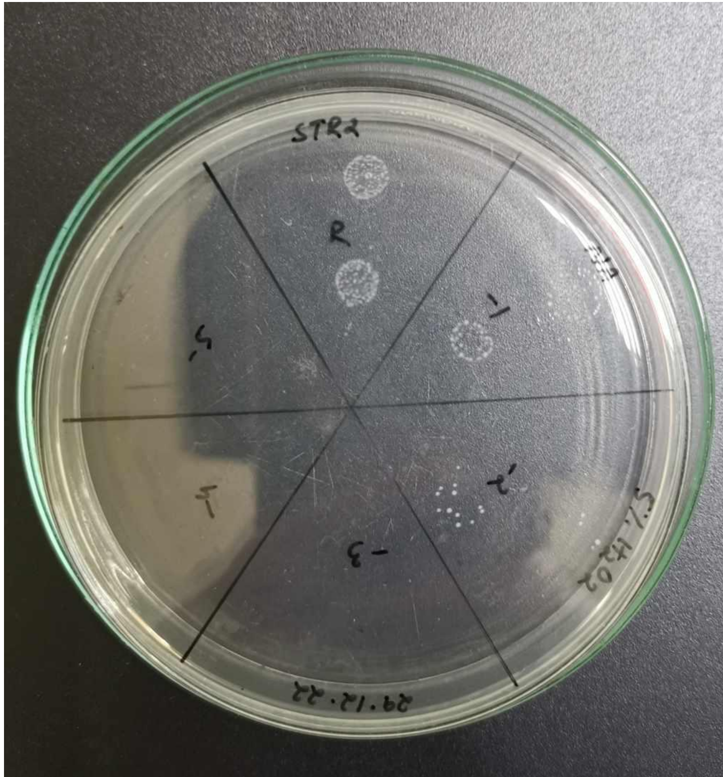
Fig: Graphs of Sodium Hypochlorite

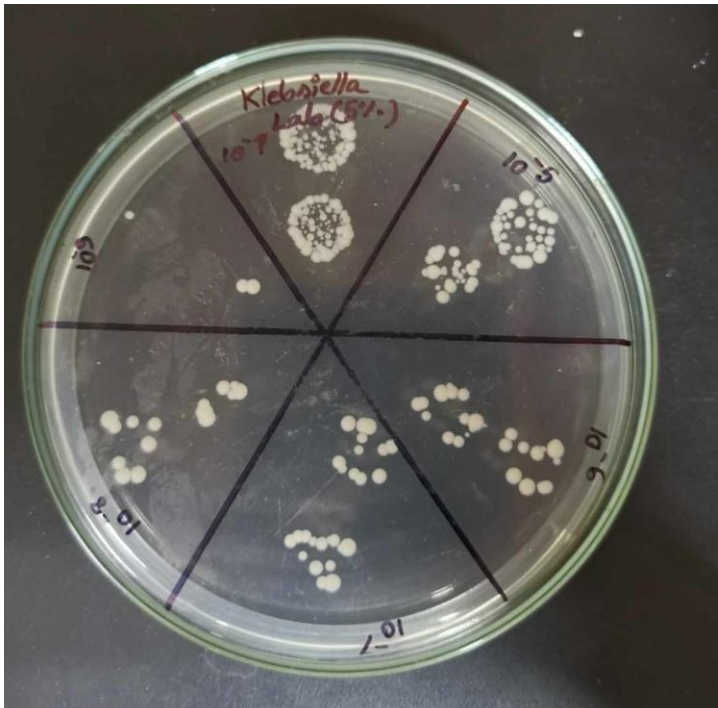
Appendix 2

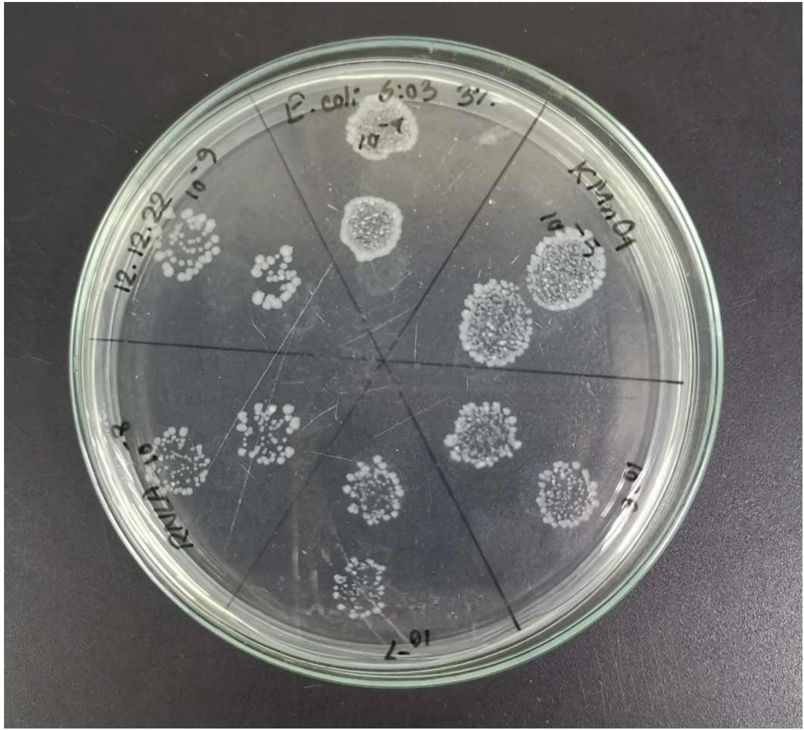
Drop Assay results






STR2
R
29.12.22


Klebsiella
10-5
10-6
10-7
10-8



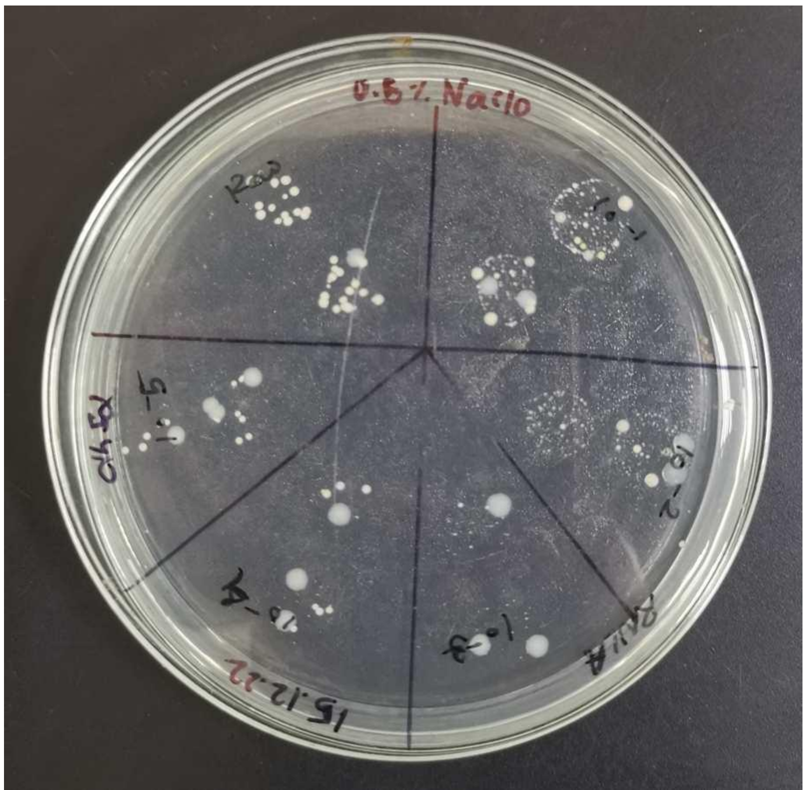


Fig: Plates from drop assay