

Study to demonstrate the efficacy of the VTX Catalyst and hydrogen peroxide combination as a disinfectant.

Hypothesis

The production of hydroxyl radicals in the combination of VTX and hydrogen peroxide is a formidable disinfectant that will prove to be superior to hydrogen peroxide alone.

Experimental Design

A study was conducted to test the efficacy of utilizing VTX as a bacterial disinfectant.

A consortium of bacteria were grown within a one liter fermentation vessel to a cell density estimated to be in excess of 1×10^8 colony forming units (CFUs) per milliliter. The consortium were grown within the fermentation vessel utilizing petroleum diesel range organics as a carbon food source in a nutrient broth containing dipotassium phosphate, ammonium chloride, ammonium nitrate, ferric sulfate, magnesium sulfate, sodium bicarbonate, calcium chloride and water. Once inoculated the vessel was continuously aerated. The diesel range carbon food source was added as needed during a growth period of 60 days.

The bacterium utilized were identified as common, non-pathogenic facultative soil bacteria as given below.

<i>Pseudomonas putida</i>	<i>Alcaligenes xylosoxydans</i>
<i>Corynebacterium aquaticum</i>	<i>Agrobacterium tumefaciens</i>
<i>Arthrobacter crystallopoietes</i>	<i>Ochrobactrum anthropi</i>
<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas alcaligenes</i>

Ten one-liter Erlenmeyer flasks were filled to the one liter level with de-ionized distilled water. Alkalinity was added to each test flask to achieve carbonate alkalinity levels of 100 ppm as calcium carbonate. The flasks were autoclaved for 30 minutes at 121°C as per the method outlined in Standard Methods for the Examination of Water and Wastewater, 19th edition, American Water Works Association. The flasks were inoculated with one milliliter each with bacterium harvested from the continuously mixed fermentation unit.

Each of the ten flasks were treated in the following manner

<u>Flask Number</u>	<u>35% Hydrogen Peroxide Added</u>	<u>VTX</u>
Flask 1 - R1	None	None
Flask 2 - R2	None	None
Flask 3 - C1	0.05 mls	None
Flask 4 - T1	0.05 mls	0.05 mls
Flask 5 - C2	0.1 mls	None
Flask 6 - T2	0.1 mls	0.1 mls
Flask 7 - C3	0.5 mls	None
Flask 8 - T3	0.5 mls	0.5 mls
Flask 9 - C4	1.0 mls	None
Flask 10- T4	1.0 mls	1.0 mls

Treated flasks receiving hydrogen peroxide and/or VTX were swirled after receiving the dosage for 10 seconds to achieve a uniform mixture.

Once the flasks were treated they were allowed to sit for 30 minutes at room temperature. Each flask was swirled for 10 seconds before samples were collected in the appropriate sterilized vessels and placed on ice for shipment to the analytical lab under EPA recognized chain-of-custody protocol.

Each sample was tested for Heterotrophic Bacterial Plate Counts (HPC) in colony forming units (CFUs) per milliliter.

Results

The results for Raw, Hydrogen Peroxide Only and Hydrogen Peroxide/Catalyst Treatment were as follows:

<u>Sample Identification</u>	<u>Heterotrophic Plate Count</u>
Raw Untreated Sample	
R1	>57,000 CFU/ml
R2	>57,000 CFU/ml
Hydrogen Peroxide Only	
C1	720 CFU/ml
C2	630 CFU/ml
C3	10 CFU/ml
C4	<10 CFU/ml
Hydrogen Peroxide/ VTX	
T1	10 CFU/ml
T2	<10 CFU/ml
T3	<10 CFU/ml
T4	<10 CFU/ml

Conclusions

It is apparent that hydrogen peroxide alone successfully reduced the heterotrophic bacterial counts substantially from > 57,000 CFUs/ml seen in the raw untreated samples. However, at 0.05 and 0.1 milliliters of hydrogen peroxide there remained 720 and 630 CFUs/ml, respectively. At the 0.5 ml dosage of peroxide the bacterium were at the low end of detection at 10 CFUs/ml. It was apparent that the bacteria were not detectable at the highest level of treatment in sample C4.

Treatments of hydrogen peroxide and VTX were uniformly effective at all dosages. Only the lowest dosage of 0.05 mls (Sample T1) retained a countable level of bacterium at the very low end of detection at 10 CFUs/ml. As compared to the hydrogen peroxide treatments at C1 and C2, the hydrogen peroxide and catalyst treatments were considerably improved leading to the conclusion that the hydrogen peroxide and VTX combination was a much better disinfectant than hydrogen peroxide alone.