# Ozone Inactivation of Microorganisms: Kinetics and Mechanisms

Ahmed Yousef

Professor of Food Microbiology
Ohio State University

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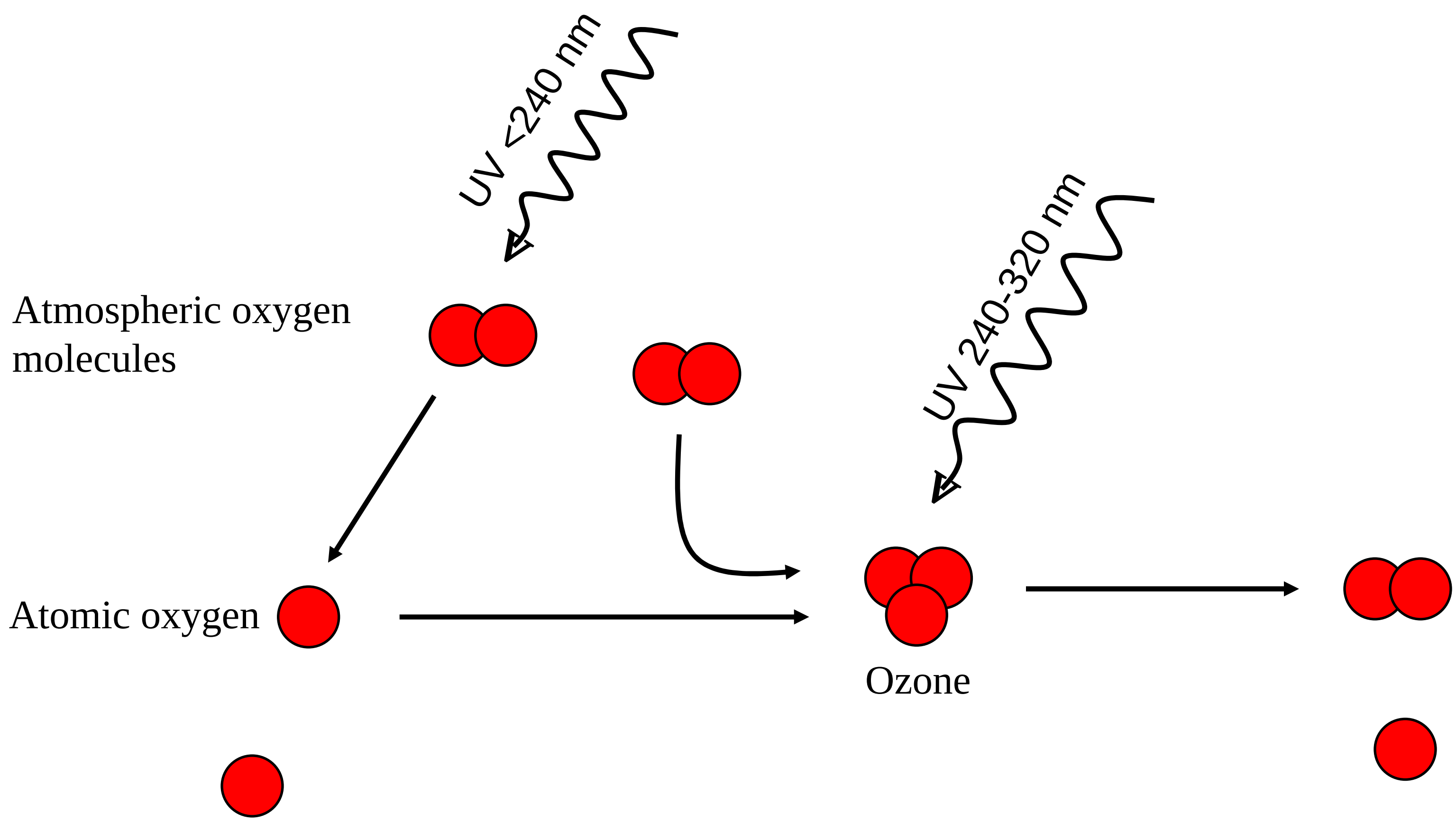
# What's Ozone?

- **Tri-atomic oxygen ($O_3$)**
- **Molecular weight of 48**
- **Bluish gas (at high concentrations)**
- **Pungent characteristic odor**
- **Low solubility in water**
- **Half-life:**
  - **Gas: ~12 hr (at ambient)**
  - **Aqueous: Short, varies by medium**

# Ozone Formation and Decomposition in the Stratosphere (Chapman Mechanism)



# Generation of Ozone for Food Applications

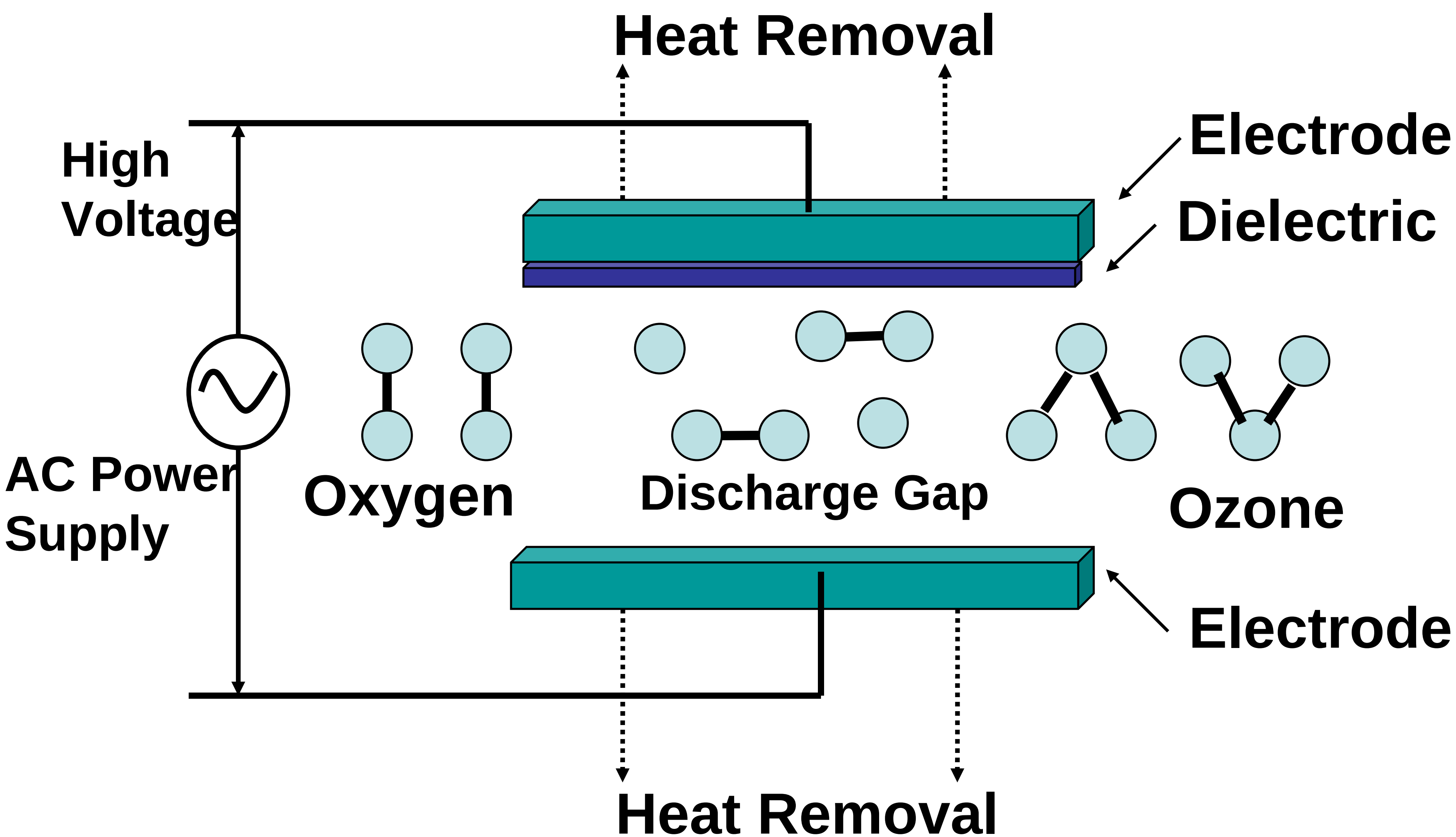
## Method

- Corona discharge
- Electrochemical
- Ultraviolet radiation

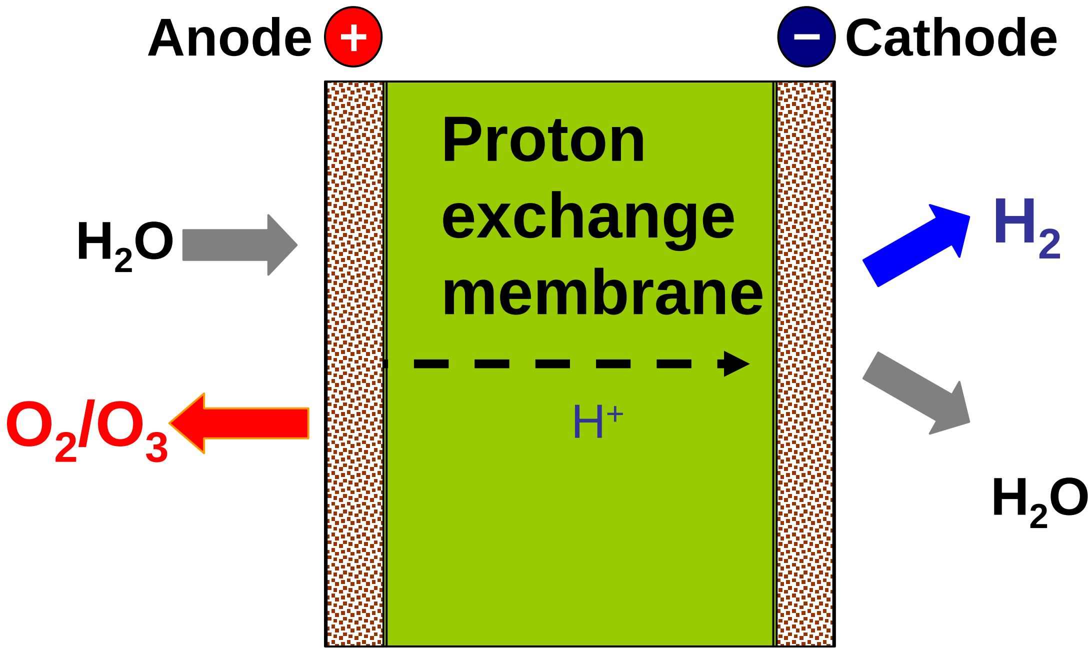
## Consumables

- Air
- Oxygen gas
- Water

# Ozone Generation by Corona Discharge



# Ozone Generation by Electrochemical Process



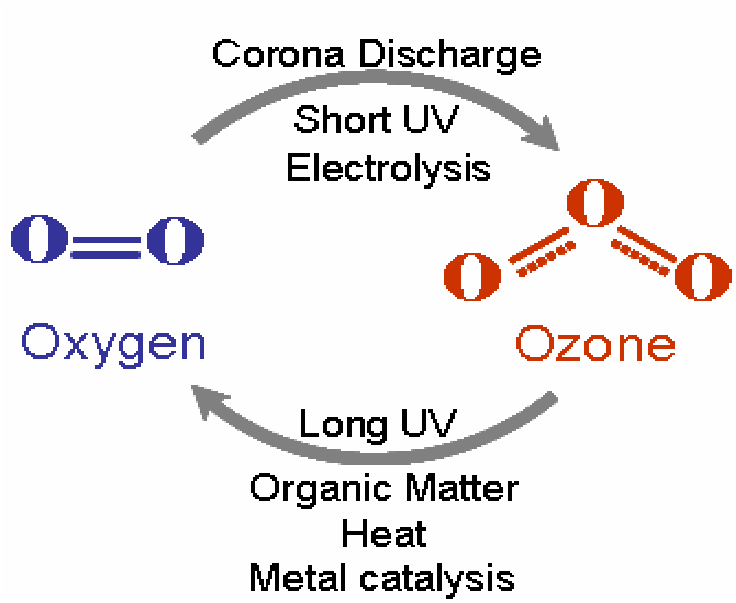

http://www.lynntech.com/pdf/1lbgenerator.pdf

# Ozone Decomposition and Disposal

- **Destruction of excess ozone in work environment**
- **Destruct units:**

  - Heat

  - Catalysts




- **Small amounts**

  - May dispose of in the atmosphere

***For ozone factsheet, visit {http://ohioline.osu.edu/fse-fact/0005.html}***

# Inactivation Kinetics

**Ozone kills diverse bacteria**

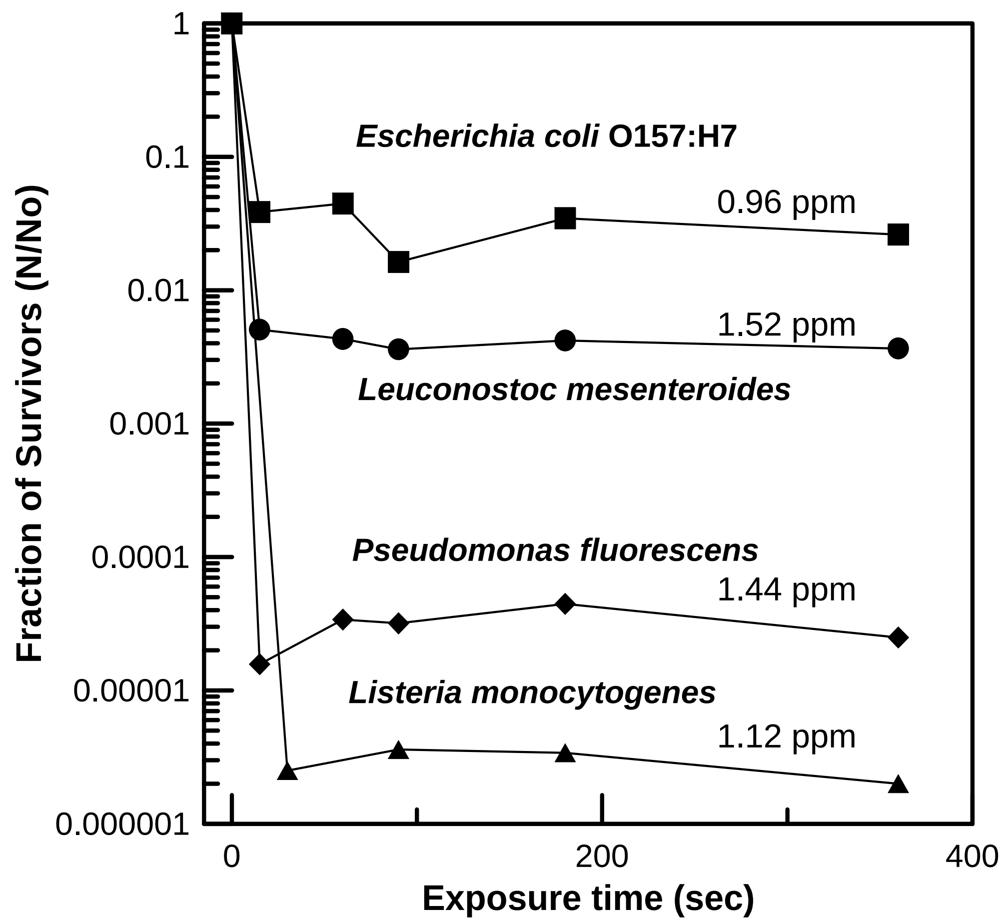
Spoilage and pathogenic bacteria are inactivated

**Rapid inactivation**

Ozone kills bacteria in less than 30s

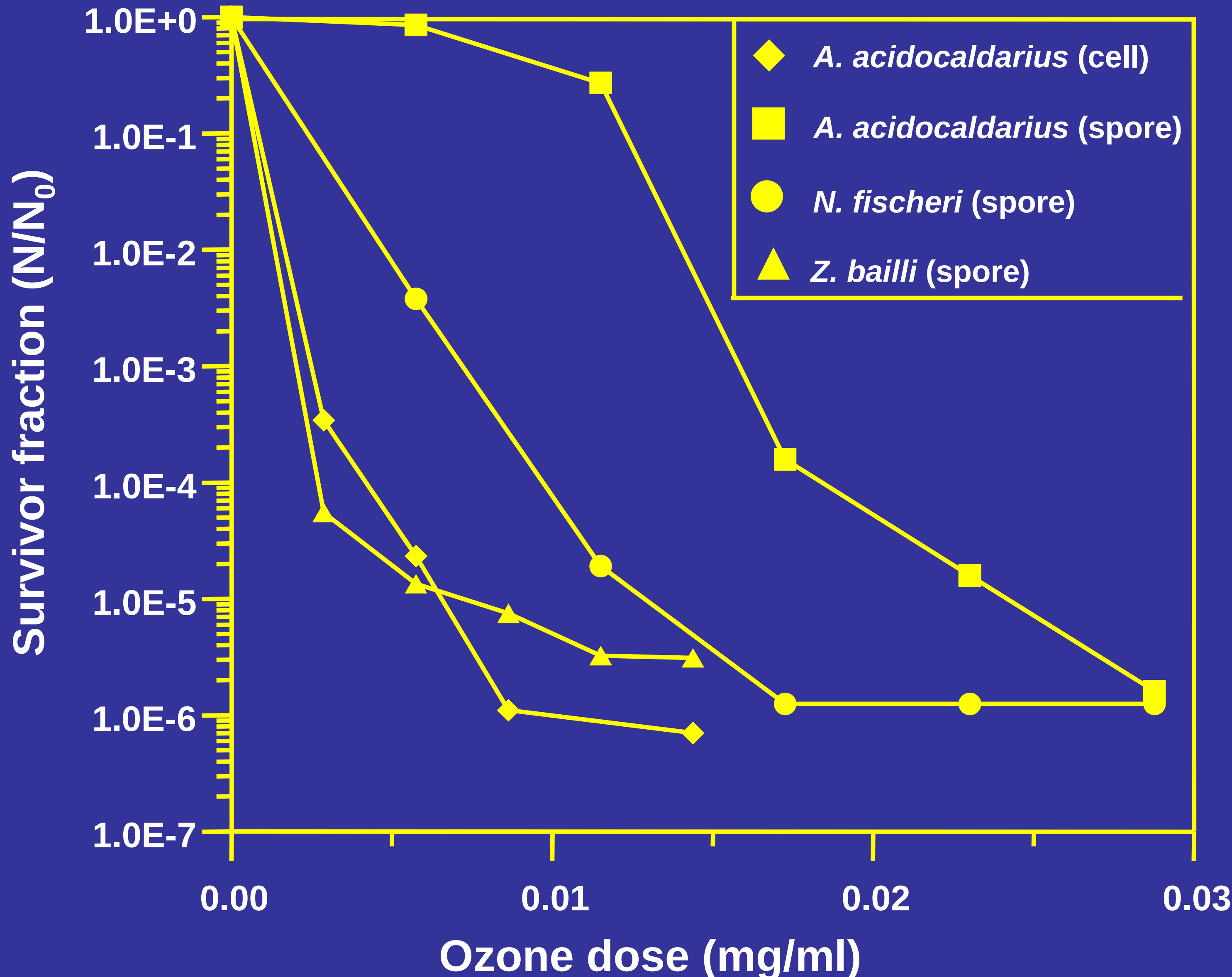
**Effective at low concentrations**

~1ppm ozone kills up to 6 logs




## Inactivation of food-transmitted microorganisms (vegetative cell in pure suspensions) by aqueous ozone

(Kim & Yousef, 2000)




**Inactivation of bacterial and fungal spores suspended in water by ozone**
**Initial count: $6.4 \times 10^6$ - $1.5 \times 10^7$ cfu/ml (Khadre et al., 2001)**

**An ozone dose (mg gas ozone/mL sample)** =

Ozone concentration in gas (mg/L) × flow rate (mL/min) × treatment time (min)/volume of spore suspension (mL).

**(We apologize for the inconvenience)**

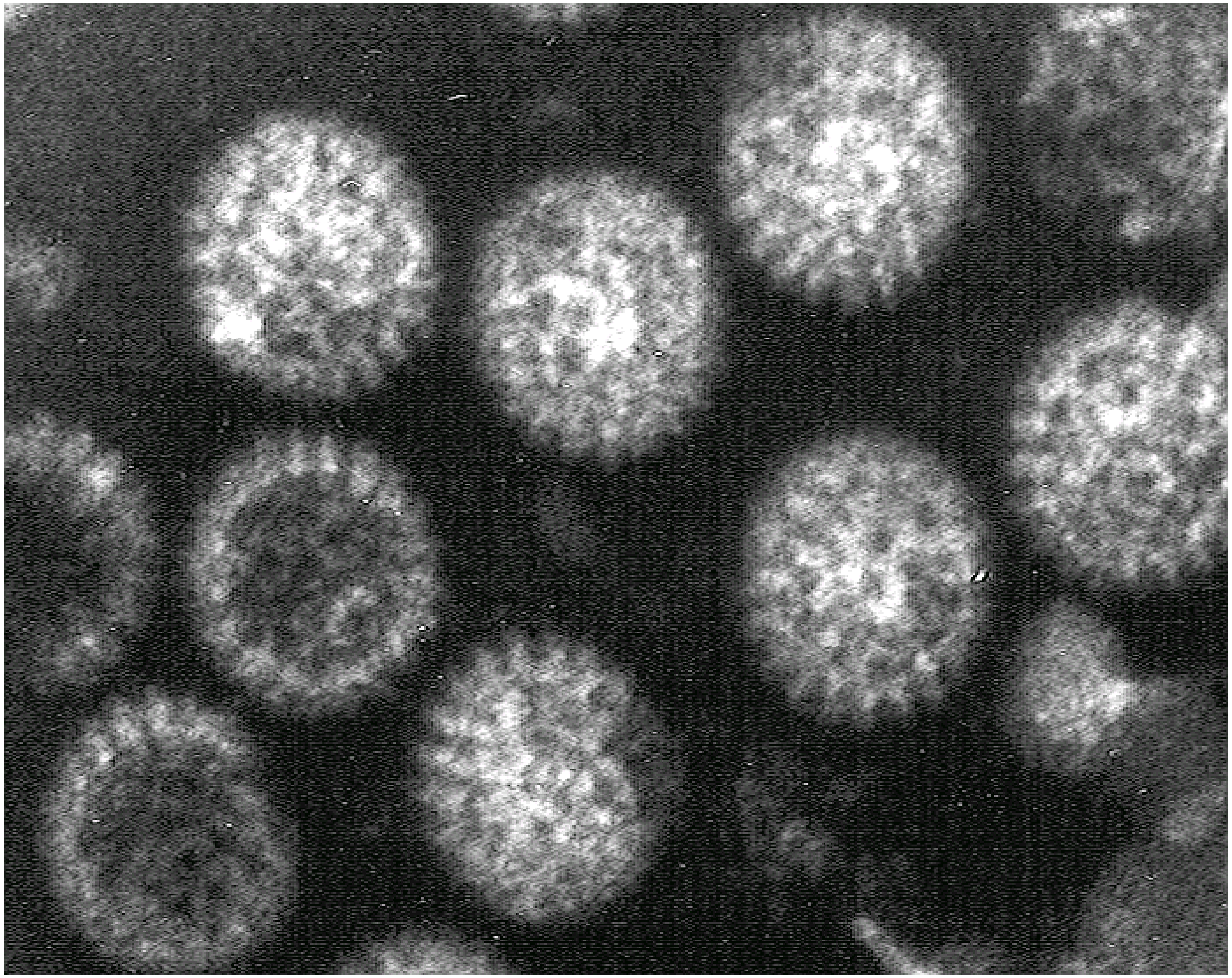
## Treatment of *Clostridium botulinum* spores with aqueous ozone for 1 min

| Treatment | Viable spores/ mL |
| --- | --- |
| Control (0 ppm) | $3.6 \times 10^7$ |
| 12 ppm | < 1 (estimated) |
| 26 ppm | < 1 (estimated) |

## Decrease in spore count ($log_{10}$/ml) with exposure to ozone (0.22 mg ozone/20 ml mixture) or hydrogen peroxide (2000 mg $H_2O_2$/20 ml mixture) for 1 min at 22°C

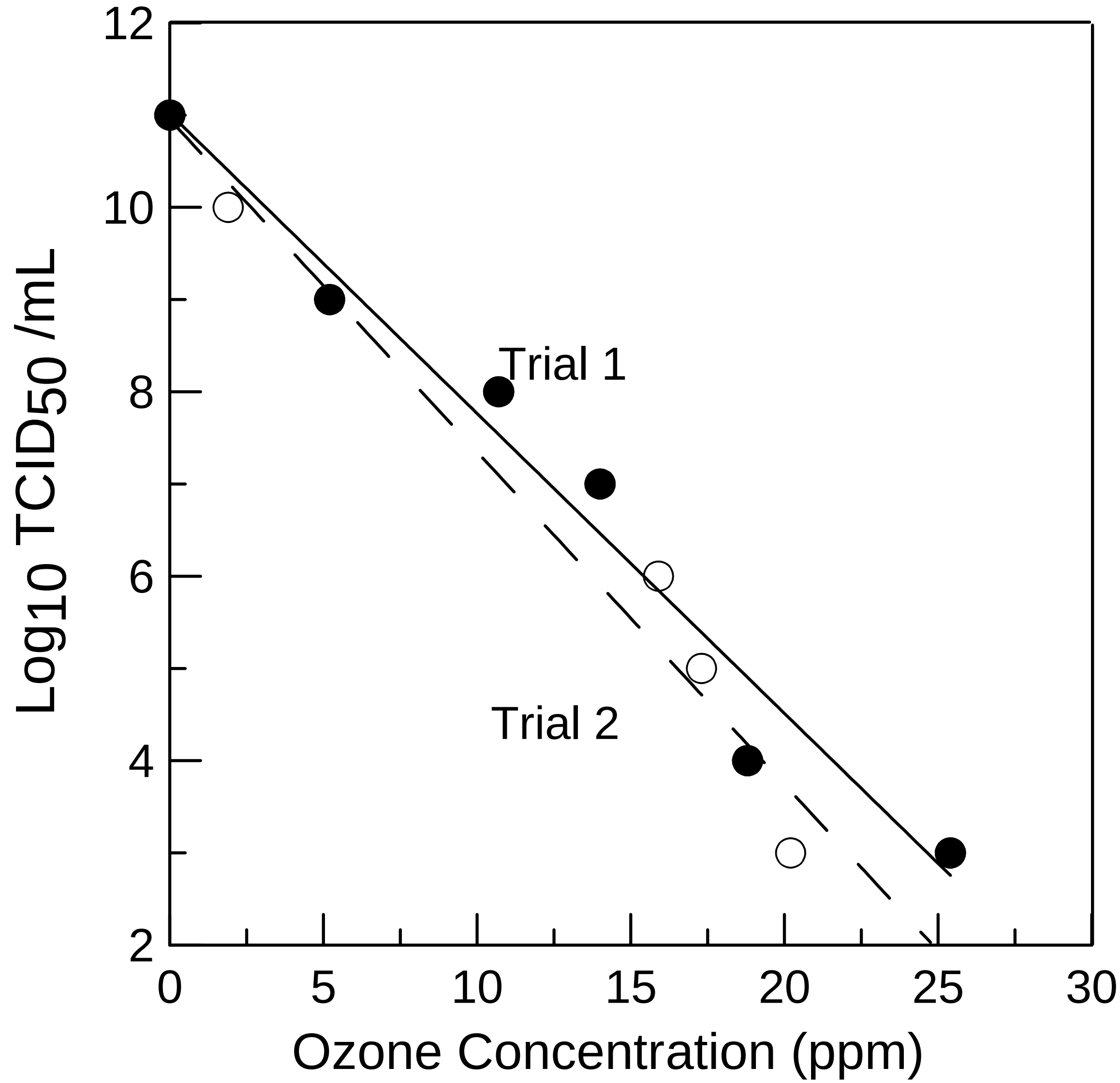
***(Khadre & Yousef, 2001)***

| Spore | $O_3$ | $H_2O_2$ |
|---|---|---|
| *B. cereus* OSU11 | 6.1 | 1.6 |
| *B. megaterium* OSU125 | 2.1 | 0.93 |
| *B. polymyxa* OSU443 | 1.9 | 0.58 |
| *B. stearothermophilus* OSU24 | 1.3 | 0.64 |
| *B. subtilis* OSU494 | 2.7 | 0.32 |
| *B. subtilis* OSU848 | 4.8 | 1.2 |
| *B. subtilis* ATCC 19659 | 6.1 | 0.64 |
| *B. subtilis* vary Niger ATCC 9372 | 5.7 | 1.3 |



**Scanning electron micrograph of rotavirus particles after release from MA 104 cell culture**

*Khadre and Yousef, 2002*




**Changes in infectivity of rotavirus Wa Wooster, measured as $TCID_{50}$/mL at different concentrations of ozone in aqueous solution at 25°C.**

*Khadre and Yousef, 2002*

## What do these kinetic data mean?

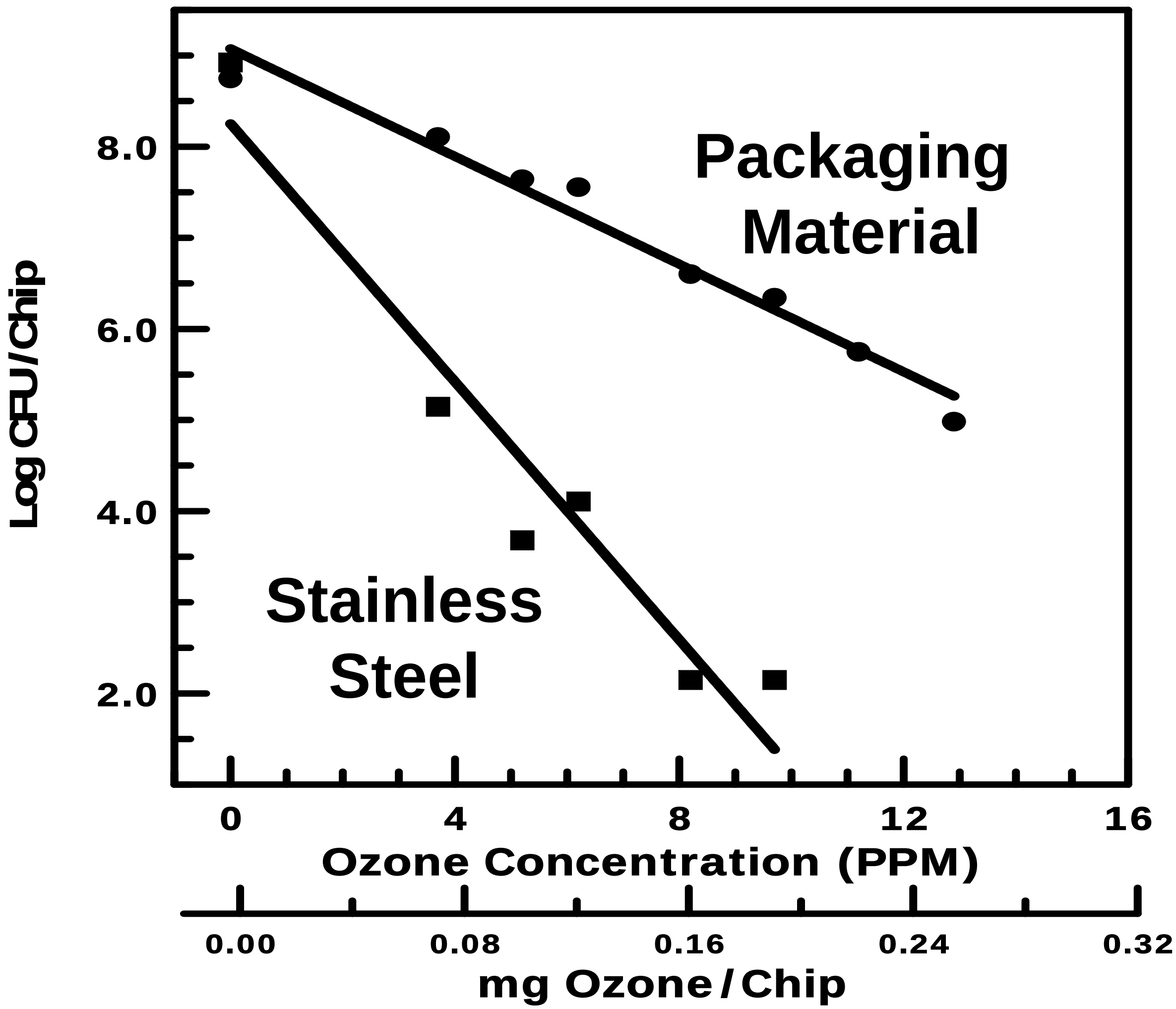
- Lab research vs. Real World
- Testing different scenarios

  - Cell suspension (planktonic) vs. biofilm
  - Equipment vs. package surface
  - Medium more complicated than pure water

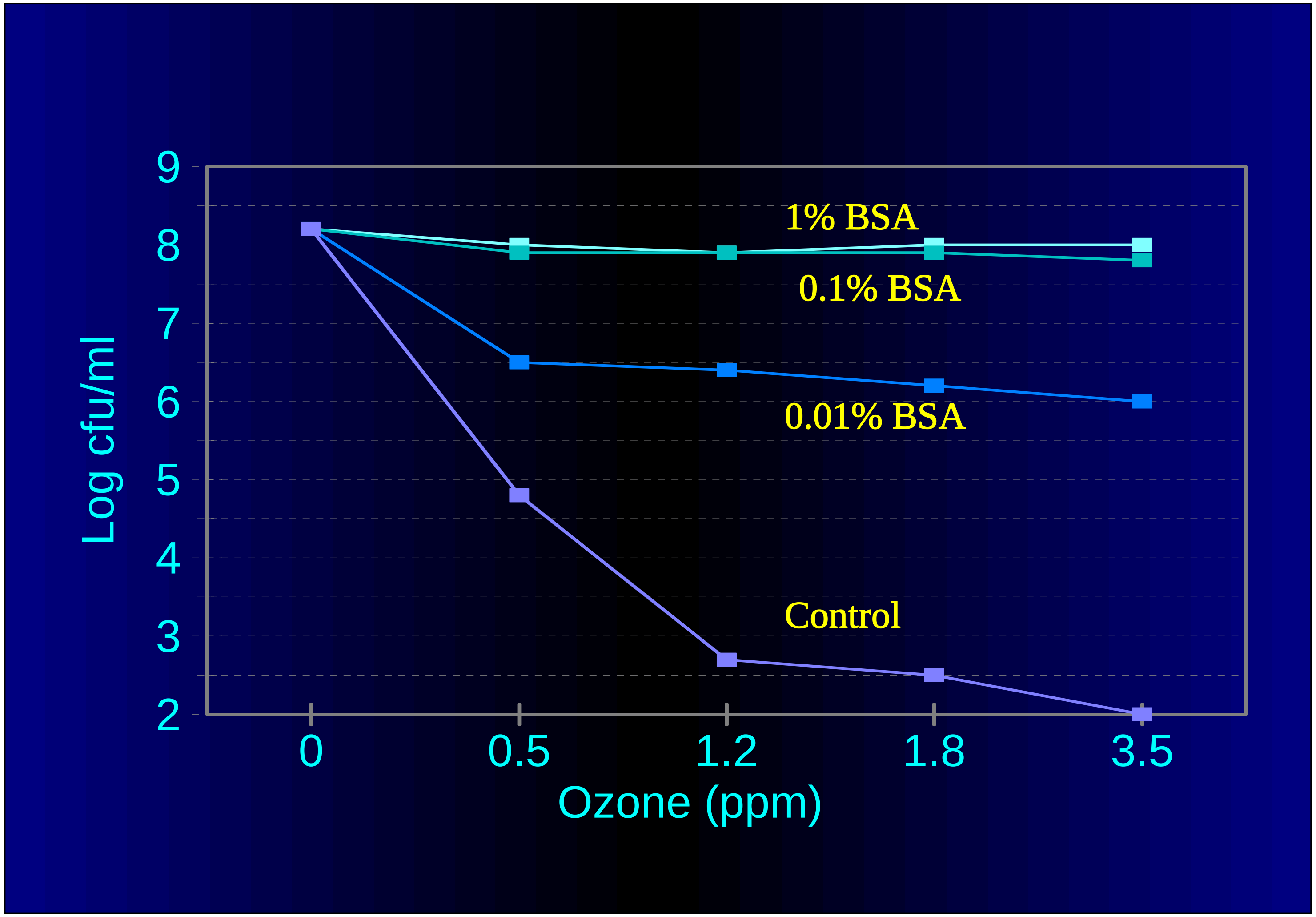
# Efficacy Against Biofilm-Repeated Exposure

Count of *Pseudomonas fluorescens* as a biofilm or a dry film on chips (12.9 $cm^2$) of a multilaminated packaging material after repeated exposure to1-min treatments with ~0.1 mg ozone/chip using 3.6 ppm aqueous ozone (Khadre & Yousef, 2000).

| No. of Exposures | Biofilm | Dry film |
|---|---|---|
| 0 | $3.5x10^8$ | $7.2x10^8$ |
| 1 | $3.2x10^6$ | $6.4x10^3$ |
| 2 | $2.7x10^5$ | <1(est) |
| 3 | $2.2x10^5$ | |
| 4 | $1.2x10^5$ | |
| 5 | $6.0x10^2$ | |




Inactivation of 24-hr biofilm of *Pseudomonas fluorescence* on chips ($12.9\text{-cm}^2$) of packaging material and stainless steel when exposed to different doses of ozone (Khadre & Yousef, 2000)




**Ozone lethality against *Escherichia coli* O157:H7 in the presence of organic load (BSA). *Restaino et al., 1995; Achen, 2000***

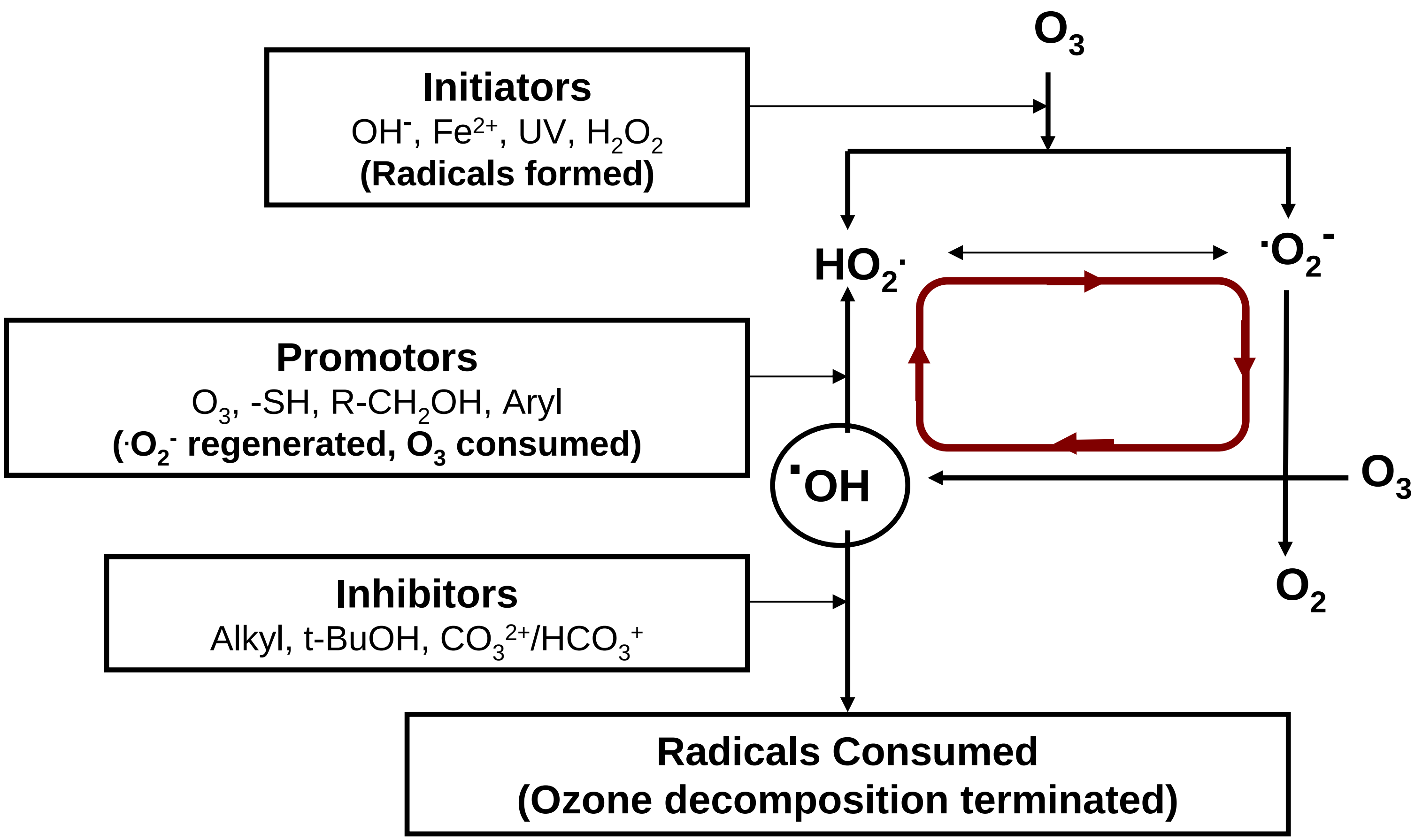
# Inactivation Mechanism

# Oxidation Potential of Selected Oxidizing Agents

| Species | Oxidation Potential (Volts) | Relative Oxidative Power[a] |
|---|---|---|
| Ozone | 2.08 | 1.53 |
| Hydrogen peroxide | 1.78 | 1.31 |
| Hypochlorite | 1.48 | 1.09 |
| Free chlorine | 1.36 | 1.00 |
| Hypobromite | 1.33 | 0.98 |
| Chlorine dioxide | 0.95 | 0.70 |

[a] relevant to chlorine

*Water Quality Association Ozone Task Force. 1997. Ozone for Point-of-Use, Point-of-Entry, and Small System Water Treatment Applications: A Reference Manual.Water Quality Association. Lisle, IL, 2-4.*




# Ozone decomposition, free radical formation and advanced oxidation processes

*(Khadre et al, 2001)*

# Inactivation Mechanism

**Oxidative power**

Molecular ozone (Hunt & Marinas, 1997)

Singlet, free radicals (Kanofsky & Sima, 1991)

# Inactivation Mechanism (Cont'd)

**Reaction with:**

| | |
|---|---|
| Cell membranes | (Giese & Christenser, 1954) |
| Dehydrogenases | (Ingram & Haines, 1955) |
| DNA | (Scott, 1975) |
| RNA | (Kin et al., 1980) |

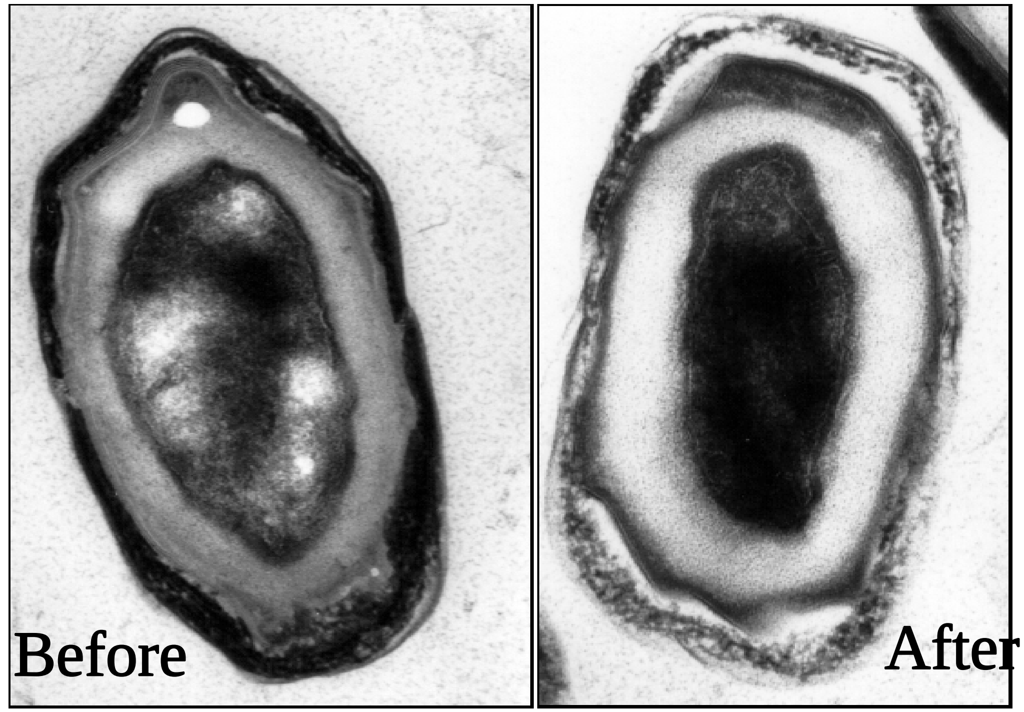
# Ozone action on bacterial spores

**Ozone at 5 ppm**

Damages spores coats (see the electron microscopic pictures).

**Ozone at >5ppm**

Total inactivation of spores (data not shown)




Khadre, M. A. and Yousef, A.E. 2001. Sporicidal action of ozone and hydrogen peroxide, a comparative study. Int. J. Food Microbiol. 71:131-138.

## Target in spore

## Inner membrane!

- Inner membrane damage is the probable killing mechanism for ozone
  *(Young, 2004)*

- Oxidizing agents may have targeted proteins, not lipids, in the spore’s inner membrane
  *(Cortezzo et al., 2004)*.

# **Future Directions**

Combination Treatments

*(if justifiable)*

# D-values* (min) of spores treated with ozone

| *Treatment* | *Temperature (°C)* | | |
|---|---|---|---|
| | 85 | 90 | 95 |
| ***Control (no ozone)*** | 294.1 | 74.6 | 27.0 |
| ***Ozone-treated (before heating)*** | 26.3 | 9.3 | 4.0 |

*Kim et al., 2002*

*** The smaller the D-value, the greater the sensitivity to heat**

# Conclusions

- Ozone inactivates microbial cells rapidly and effectively.
- Spores of *Bacillus* and *Clostridium* species, compared to vegetative cells, require higher ozone concentrations to be killed.
- Ozone damages spore outer coats but membrane damage is probably the cause ozone sporicidal action.
- Bacterial spores become sensitive to heat when pre-treated with sublethal levels of ozone.
- Direct use of ozone in liquid foods and on food surfaces with large ozone demand may not be recommended.