TURI (Toxics Use Reduction Institute)

Applications of ozone in biopharmaceutical industry A cleaner greener approach to cleaning and sterilization

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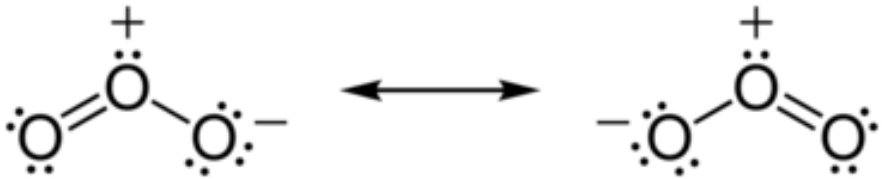
OUTLINE

- Introduction to Ozone
- Ozone based Cleaning In Place(CIP)
- Ozone based Sterilization In Place(SIP)
- Other Potential Applications
- Impact of Technology

OZONE

- **Ozone** (O_3), is a triatomic allotrope of oxygen.
- First identified as a distinct chemical species in 1839.
- Bent molecule with C_{2v} symmetry



Tanaka, Takehiko; Morino, Yonezo "Coriolis interaction and anharmonic potential function of ozone from the microwave spectra in the excited vibrational states". *Journal of Molecular Spectroscopy* 33: 538–551.
Mack, Kenneth M.; Muenter,"Stark and Zeeman properties of ozone from molecular beam spectroscopy". *Journal of Chemical Physics* 66: 5278–5283.

OZONE

- WHY OZONE?
  - Chemical Standpoint
    - Strongest broad spectrum microbial treatment
    - Completely green no residues, readily converts to oxygen

Biocidal Agent	Oxidation Potential(v)	Oxidation Capacity
Ozone(O3)	-2.07	2e-
CH3COOH (Peracetic acid)	-1.81	2e-
H2O2(Hydrogen Peroxide)	-1.78	2e-
NaoCl (Sodium Hypochlorite bleach)	-1.49	2e-
ClO2	-0.95	5e-

Aqueous Ozone

- Ozone in water

INITIATION

$$O_3 + OH^- \rightarrow HO_2^- + O_2$$

-2.67 V

PROPOGATION/ TERMINATION

$$HO_2^- + O_3 \rightarrow O_3^- + HO_2$$
$$HO_2 + OH^- \rightarrow O_2^- + H_2O$$
$$O_2^- + O_3 \rightarrow O_3^- + O_2$$
$$O_3^- + H_2O \rightarrow OH + O_2 + OH^-$$
$$O_3^- + OH \rightarrow O_2^- + HO_2$$
$$O_3^- + OH \rightarrow O_3 + OH^-$$
$$OH + O_3 \rightarrow HO_2 + O_2$$
$$HO_2 = O_2^- + H^+$$
$$HO_2 + OH^- \rightarrow O_2^- (+H_2O)$$
$$H_2O_2 = HO_2^- + H^+$$

$$O_3^- + OH \rightarrow O_3 + OH^-$$

Urs Van Gunten "Ozonation of drinking water: Part I Oxidation Kinetics and product formation" Water research 37(2003) 1443-1467

Aqueous Ozone

- Industrial implementation Standpoint
  - Commercially available

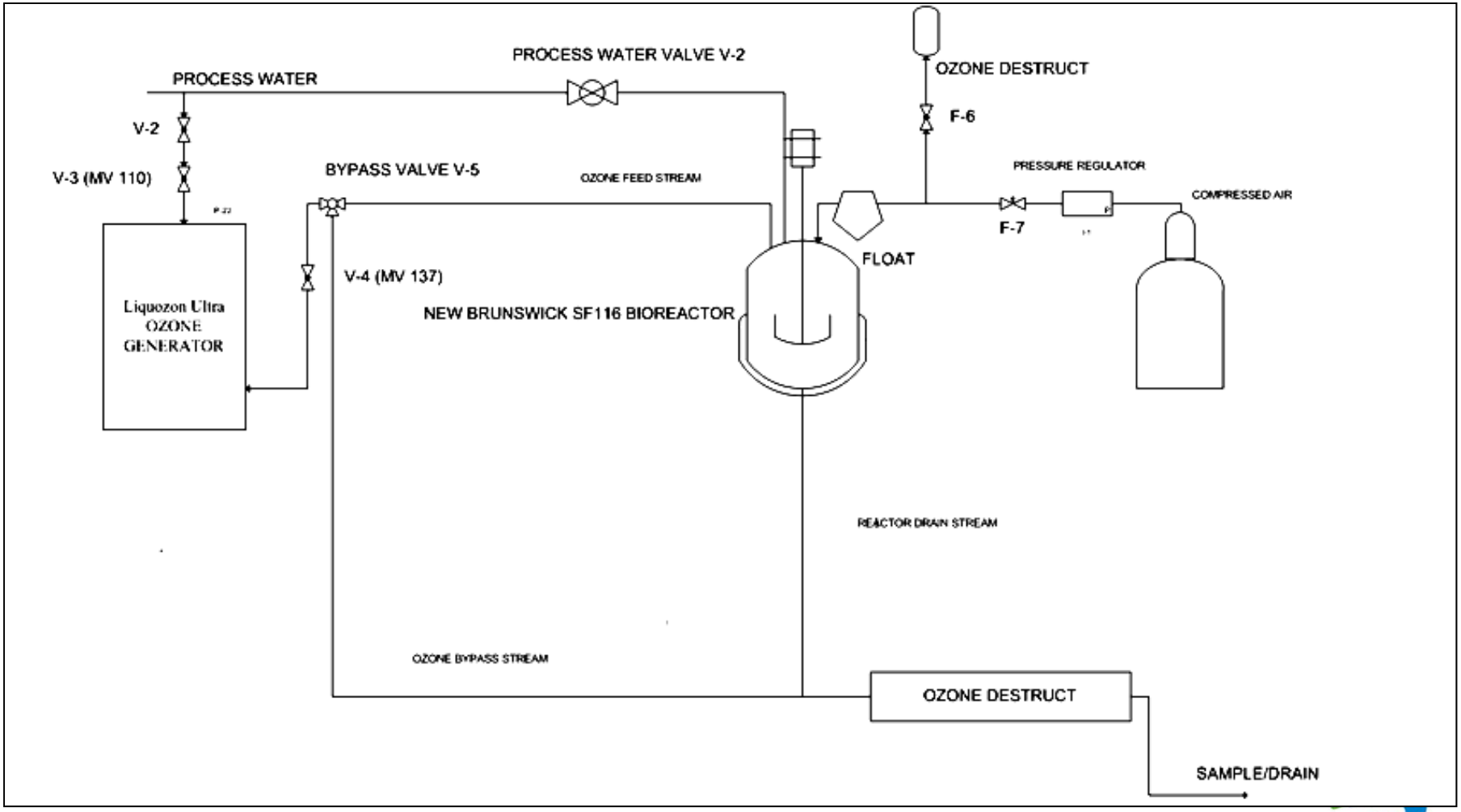
    MKS Instruments' LIQUOZON® *Ultra* ozonated water system
  - FDA approved anti-microbial agent (June 21, 2001)
  - Established usage and scale up in food and beverage industry
  - OSHA defined exposure norms
  - Established sensor technology for detection

CLEAN IN PLACE

- **Clean-in-Place** (**CIP**) is a method of cleaning the interior surfaces of vessels, piping, process equipment, and associated fittings, without dismantling.
- Key parameters
  - **T ime**
  - **A ction**
  - **C oncentration**
  - **T emperature**

George Verghese "Selection of cleaning agent and parameters for CGMP Processes". Proceedings of INTERPHEX Conference March 17-19 2009

CLEAN IN PLACE



OZONE BASED CIP

CONVENTIONAL CIP PROCEDURE

PROCESS WATER PRERINSE 15 MIN 60°C

↓

CAUSTIC WASH -15 MIN 60°C

↓

PROCESS WATER RINSE 15 MIN 60°C

↓

ACID RINSE RINSE 15 MIN 30°C

↓

PROCESS WATER RINSE 15 MIN 60°C

↓

FINAL RINSE 15 MIN 60°C

OZONE BASED CIP PROCEDURE

2 ppm OZONATED WATER PRERINSE 15 MIN 27°C

↓

CIP 100 (STERIS)(3%) 15 MIN 27°C

↓

2 ppm OZONATED WATER POST CIP 100 -15 MIN 27°C

↓

2 ppm OZONATED WATER FINAL RINSE 15 MIN 27°C

CIP RESULTS SUMMARY

SAMPLING TECHNIQUE	ANALYTICAL TECHNIQUE	RESULT
DIRECT SURFACE	VISUAL OBSERVATION	No residue
DIRECT SURFACE-SWAB	HPLC (Shiga toxin B)	<17.6 µg/ml
	UV SPECTROPHOTOMETRY (200-300nm)	<.05
	PLATE COUNT*	NIL
FINAL RINSE	HPLC	<17.6 µg/ml
	UV SPECTROPHOTOMETRY (200-300nm)	<.05
	PLATE COUNT (CFU)*	NIL
	pH	6.4
	TOC (ppm)	.169 ppm
	CONDUCTIVITY (mS/cm)	.002 mS/cm

*Starting Culture 5.00E+07 CFU

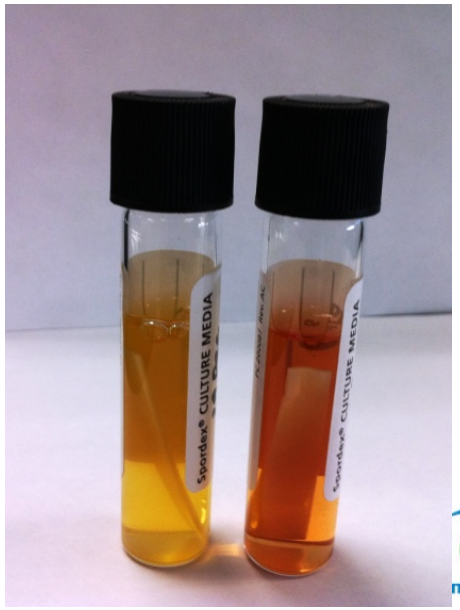
STERILIZATION IN PLACE (SIP)

- Sterilization In Place of Bioreactors and Process vessels is being evaluated
- Preliminary results are promising using high humid conditions with gaseous ozone and aqueous ozone
- Unlike chlorine based sterilants does not result in formation of Trihalomethanes (THMs) which are potential mutagens and carcinogens

STERILIZATION IN PLACE (SIP)

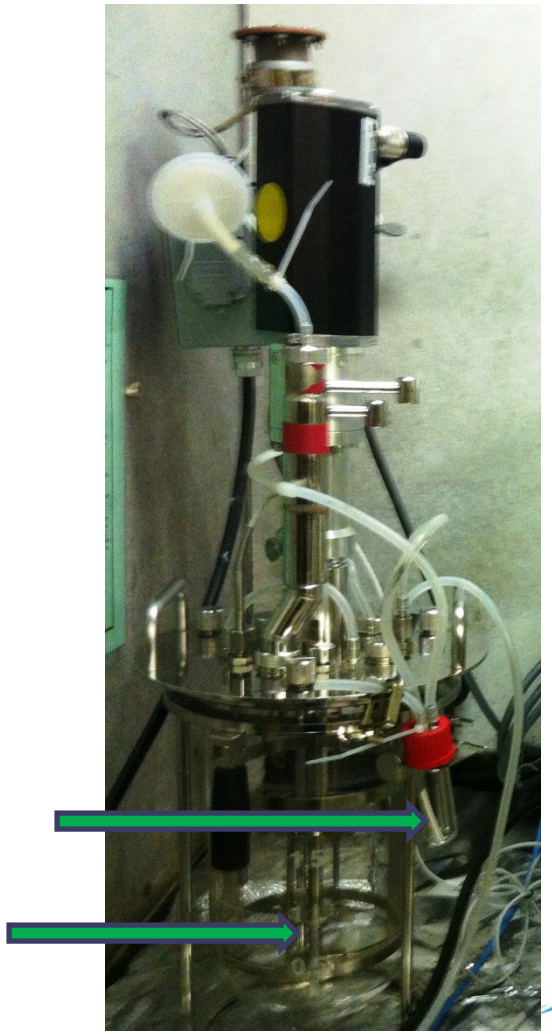
Biological indicator: **STERIS Spordex NA037**

- Spore strips Inoculated with single spore species E6 population
  (Geobacillus stearothermophilus, ATCC 7953)
- Growth media is modified soybean casein with pH indicator
- Post incubation show color and turbidity change representing presence of spores
- 6 log reduction test meets ISO/AAMI requirements



STERILIZATION IN PLACE (SIP)

- Ozone concentration: 22 mg/L
- Flowrate: 4 lpm
- Reactor volume: 2L
- Spore strip location
  - Core of reactor
  - Sample tube



STERILIZATION IN PLACE (SIP)

OZONE PHASE	CONTACT TIME (MIN)		
	90 MIN	120 MIN	150 MIN
DRY GASEOUS OZONE	-	-	-
HUMID OZONE	-	-	+
OZONATED WATER	-	+	+

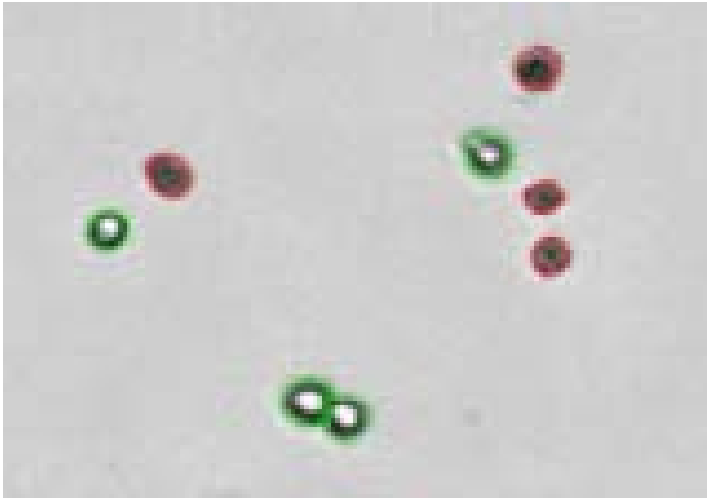
- **IMPLIES INCOMPLETE INACTIVATION/ STERILIZATION**

+ **IMPLIES STERILITY ACHIEVED**

POTENTIAL APPLICATIONS

Batch Decontamination

- Cell line : Chinese hamster ovary (CHO) cells
- Exposure: 16 mg/L overlay
- Flowrate: 3.5 lpm
- Cell necrosis tracked by Cell counter



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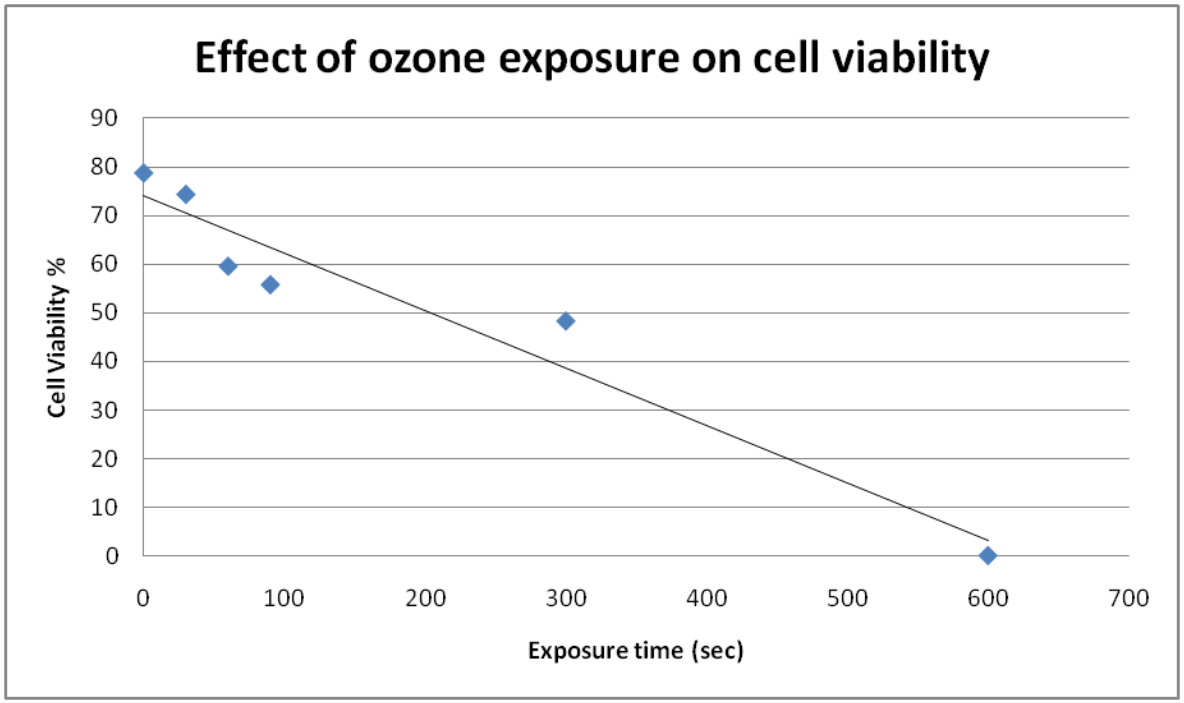

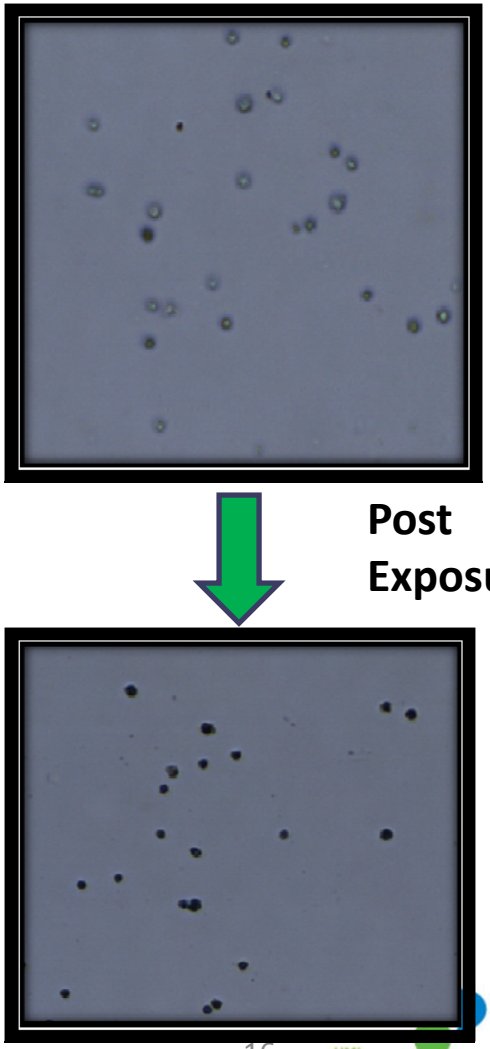

IMPACT OF TECHNOLOGY

- Alternative to conventional CIP and SIP
- Environmentally benign cleaning and sterilization process
- Significant energy and water consumption reduction
- No high pressure and temperature requirement for biopharmaceutical equipment

ACKNOWLEDGEMENTS

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- MKS Instruments
- MBMC
- STERIS

University of Massachusetts Lowell

EMERGING TECHNOLOGIES AND INNOVATION CENTER