ENGINEERING PHARMACEUTICAL INNOVATION

Best Practice for the Use of Ozone in Life Science Applications

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Introduction

- Ozonation is an attractive choice to replace heat shock and chemical sanitization in high purity water production.

Overview

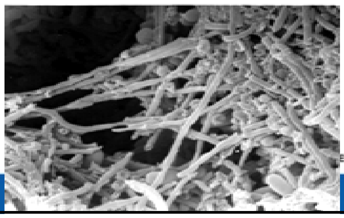
- Water purification challenges
- Issues surrounding the use of ozone in water purification systems
  - Ozone technical parameters, regulatory barriers, materials of construction, and safety considerations
- Practical examples of the successful implementation of ozone-based water purification in pharmaceutical applications
  - Intermittent sanitization of water systems
  - Indirect sanitization via ozone Total Organic Carbon (TOC) control
  - Process sanitization as the final CIP step
- Designing an Ozone water purification system for pharmaceutical applications

Purified Water Facts

- **Purified Water is too often conducive to microbiological growth**
- 500 ppb TOC is permitted
- Conductivity is only < 1.3 uS
- 100 cfu/ ml is the max allowed

BIOFILM in Water Systems

- A thin layer of bacteria and organic matter that occurs under the viscous boundary layer, at the interface between the bulk water phase and solid system components, such as piping, filters and resins.



Parameters Effecting Biofilm Growth

- Prevent Microbial Colonization of Loop
  - System Design Philosophy
  - Ozone treatment
- Heat
- Materials of Construction
  - Metals may provide scarce limiting nutrients
- Nutrient Deprivation
  - In extreme nutrient deficient environments, bacteria will not attach to surfaces. If they do not attach, cannot form biofilm. - Costerton

Heat sanitization does not remove biofilm build up

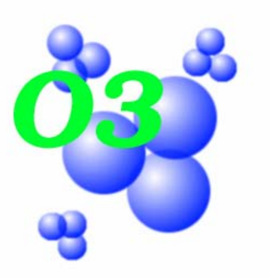
- Heat has been proven to be an effective means of controlling overall system bio-colonization levels. However, heat sanitization does not remove biofilm.
- Controlling Total Organic Carbon (TOC) is essential to preventing biofilm proliferation.
- Hot water and chemical sanitization of problem water systems may exacerbate problems
- TNTC spikes are common after sanitization
  - This is likely the re-colonization of biofilm

Sanitization Strategy

- The pharmaceutical and semiconductor industries utilize differing approaches for biofilm control
- Pharmaceuticals tend to rely upon heat
- Semiconductors utilize nutrient deprivation via exceedingly high water quality

Why Ozone?

- Powerful reactant
  - Ozone disinfects more powerfully than other chemical disinfectants, 3000 times stronger than Chlorine
  - Fast reaction rate
  - Oxidizes and removes biofilm
- “Green chemical”
  - Generated in point of use from air or oxygen
  - No storage and transportation
  - Decays to oxygen (2 O3 ⇒ 3 O2)
- Lower water consumption & disposal cost
  - Reduces chemical sanitizing or energy costs
  - Ozone reduces overall water consumption
    - Fewer rinsing steps required
  - Ozone is safe with no disposal costs
    - Ozone decomposes back into O2 as a byproduct
  - Ozone improves uptime potential
    - What can take up to 8 hours with Steam or chemical can be done in 15-20mins with Ozone
  - Ozone reduces overall water consumption
    - Safe with no disposal costs
  - Reduces overall operating costs



Common Organisms that are Killed by Ozone:

- **Bacteria**

  Achromobacter butyri NCI-9404
  Bacillus cereus
  Bacillus subtilis
  B. stearothermophilus
  Clostridium botulinum
  C. sporogenes
  Cryptosporidium
  Coliphage
  Escherichia coli
  Listeria
  Micrococcus spharaeroides
  Mycobacterium leprae
  Phytomonas tumefaciens
  Pseudomonas aeruginosa
  Pseudomonas putida
  Salmonella paratyphi
  Spirllum rubrum
  Staphylococcus albus
  Vibrio comma
  V. parahaemolyticus
- **VIRUS**

  AIDS
  Adenovirus (type 7a)
  Bacteriophage (E.coli)
  Coxackie A9, B3, & B5
  Cryptosporidium
  Echovirus 1, 5, 12, &29
  Encephalomyocarditis
  Hepatitis A
  GD V11 Virus
  Onfectious hepatitis
  Influenza
  Legionella pneumophila
  Polio virus (Poliomyelitus) 1, 2 & 3
  Rotavirus
  Vesicular Stomatitis
- **FUNGUS & MOLD SPORES**

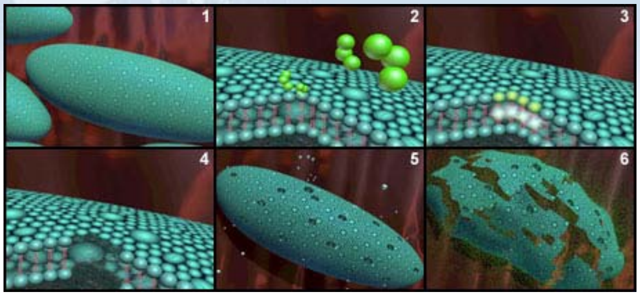
  Aspergillus glaucus (bluish-green)
  Aspergillus terreus, saitoi & oryzac
  Botrytis allii
  Colletotrichum lagenarium
  Fusarium oxysporum
  Grotrichum
  Mucor recomosus A & B (white-gray)
  Oospora lactis (white)
  Penicillium cyclopium
  Rhizopus nigricans (black)
- **PROTOZOA**

  Paramecium
  Nematode eggs
  Chlorella vulgaris (Algae)
  All Pathogenic and Non-pathogenic forms of Protozoa
- **FUNGAL PATHONGENS**

  Alternaria solani
  Botrytis cinerea
  Fusarium oxysporum
  Monilinia fruiticola
  Pythium ultimum
  Phytophthora erythroseptica
  Rhizopus stolonifera
  Sclerotinia sclerotiorum
- **YEAST**

  Baker's yeast
  Candia albicans-all forms
  Common yeast cake
  saccharomyces cerevisiae

How Ozone Attacks Bacteria



- Ozone oxidizes contaminants directly or through the formation of hydroxyl radicals
- When added to water, Ozone increases the redox potential of water
    - When Ozone decomposes in water, it generates the free radicals (1) hydrogen peroxy (HO2) and (2) hydroxyl (OH)
    - The radicals oxidize organic compounds in the water
- Ozone also serves as a very powerful disinfectant that destroys bacteria, viruses, fungi, algae, yeast, mold, and parasites

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Controlling TOC leads to Controlling microbial growth

- Two key steps in the post treatment are TOC reduction and the avoidance of system bio-colonization. Ozone can do both.
- Controlling the TOC well below 20 ppb typically results in controlling the microbial level at or less than 1 cfu/100 ml.
- The USP specification for TOC is 500 ppb and for bacteria count is 100 cfu/ml both of which are not realistic for a trouble free water system.

Nutrients Deprivation

- Besides the reduction of living micro-organisms, Ozone reduces TOC, the nutrients for micro-organisms.
- Ozone will destroy most bacteria in seconds by lysis of the cell wall.
- Ozone is a very effective sanitant with cell destruction kinetics orders of magnitude higher than chlorine.

Microbiological Integrity of High Purity Product Water

- You can only restrict the growth of microorganisms in a high purity (low nutrient) environment.
- The Post treatment and Distribution Loop are the highest purity environments in the water system.
- You cannot get control until you get to high purity (in distribution)

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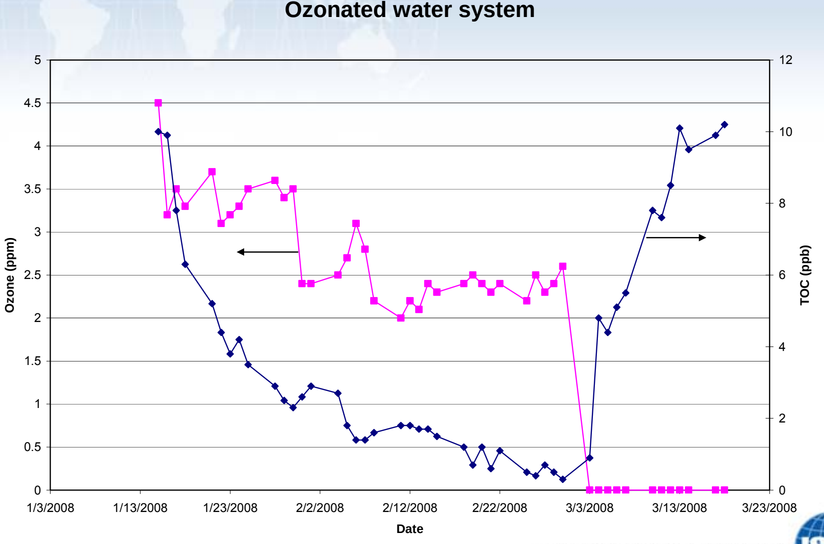
Practical Case Studies

Case Study 1

- A 500 gallon DI water system was idle for 6 years
  - Algae and bacteria took over the system.
  - After cleaning the storage tank, system was started up.
  - Bacteria count was too numerous to count, the TOC level was over 5000 ppb.
- Ozone was injected into the water
  - within less than a day the TOC was reduced to < 5 ppb and eventually to < 2 ppb, with the resistivity of 18.2 mΩ.
  - The water was tested for bacteria counts and the results were stunning, <1 CFU/ 100 ml.
- Even with ozone off for 3 weeks and without further sanitation, the water and distribution system maintained a bacteria count of < 1 CFU per 100 ml.

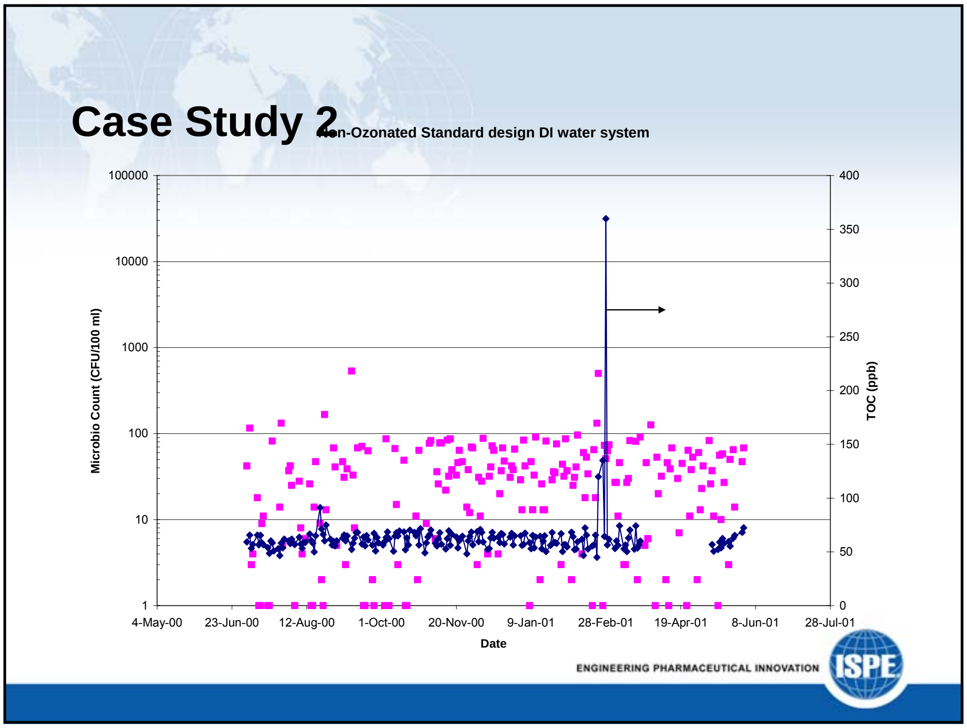
CASE STUDY 1
The efficacy of ozone in TOC reduction



Case Study 2
Unrealistic USP Specifications

- TOC and Bacteria counts of a properly maintained standard system of good design was measured for a period of 14 months
- The TOC level was between 50 to 70 ppb, the bacteria count averaged less than 100 cfu/100ml
  - Much better than the USP recommended specification of 500 ppb TOC and 100 cfu/ml
- The system exhibited biofilm and bacteria problems in the long term

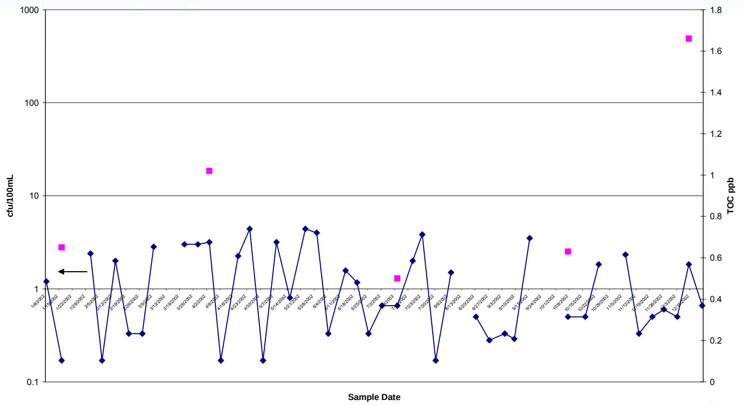
Case Study 2



Case Study 2
Unrealistic USP Specifications

- This system was modified to control the TOC level.
- Controlling the TOC level to less than 2 ppb reduced the bacteria count to less than 10 cfu/100 ml with no sanitization.

Case Study 2



Standard Plate Count for Ambient Temperature Purified Water System

Average TOC content of HP water < 2ppb

Average of 6 samples collected weekly

8 out of 300 samples were discarded

Ozone System Design Considerations

Ozone Manufacturing Methods

- Oxygen source
  - Presser swing absorption (PSA)
  - Piped oxygen gas or Oxygen cylinders
- Ozone generation by Corona Discharge
  - Oxygen: 85%- 80%
  - Ozone: 15% - 20 %
  - (lower ozone concentrations are possible as required)
  - Nitrous oxides: Trace PPM
- Typical ozone concentrations
  - 2-10% by weight if generated by air
  - 10-20% if generated from oxygen

Ozone Manufacturing Methods

- Ozone generated from air is contaminated with corrosive Nitric acid due to high N2 content of Air.
- This is a concern in 316 Stainless Steel distribution loops.
- Ozone may be generated from almost pure Oxygen supplied by house piped gas or cylinders. Traces of nitrogen are still recommended to facilitate the ozone generation process. The resulting nitric acid formation is so low it is virtually negligible.
- Ozone is 13X more soluble in water than oxygen, resulting in some portion of the ozone going into solution while the oxygen and excess ozone is removed by a phase separation process.

Dissolving Ozone into water

- Typically mixing and dispersal methods for introducing the ozone into water are:
  - Venturi or injector
  - Static mixer
  - Tank sparger
- Ozone is 13X more soluble in water than oxygen, resulting in some portion of the ozone going into solution while the oxygen and excess ozone is removed by a phase separation process.

Methods of Ozone Destruction

- In water
  - UV irradiation denatures ozone to oxygen
  - Heat
  - Granular Activated Carbon (GAC)
- In Gas (vent on storage tanks, ozone generator off gas)
  - Manganese Dioxide Catalyst
  - Heat

Ozone Measurement

- On line measurement is required for effective ozone level control
- Where to measure Ozone is application dependant
  - Effective dose
  - Effective Removal
  - Ambient safety

Materials of Construction

- Materials of construction in contact with ozone are of primary importance
- Recommended piping materials for use with ozone gas include
  - Teflon, galvanized steel, glass, Stainless Steel
- Recommended piping materials for use with ozonated water include
  - PVDF, CPVC, Stainless Steel, and in lower concentrations PVC
- Other material recommendations:

• Gaskets	EPDM (up to 100 F)
• Diaphragms	PVDF and EPDM
• Tanks	FRP and 316 Stainless Steel
• Filters	Teflon
• Sensors	Check with manufacturer

Materials Not Recommended for Use with Ozone

- Materials that are NOT recommended in contact with ozonated water include
  - rubber
  - nylon
  - polypropylene

Rouge and Ozone

- Ozone itself is not corrosive to 316 Stainless Steel and does not result in rouging.
- Rouging of 316 Stainless Steel systems in use with some ozone systems is typically resulted from ozone contaminated with carbonic acid (Carbon Dioxide).

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Ozone Sanitization Strategies

Three Effective Applications of Ozone

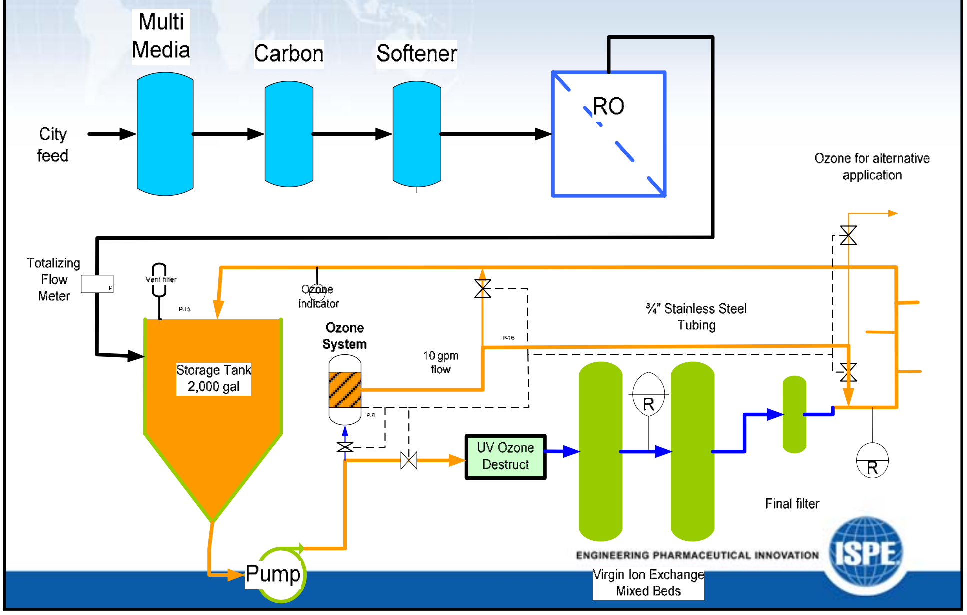
- Sanitization of Distribution Loops on Demand
- Passive Microbial Control via TOC Reduction
- Process Sanitization
  - CIP/SIP
  - Vivarium Water

Intermittent Sanitization of Water Systems

- Ozone used for sanitization of distribution loops on an intermittent basis should not exceed 20 -200 ppb for 15 minutes max.
- No deleterious effects to piping and elastomers may be expected other than the desired sanitization.
- Higher ozone dosages (1 ppm) and longer contact times will permit the removal of biofilm in pipes of appropriate materials.

Client :
Facility:
Date :

Distribution Loop Sanitization



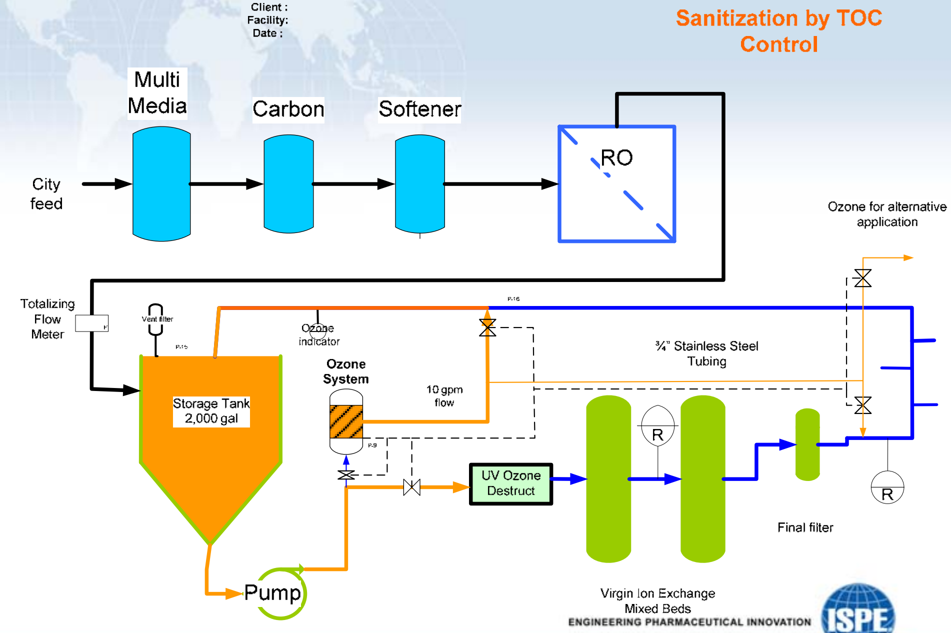
Indirect sanitization via Ozonated TOC control

- **The most attractive use of ozone is to <u>prevent</u> the contamination of the high purity water with TOC.**
- **While passive ozonation may be used to directly sanitize a distribution system, quickly and efficiently *preventative* use of ozone is recommended.**
- **Very low TOC (< 5 ppb) and passive microbial control (typically < 5 cfu/ 100 ml) in**

Indirect sanitization via Ozonated TOC control

- **Ozone is essentially the only way to achieve sub ppb TOC levels in high purity water.**
- **Ozone may now be used "outside of the distribution loop" to control TOC, and thereby impart microbial control to the product water in distribution. This approach sidesteps questions of "added substances" from a regulatory standpoint, as the ozone is not in distribution or in the product water.**

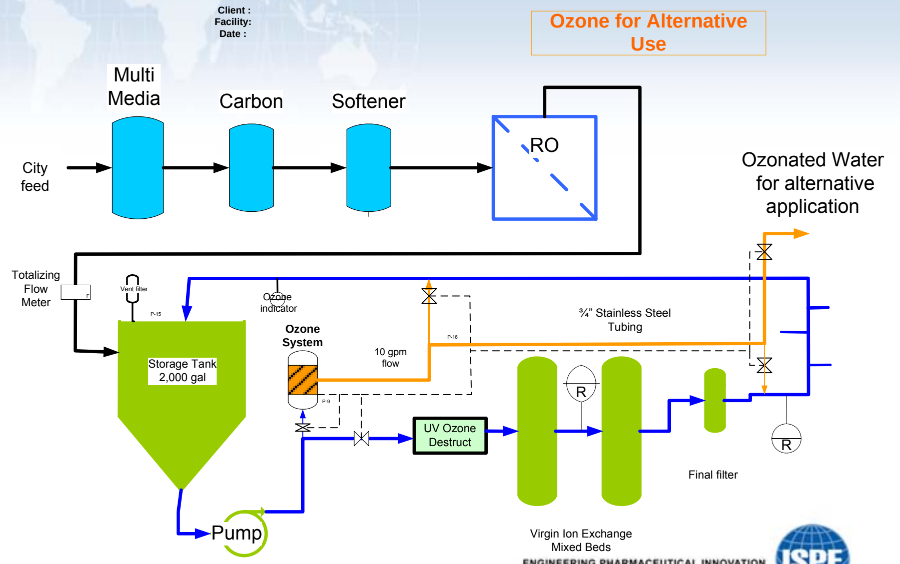
Indirect Ozone Sanitization by TOC Control



Ozonated Water for Alternative Applications

- The ozone system may be used to provide ozonated water for :
- Vivarium water sanitization
- Cage and rack washing
- Process CIP/SIP
- Vessel sanitization and fill
  - No water waste

Ozone for Alternative Use



Water System Design Controls Biofilm

- Properly configured post treatment equipment prevents the inoculation of the distribution loop
- The “High Purity” environment prevents the proliferation of organisms in the distribution piping and points of use

Conclusion

- Controlling Total Organic Carbon (TOC) is essential to a well designed DI water system.
- Ozonation and ozone treatment reduces TOC and bacterium count.
- Ozonation is an effective and economical treatment method.

References

- **The Application of Ozone and its use in Water Systems for Pharmaceutical Production** By **Gary Zoccolante**, Penfield, 7/16/92
- US Filter Watercourse Notes “**High Purity Water Systems for Biopharmaceutical Applications**” by Gary Zoccolante, Technical Director, US Filter, Cambridge MA, 2000
- **Biofilms in Pharmaceutical Waters,** Frank Riedewald**,** Pharmaceutical Engineering November, 1997