



Environmental friendly technologies

## OZONE ENRICHED WATER FOR DISINFECTION OF

### Tanks

### Production Area and Equipment

### Pipelines

### RSW Systems

### Food Washing

### Food Safety

Food safety is of paramount importance in the food processing industry. Because of the incidence of food contamination along the entire chain of production, the need for disinfection in multiple forms and at multiple points is necessary. Growing concerns with chemical use on products and in wastewater, coupled with the need to conserve or reuse water are supporting the need for alternative sanitation technologies. Ozone is emerging as the anti-microbial technology for the 21st century and is well suited to the multiple intervention approach that is being taken today by the food industry to improve sanitation and food safety.

### Ozone enriched water for Surface Sanitation

Ozone is a strong oxidizing agent which makes a major contribution towards a cleaner environment and food safety. Ozone affects microbial membranes and denatures metabolic enzymes. Ozone-enriched water kills microbes effectively and is an effective biocide against:

- Bio - Film
- Bacteria
- Viruses
- Fungi ( yeast, mold and their spores )
- Protozoa ( including cysts )

Ozone- enriched water can be sprayed directly on floors, drains, walls, wet-table equipment, tanks & piping (externally or internally) and clean rooms via a mobile or centralized system with handheld, dropdown or low pressure sprayers.

Based on industry experience, the amount of time necessary to attain sanitation depends on the amount of debris found in the area to be sanitized. A general cleaning to remove heavy surface dirt is necessary to expedite the ozone sanitation process. Time estimates for proper sanitation are difficult to predict, therefore Normex recommends that the end-user uses their own sanitation efficacy testing protocol to determine necessary spray time.



## Antimicrobial Action

- Causes irreversible damage to the fatty acids in the cell membrane (e.g. phosphatidylethanolamine) and cellular macromolecules, e.g. DNA.
- Biphasic death curve: an initial rapid inactivation stage followed by a slower inactivation stage.
- 3000 times faster than chlorine
- Bactericidal effect on Salmonellae, Staphylococcus, E. coli, etc.
- Rapid and effective sporicide (Bacillus and Clostridium spores)

## Antimicrobial Efficacy Results\*\*

Organism	Applied O <sub>3</sub> Dose	O <sub>3</sub> Dose at Nozzle (ppm)	Exposure Time (min)	Reduction
Trichophyton mentagrophytes ( ATCC 9533 )	3.0 ppm	1.85-2.25	3	6 log ( 99.9999 % )
Salmonella choleraesuis ( ATCC 10708 )	3.0 ppm	1.85-2.25	10	6 log ( 99.9999 % )
Staphylococcus aureus ( ATCC 6538 )	3.0 ppm	1.85-2.25	5	6 log ( 99.9999 % )
Pseudomonas aeruginosas ( ATCC 15442 )	3.0 ppm	1.85-2.25	30 sec	6 log ( 99.9999 % )
Campylobacter jejuni ( ATCC 33250 )	3.0 ppm	1.85-2.25	3	4 log ( 99.99 % )
Listeria monocytogenes ( ATCC 7644 )	3.0 ppm	1.85-2.25	3	4 log ( 99.99 % )
Aspergillus flavus ( ATCC 9296 )	3.0 ppm	1.85-2.25	5	4 log ( 99.99 % )
Brettanomyces bruxellensis ( ATCC 10560 )	3.0 ppm	1.85-2.25	3	4 log ( 99.99 % )
Escherichia coli * ( ATCC 11229 )	3.0 ppm	2.1	30 sec	5 log ( 99.999 % )

Efficacy studies were conducted by the National Sanitation Foundation (NSF) according to: \*\*AOAC Official Method 961.02; Germicidal Spray Products as Disinfectants Test and \*AOAC Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants.

## Internal Sanitation of Tanks & Piping Systems

For internal sanitation ozone-enriched water is directly injected into a facility's fluid distribution network and circulated for a set duration of time. Overall chemical costs are reduced or eliminated, and overall system deterioration is reduced when using ozone enriched water rather than traditional anti microbial chemicals or hot water.

### Benefits

Ozone enriched water has the benefit of replacing the chemicals and hot water typically used in cleaning the inside of pipelines, tanks and external surfaces. The benefits are:

- Ozone reduces chemical sanitation cost
- Ozone disinfects more powerfully than most chemical disinfectants
- Micro-organisms can not build up an ozone tolerance.
- Ozone used properly cannot endanger the environment

### Other advantages using Ozone enriched water are:

- High environmental profile as ozone leaves no chemicals or residual by-products to spoil product quality



- Ozone management systems eliminates inhalation worries
  
- Ozone is not harmful to the environment as chlorine
- Ozone is safer than chlorine or sulphur dioxide
- Ozone is generated "on-site" and does not have to be purchased or stored
- Ozone does not have to be measured daily
- Ozone at low levels can be measured with a simple ORP meter
- Ozone at high levels can be measured with a dissolved ozone monitor

## Direct Food Contact

Ozone enriched water is in the US and in many other countries used for direct contact on fruits and vegetables, raw and ready to eat meat and poultry, fish and commercial eggs. The benefits of ozone enriched water far outweigh chemical enriched water traditionally used. Testing performed by the National Sanitation Foundation, NSF, (under AOAC Methodology), shows that, ozone dissolved in water can provide a 6 log reduction in as low as 30 sec. (depending on micro organism) the clew lies in the ozone dosage and holding time. Ozone enriched water leaves no chemical residue on your product and actually assist in removing pre-wash residues in some products. Ozone systems can easily be integrated into most existing conveyer lines. Our systems provide low pressure spray, and can be easily integrated into existing wash lines.

## Desinfecta™

The Normex surface sanitation system "Desinfecta™" recirculates ozone enriched sea/freshwater for disinfection purposes. It is designed to charge a given tank of water with ozone for safe and effective disinfection of external or internal surfaces. The water circulates in a tank and ozone is added until it reaches the required ORP value for disinfection.

**Normex** can through its **Desinfecta™** systems deliver ready to use units for sanitation of surfaces or customised units according to sanitation needs to suit the individual project.

## US Government Regulatory Status

**USDA/FSIS – December, 2001** – "The use of ozone on meat and poultry products, including treatment of ready-to-eat meat and poultry products just prior to packaging, is acceptable," and that there are "no labelling issues in regard to treated product."

**FDA/CFSAN** – June 26, 2001- Final Rule published in Federal Register ( 21 CFR Part 173, Docket NO. 00F-1482) " The FDA amends the food additive regulations to provide for the safe use of ozone in gaseous and aqueous phase as an anti-microbial agent on food, including meat and poultry."

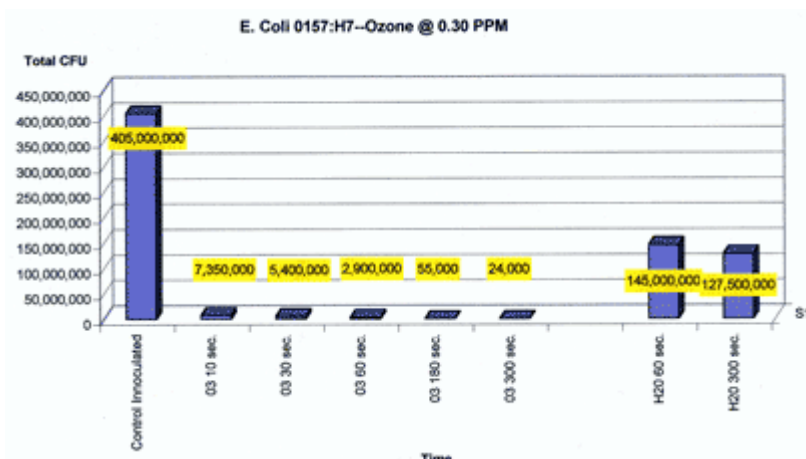
**USDA/AMS** – December, 2000 – Ozone is listed in the National Organic Programme Final Rule (Subsection 205.605 (b) (20) – Non-agricultural (non organic) substances allowed as ingredients in or on processed products labelled as" organic" or "made with organic (specified ingredients or food group(s))".

**FDA** – November 5, 1982 – Final rule published – O3 declared GRAS for treatment of bottled water, (2) CFR §184.1563)

# Cal Poly State University Ozone Research Results Summary

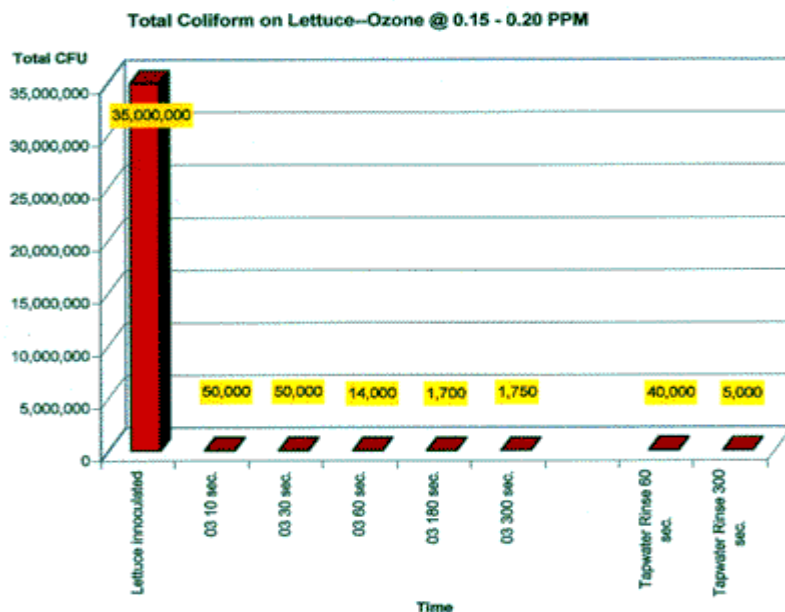
## E.Coli 0157:H7 on Lettuce

Microorganism	E. Coli 0157:H7
Medium	Lettuce
Ozone Concentration in Solution	0.30 PPM
Reduction	4-Log Cycle Average at 180 Seconds
Percent Reduction	99.9999672% @ 0.30 PPM Ozone



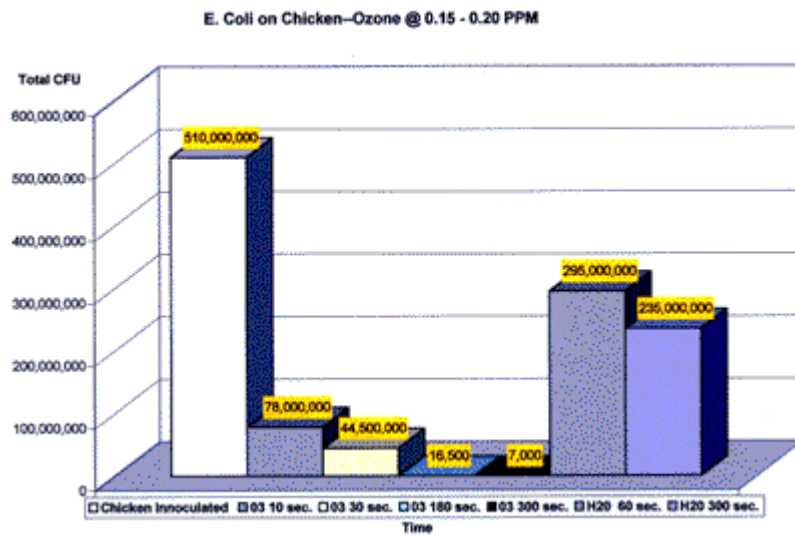
## Total Coliform on Lettuce

Microorganism	Total Coliforms
Medium	Lettuce
Ozone Concentration in Solution	0.15 - 0.20 PPM
Reduction	3 to 4-Log Cycle Average at 180 Seconds
Percent Reduction	99.999992% @ 0.15 PPM Ozone



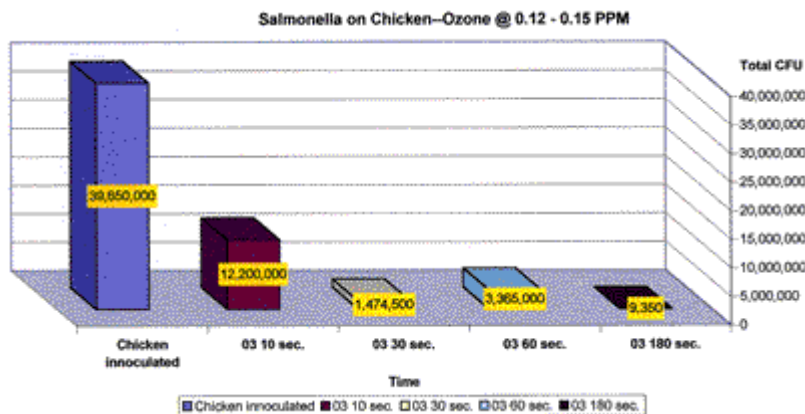
### E. Coli on Chicken

Microorganism	<b>E. Coli</b>
Medium	<b>Chicken</b>
Ozone Concentration in Solution	<b>0.15 - 0.20 PPM</b>
Reduction	<b>4-Log Cycle Average at 180 Seconds</b>
Percent Reduction	<b>99.99993% @ 0.15 PPM Ozone</b>

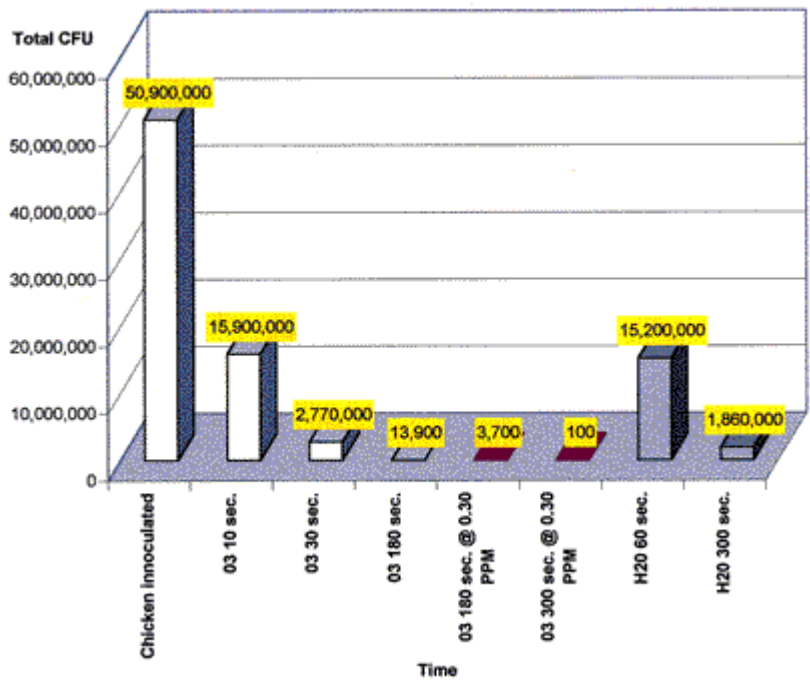


### Salmonella on Chicken

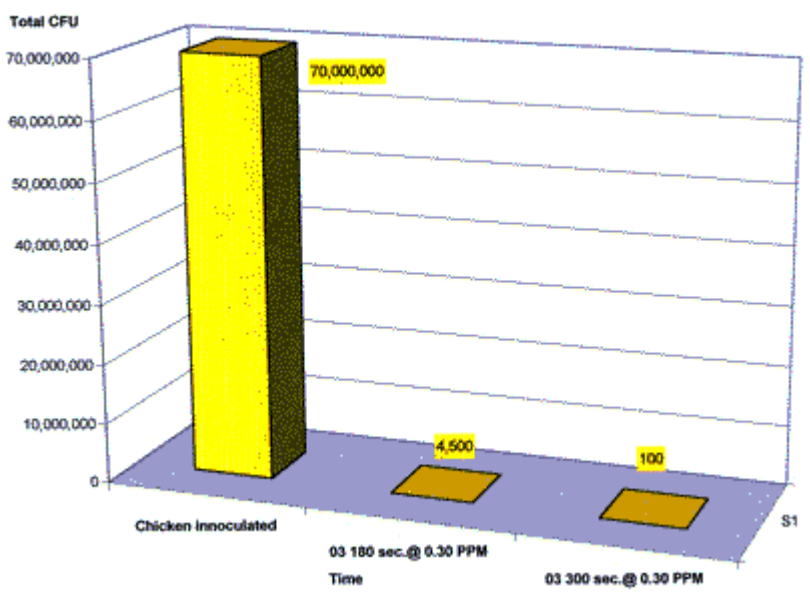
Microorganism	<b>Salmonella</b>
Medium	<b>Chicken</b>
Ozone Concentration in Solution	<b>0.15 - 0.20 PPM and 0.30 PPM</b>
Reduction	<b>3 to 4-Log Cycle Average at 180 Seconds</b>
Percent Reduction	<b>99.999% @ 0.15 PPM Ozone 99.999966% @ 0.3 PPM Ozone</b>



Salmonella on Chicken--Ozone @ 0.12 - 0.15 & 0.30 PPM

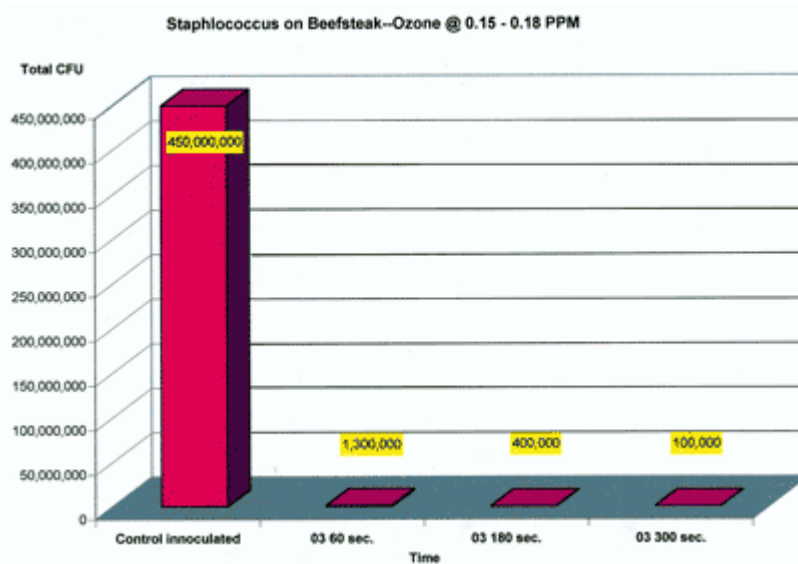


Salmonella on Chicken--Ozone @ 0.30 PPM



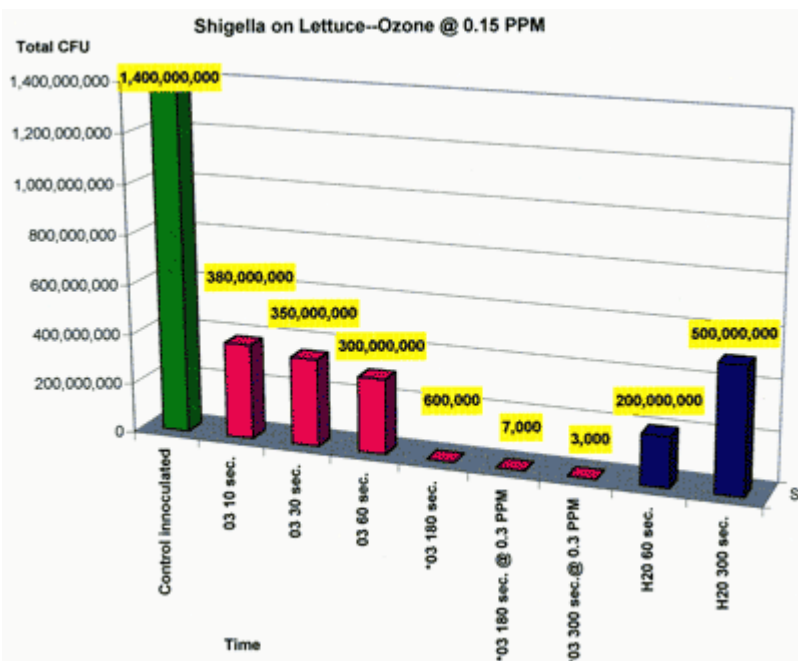
### Staphylococcus on Beefsteak

Microorganism	Staphylococcus
Medium	Beefsteak
Ozone Concentration in Solution	0.15 PPM
Reduction	3-Log Cycle Average at 180 Seconds
Percent Reduction	99.97778% @ 0.15 PPM Ozone



### Shigella on Lettuce

Microorganism	Shigella
Medium	Lettuce
Ozone Concentration in Solution	0.15 and 0.30 PPM
Reduction	3 to 4-Log Cycle Average at 180 Seconds
Percent Reduction	99.957% @ 0.15 PPM Ozone 99.9995% @ 0.3 PPM Ozone



# Use of ozone in food industries for reducing the environmental impact of cleaning and disinfection activities

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## Introduction

The interest in ozone as an alternative to chlorine and other chemical disinfectants in cleaning and disinfection operations is based on its high biocidal efficacy, wide antimicrobial spectrum, absence of by-products that are detrimental to health and the ability to generate it on demand, 'in situ', without needing to store it for later use.

It also has the significant advantage of being an environmentally friendly technology that reduces the company's environmental costs and facilitates their compliance with statutory obligations.

This advantage is usually underestimated by food companies, but the new environmental legislation emerging in Europe, especially the IPPC Directive 96/61/EC, will drive a change in the food industry in the next years that will increase the interest in the use of ozone. It should be taken into account that cleaning and disinfection operations are responsible for the greatest environmental impacts (water and energy consumption, wastewater, etc.) in a number of food processing plants.

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The Spanish technological centre *ainia* is the leader of the OZONECIP project which has been recently funded by the EU LIFE Programme (LIFE 05 ENV/E/000251). This project will not only evaluate the use of ozone as a powerful disinfectant for machinery and equipment, and sanitisable surfaces in general, but also analyse the environmental advantages of ozone and its potential consideration as a Best Available Technology (BAT) for cleaning and disinfection in food processing plants.

## Ozone as a disinfectant agent

Effect of the medium on the bactericidal efficacy of ozone

Ozone effectiveness against micro-organisms depends not only on the amount applied, but also on the residual ozone in the medium. Residual ozone is the concentration of ozone that can be detected in the medium after application to the target surface. Both the instability of ozone under certain conditions and the presence of ozone-consuming materials affect the level of residual ozone available in the medium. It is important, therefore, to distinguish between the concentration of applied ozone and residual ozone necessary for effective disinfection. It is advisable to monitor ozone availability during treatment.

Pure water has the lowest ozone demand. Impurities react with and consume the applied ozone. Depending on the type of substance, the demand will be greater or less. For example, according to one study, the residual ozone in ozonated water containing 20 ppm of Bovine Serum Albumin (BSA) was significantly lower than in deionised water or water with 20 ppm of soluble starch. As a result, the biocidal efficacy of ozone was not affected by the starch but was significantly reduced by the BSA (Restaino, Frampton, Hemphill, & Palnikar, 1995).

There is no consensus on the effect of temperature on the biocidal efficacy of ozone. A fall in the temperature of the aqueous medium increases ozone solubility and stability, augmenting its availability in the medium and, consequently, its efficacy. A rise in temperature, on the other hand, increases the proportion of micro-organisms destroyed by disinfectants. Consequently, the simultaneous contribution of these two factors (solubility/stability and reactivity) to ozone efficacy can vary with experimental conditions, making it difficult to predict the influence of temperature on a particular application.

High relative humidity is required for micro-organisms to be inactivated by ozone gas. The optimum level is



90–95% RH, below 50% the bactericidal effect disappears (Kuprianoff, 1953).

#### Sensitivity of microbes to ozone

Ozone is a powerful broad-spectrum antimicrobial agent that is active against bacteria, fungi, viruses, protozoa, and bacterial and fungal spores (Khadre, Yousef, & Kim, 2001). Inactivation by ozone is a complex process that attacks various cell membrane and wall constituents (e.g. unsaturated fats) and cell content constituents (e.g. enzymes and nucleic acids). Both molecular ozone and the free radicals produced by its breakdown play a part in this inactivation mechanism but there is no consensus on which of them is more decisive. The micro-organism is killed by cell envelope disruption or disintegration leading to leakage of the cell contents. Disruption or lysis is a faster inactivation mechanism than that of other disinfectants which require the disinfectant agent to permeate through the cell membrane in order to be effective.

As regards the spectrum of action, each micro-organism has an inherent sensitivity to ozone. Bacteria are more sensitive than yeasts and fungi. Gram-positive bacteria are more sensitive to ozone than Gram-negative organisms and spores are more resistant than vegetative cells.

Some bacteria have innate chlorine resistance, including bacterial spores and *Cryptosporidium* (Holah, 2003). Micro-organism resistance to other disinfectants have also been observed, though at concentrations significantly below in-use concentrations such as that of *Listeria monocytogenes* to quaternary ammonium sanitizers (Lemaitre, Echchannaqui, & Michant, 1998).

Due to the mechanism of the ozone action, which destroys the micro-organism through cell lysis, it cannot lead to micro-organism resistance.

#### Tainting

There is growing concern about the residual presence of dangerous by-products from chlorine and other chemicals when used as disinfectants in the food industry (Richardson et al., 1998). If water contains organic nitrogen or free ammonia, chloramines are formed. These cause odours and their possible carcinogenic effect is under study. If small amounts of phenols are present, chlorophenols can form, giving the water medicinal odours and tastes. Trihalomethanes (THM), which are potentially carcinogenic, also appear in drinking water that has been chlorinated. This is one of the reasons for the food industry's interest in finding alternative disinfectants that do not cause this problem. Ozone is of great interest as a disinfectant, as it breaks down quickly into oxygen without leaving residues. When it reacts with organic compounds the possible breakdown by-products are aldehydes, ketones or carboxylic acids, which do not present health problems.

#### On-site production

Ozone is generated on site and does not need to be stored for later use. The ozone production method most

commonly used in commercial equipment is corona discharge. This method can employ dry air, oxygen, or a combination of the two. Ozone is generated by passing the feed gas between two closely spaced electrodes (one of which is coated with a dielectric material) under a 10 kV current. A discharge occurs when the gas becomes partially ionised, resulting in a characteristic violet glow when air is used as the feed gas (if oxygen is used the violet color is seldom observed) (EPRI, 2000).

#### Toxicity

The toxicity of ozone varies, depending on its concentration and the length of exposure. Symptoms resulting from exposure to ozone at 0.1–1.0 ppm include headaches, dry throat, irritation to the respiratory system and smarting eyes. Exposure to 1.0–100 ppm can cause asthma-like symptoms such as tiredness and loss of appetite. Short exposure times at high concentrations can cause throat irritation, haemorrhage and pulmonary congestion.

In the United States, the current permissible level for ozone exposure in the workplace environment is 0.1 ppm, as adopted by the Occupational Safety and Health Administration (OSHA). This is the concentration at which a susceptible individual may be continuously exposed to ozone under normal working conditions for 8 h a day or 40 h a week without adverse effects. The short-term exposure limit is 0.3 ppm: short-term means exposure for less than 15 min not more than 4 times a day, with intervals of at least 1 h between each short-term exposure (Prior and Rice, 2000).

Ozone is, therefore, a toxic gas that must be monitored in the workplace when it is used to disinfect equipment and installations. Nowadays, a wide variety of ozone sensors are commercially available to monitor levels in the working environment. They are usually UV analysers, equipped with a cell that measures concentrations from 0.1 to 100 ppm v/v, that trigger an alarm as soon as the ozone concentration rises above 0.1 ppm.

Safety aspects must always be taken into account, particularly when ozone is used in gas form in cold stores, rooms or closed spaces. In these situations, concentrations must be precisely monitored at different critical points and appropriate safety intervals before opening must be established in order to avoid personal health risks.

When ozone is dissolved in water for use as a disinfectant it is accompanied by excess undissolved gas, as no ozone transfer system is 100% efficient. The excess ozone must therefore be destroyed or converted back into oxygen before being released into the atmosphere. Small heated catalyst ozone scrubbers are usually installed for this purpose.

#### Interaction with materials

Ozone interaction with the equipment and surfaces to be cleaned and disinfected is a key factor that must be taken into consideration, essentially because of the corrosion it may cause, but also because the ozone loses its effectiveness.

Table 1. Summary of studies of surface disinfection using ozone				
Application	Treatment	Micro-organism	Results	Author, year
Dairy biofilms on stainless steel surface	Ozonated water, 0.5 ppm for 10 min	<i>Pseudomonas fluorescens</i> and <i>Alcaligenes faecalis</i>	5.6 and 4.4 log reduction, respectively	Greene, Few, Joao, & Serafini, 1993
CIP system	Ozonated water	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> and <i>Candida albicans</i>	99% microbial count reduction	Lagrange, Reiprich, & Hoffmann, 2004
Mixing kettle, table top and shroud (all stainless steel)	Ozonated water, 2 ppm at 10 gpm for 1 min	Unspecified	Microbial plate count reduction ranging from 63.1 to 99.9% (depending on surface)	Hampson, 2000
'High-traffic' and 'low-traffic' floor areas	Ozonated water, 2 ppm at 10 gpm for 1 min	Unspecified	Microbial plate count reductions 67.0–95.6% and 84.3–99.9%, respectively	Hampson, 2000
Plastic shipping container	Ozonated water, 2 ppm at 10 gpm for 1 min	Unspecified	Microbial bioluminescence assay reduction 68.8–97.4%	Hampson, 2000
Stainless steel surfaces	2 ppm ozone gas at atmospheric pressure, 22 °C and 77% HR for 4 h	<i>Escherichia coli</i> , <i>Serratia liquefaciens</i> , <i>Staphylococcus aureus</i> , <i>Listeria innocua</i> and <i>Rhodotorula rubra</i>	Reduction ranging from 7.56 to 2.41 log values	Moore, Griffith, & Peters, 2000
Stainless steel surfaces in the presence of UHT milk	2 ppm ozone gas at atmospheric pressure, 22 °C and 77% HR for 4 h	<i>Escherichia coli</i> , <i>Serratia liquefaciens</i> , <i>Staphylococcus aureus</i> , <i>Listeria innocua</i> and <i>Rhodotorula rubra</i>	Reduction ranging from 5.64 to 1.65 log values	Moore et al., 2000
Stainless steel surfaces	2 ppm ozone gas in bioaerosol chamber at 20 °C and 50% HR for 1 h	<i>Micrococcus luteus</i>	2–3 log reduction	Bailey, Young, Fielding, & Griffiths, 2001
Surfaces	2 ppm ozone gas, 2 h exposure	Unknown	2 log reduction	Taylor & Chana, 2000
Equipment, walls, floors, drains, tables and conveyors, previously well-cleaned	Ozonated water, 3.0–3.5 ppm	<i>Trichophyton mentagrophytes</i> , <i>Salmonella choleraesuis</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Campylobacter jejuni</i> , <i>Listeria monocytogenes</i> , <i>Aspergillus flavus</i> , <i>Brettanomyces bruxellensis</i> , <i>Escherichia coli</i>	Log reduction ranging from 6 to 4	Boisrobert, 2002

of wastewater is cleaning water. This is used for equipment cleaning, e.g. line purging at product change-over, start-up, shut-down and change-over of HTST pasteurisation units as well as some product washing.

Breweries use significant amounts of water and energy and produce wastewater and solid residues. The typical consumption levels of fresh water and emission levels of wastewater for German breweries are 3.7–4.7 hl/hl beer sold and 2.2–3.3 hl/hl beer sold, respectively [Germany BAT Reference Document, 2002].

Suspended solids in the wastewater originate from the discharge of by-products, diatomaceous earth, e.g. kieselguhr, and possible label pulp from the bottle cleaner. Nitrogen originates mainly from detergents used for tank cleaning, from the malt and from additives. Phosphorus may come from the cleaning agents used. Large variations in pH may occur due

to the use of acids and caustic for the cleaning of process equipment and returnable bottles. Heavy metals are normally present in very low concentrations. Wear of the machines, especially conveyors in packaging lines, may be the source of nickel and chromium.

In the wine industry, wastewater is generated in nearly all process steps, e.g. cleaning of containers, reactors and filters. The highest concentrated wastewater is produced during fermentation, fining and ageing/racking due to the washing out of the sediments, marcs and lees. The semi-solid fractions can be separated for further dewatering, drying, processing or disposal rather than being washed with water, due to their high organic load.

Wine bottles are cleaned before filling, and consequently washing water enters the wastewater treatment plant or is recycled. Even after the recovery process, the wastewater

shows an acidic character (pH 4–6) except when caustic solutions are used in the elimination of tartrate or during the conditioning of bottles. The most polluting wastewater during wine production is generated during the fermentation and racking (especially first racking) operations.

Adopting ozone in cleaning and disinfection processes can bring various advantages over commonly employed disinfectants. Ozone breaks down quickly into oxygen without leaving undesirable residues. This is an advantage both from the point of view of food safety and to improve the quality of wastewaters by avoiding the presence of harmful chlorine compounds. Replacing chemical products with ozone also lowers the concentration of salts and, therefore, the electrical conductivity of discharges.

The use of ozone can save water in comparison to other biocides, as it is faster-acting. Additionally, since it does not leave residues it does not require a final rinse to remove any residual disinfectant that might remain in the treated medium.

Another advantage, provided adequate microbiological controls are implemented, is that the ozonated water that has been used for disinfection can potentially be re-used for the initial cleaning stages, either directly or after re-ozonation to attain the required quality.

Wastewaters are oxygenated by ozone conversion, so ozone use will improve the performance of aeration tanks and biological wastewater treatment processes. This is also an advantage from the point of view of reducing odour generation.

Ozone use also provides energy savings as it is normally used at low temperatures. Finally, as it is generated “on

rack rinse can be re-ozonated and used for the first rinse, reducing water usage and disposal costs. Before installing ozone, the company kept a large stock of 30% sodium hypochlorite. Throughout the day, 100 ppm chlorine rinses were used on equipment surfaces. Now, water containing 1 ppm ozone is used. The total cost of the system was \$ 73,800, but it has already reduced the \$ 9000 per quarter expenditure on hypochlorite.



CIP systems using ozonated water. (courtesy of Del Ozone)

Rice, Graham, and Lowe (2002) described a financial study conducted in an American food plant (20 processing lines operating 24 h a day, 300 days a year) that introduced a mobile ozonated water disinfection system. The new system enabled the processor to cut the previous four disinfection steps to only two and thereby reduce water use from 56.8 m<sup>3</sup> to 22.7 m<sup>3</sup>. The following table shows how annual savings of \$ 18,981 were achieved.

	Cost of chemical products (\$/year)	Wastewater discharges (m <sup>3</sup> /day)	Discharge tax (\$/m <sup>3</sup> )	Monthly discharge tax (\$)	Annual discharge tax (\$)	Annual total (\$)
Without ozone	6000	56.775	12,702	1802	21,635	27,635
With ozone	0	22.710	12,702	721	8654	8654
Total annual saving						18,981

the spot”, ozone removes the need to store hazardous substances which could give rise to accidents that endanger human and environmental health and safety.

### Examples at industrial scale

Plumrose USA Inc. employs ozonated water for sanitising work areas and for processing equipment used for slicing and packaging ham, turkey, chicken and other meats. The company has a centralised system that produces ozonated water on demand (28 g ozone/h) and delivers it automatically to the work areas through closed piping under low pressure. As well as using ozonated water to sanitise plastic tubs and stainless steel walk-in coolers, the company also uses ozone instead of chlorine to rinse its stainless steel transportation racks in a three-stage process. Since ozone breaks down into oxygen, ozonated water from the final

Ozone-based disinfection methods usually entail higher investment costs than methods based on other chemical disinfectant products. However, their running costs are very low as they only consume a moderate quantity of electricity. As the above example shows the savings on water, discharge taxes and chemical products can quickly recoup the higher initial outlay.

### Wineries

In Australia, ozone is being used successfully on an industrial scale as an alternative to chlorine for disinfecting the oak barrels used for ageing wine. The main advantage that is stimulating a growing interest in the use of ozone is that it is more effective for controlling certain *Brettanomyces* yeast species that cause off-tastes and other defects in wines (Day, 2004).

The corrosive effect of ozone depends on the concentration employed. At high concentrations it may corrode equipment, but such high concentrations only occur within the ozone generator or in the system that injects the ozone into the water. Most materials are compatible with ozone at moderate concentrations of 1–3 ppm ozone.

The plastics most frequently employed in the food industry perform well in the presence of ozone and their resistance to corrosion by ozone is considered good or excellent: PTFE (Teflon), PVDF (Kynar), PVC (rigid and flexible) and ECTFE (Halar) are mentioned in various publications.

Other materials that show resistance are 316L and 304L stainless steel, particularly the former, which stands up better to corrosion by ozone than by chlorine according to some authors (Green, Smith, Knight, 1999; Singh & Singh, 1999).

However, natural rubber is highly sensitive to contact with ozone, leading to total disintegration (Kim, Yousef, & Khadre, 2003). Silicone is resistant in the short-term but oxidises on extended exposure to ozone. Consequently, it is good practice to identify all the materials that could come into contact with ozone and check their potential resistance.

### Use of ozone to clean and disinfect surfaces and equipment

Ozone can be applied both as a gas and in ozonated water. Several studies have examined its efficacy by testing different treatments on various surfaces and micro-organisms and a number of these are listed in Table 1.

From the table it can be seen that moderate doses of ozone, between 0.5 ppm and 3.5 ppm, both in gas form and as ozonated water, are sufficient to achieve significant microbial reductions. These concentrations are potentially compatible with most plastic materials and certain types of stainless steel used in food sector plants.

When ozone is applied as a gas, the necessary exposure times are considerably longer (1–4 h) than for application in ozonated water (1–10 min). Theoretically, increasing the relative humidity of the space where it is applied might increase the efficiency of gaseous ozone, thus shortening exposure times.

Some recent studies have examined new methods of applying ozone by fogging ozonated water and charging it electrostatically to increase the effectiveness of this technique on vertical surfaces and undersides (Birks, 2003).

In practice, a custom-tailored study is recommended in order to design a safe, efficient cleaning and disinfection programme. This study should include an analysis of the surfaces to be treated, the best way to apply the ozone, the dose to be applied and the residual ozone level to be achieved in the medium, exposure time, microbiological analyses, etc. The cost of these studies as well as the capital cost must be taken into account, and may prove

a commercial obstacle to these methods which are still relatively unfamiliar in the sector.

The investment costs for ozonation systems are usually higher than for chlorine or other chemical products, but running costs are very low as the only requirement is the electricity to produce the ozone. Ozone disinfection methods also save water and energy, as well as wastewater treatment costs and discharge taxes.

In recent years, particularly in the United States, the introduction of ozonation equipment for food industry surface cleaning and disinfection has made significant advances. One factor that has undoubtedly boosted this advance is the recent FDA approval for ozone use in food treatment, storage and processing.

A significant point in its progress is the recent launch of the first commercial models. These are compact systems (fixed or mobile) that spray ozonated water onto open surfaces or recirculate it through CIP systems. They have received NSF recognition after passing biocidal efficacy tests using official methods.

### Environmental impact of cleaning and disinfection: potential advantages of ozone

Cleaning and disinfection are essential to maintain hygienic conditions in food processing plants. However, high water and energy use and the generation of wastewaters have a significant environmental impact.

Large quantities of water are required for cleaning and disinfection in the food industry. The wastewater profile is largely dependent on production and cleaning patterns. Wine, beer and dairy processing plants installations use considerable amounts of water with the amount depending on the type and size of equipment to be cleaned and the materials processed; 60–80% of the total water consumption is used for cleaning activities.

Cleaning and disinfection produces wastewater. This typically contains soluble organic material, FOG, SS, nitrate, nitrite, ammonia and phosphate from product remnants and removed deposited soil. It also contains residues of cleaning agents, e.g. acid or alkali solutions. In principle, the cleaning and disinfection agents that are used are discharged via the wastewater, either in their original state or as reaction products.

Wastewater may have a high or low pH due to the use of acid and alkaline cleaning solutions. The use of phosphoric and nitric acids will increase the phosphate and nitrate content of the wastewater. Badly designed systems and inadequate product removal prior to the start of cleaning may lead to large quantities of product entering the cleaning water.

Wastewater is the main environmental issue in the dairy sector (0.9–25 m<sup>3</sup>/t processed milk [European Dairy Association, 2002]). The sector uses a vast amount of water, and generates a huge amount of wastewater in maintaining the required level of hygiene and cleanliness (between 25 and 40% of the total water consumed). The largest proportion

A further, no less important advantage is that changing to ozone disinfection avoids the presence of substances such as Trichloroanisol (TCA), which is responsible for cork taint problems in many wines (Franson, 2004). Ozone is also considered to provide cost-savings as it reduces the need to buy and store chlorine.

Ozone is increasingly used in Australian wineries and various ozonation system suppliers are already marketing equipment specifically designed for this application. As some wine-makers have found, ozone doses that are effective to control microbes in the barrels do not affect the quality of the wine.

The system for cleaning and disinfecting the barrels with ozone consists of two stages. The first stage uses high pressure hot water to dissolve the tartrates and blast the barrels clean. The second is a cool rinse with ozonated water which sanitises and shrinks the pores in the oak and cools the barrels. Three factors affect how long the treatment with ozonated water should last: the type and age of the barrel, its microbial load and the concentration of ozone in the treatment water, which is typically 2.0–2.5 ppm for the equipment used in these cellars.

The use of ozone in the wine business is not confined to oak barrels. After cleaning, ozonated water is sprayed directly onto floors, sumps, walls, the inside and outside of tanks, fruit bins and other wettable surfaces in the winery.

It is also used for the disinfection stage in CIP system installations. The ozonated water is recirculated around the equipment using a closed loop of pipe or hose. The ozone is used up as it reacts with the organic matter in the equipment. When ozone is again detected in the water leaving the equipment this means that all the organic matter has been oxidised. To ensure that sterility has been achieved, ozonated water is usually left to recirculate for a few minutes longer.

Other disinfection-related wine industry applications of ozone that are emerging are: treating well water to remove micro-organisms, organic matter, iron and manganese; using ozone gas to replace SO<sub>2</sub> in barrel storage; bottle washing as in other drink industries; or treating wastewater.

### Equipment for ozone disinfection

Currently, three ozonation equipment suppliers have already received National Sanitation Foundation (NSF) registration of their systems for disinfecting surfaces with ozonated water. They are all identified in the NSF White Book™ Listing of Proprietary Substances and Nonfood Compounds (<http://www.nsf.org/usda>). As a result of this registration, food processors can consider these systems as 'USDA approved' for sanitation of food-contact and nonfood-contact surfaces. This is particularly important as it enables plants operating under the USDA poultry, meat, shell egg and egg products inspection programmes to introduce this application of ozone.

Boisrobert (2002), explains the features of two NSF-registered ozonated water surface sanitation systems and

gives the microbiological results of antimicrobial efficacy tests performed by the Toxicology Group, LLC, a division of NSF.

One of the models is a mobile system that provides a 10-gpm water spray with a 3.0–3.5 ppm ozone dose. It is designed to sanitise equipment, walls, floors, drains, tables, conveyors, containers, tanks and barrels. The other, which is also mobile, recirculates ozonated water at 35 gpm with a 3.0 ppm ozone dose through tanks ranging in size from 50 to 2500 gallons. It is designed for CIP and COP (clean-out-of-place) processes.



Mobile ozone surface sanitation system. (Courtesy of Del Ozone)

The methods used for the tests were AOAC Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants; and AOAC Official Method 961.02, Germicidal Spray Products as Disinfectants.

The micro-organisms studied had an ozone dose application of 1.85–2.25 ppm from the nozzle, except for *Escherichia coli* where the ozone dose was 2.1 ppm. The results obtained substantiate the efficacy of these systems for sanitising previously cleaned non-porous surfaces, including processing equipment, which came into contact with food.

Micro-organism	Log reduction
<i>Trichophyton mentagrophytes</i> (ATCC 9533)	6
<i>Salmonella choleraesuis</i> (ATCC 10708)	6
<i>Staphylococcus aureus</i> ATCC 6538	6
<i>Pseudomonas aeruginosa</i> ATCC 15442	6
<i>Campylobacter jejuni</i> (ATCC 33250)	4
<i>Listeria monocytogenes</i> (ATCC 7644)	4
<i>Aspergillus flavus</i> (ATCC 9296)	4
<i>Brettanomyces bruxellensis</i> (ATCC 10560)	4
<i>Escherichia coli</i> (ATCC 11229)	5

### The IPPC Directive and the best available techniques

European environmental legislation is increasingly requiring polluting industries to move to clean technologies. The most important regulation in this respect is the Integrated Pollution Prevention and Control (IPPC) Directive 96/61/EC, which has considerable relevance and far-reaching effects for all European food manufacturers.

The IPPC directive attempts to encourage the Best Available Techniques (BATs). BATs are defined as techniques that enable competitive levels of quality and productivity to be achieved and are noted for their greater environmental efficacy. This could be the case of ozone against other traditional cleaning and disinfection techniques. Therefore, the IPPC Directive could potentially lead to an increased use of ozone in EU countries.

To be considered as a BAT, a technique must be evaluated and environmental benefits associated must be demonstrated. Some EU programmes are promoting the investigation and validation of emerging clean technologies in order to include them in the European Reference Documents on BATs (BREF documents) which are used for the state members to regulate the industrial activities of the affected facilities. The European IPPC Bureau is responsible for organising an exchange of information between Member States and the industries and produces BAT reference documents (BREFs) (see web site <http://eippcb.jrc.es>).

### The LIFE PROJECT OzoneCIP

The “Ozone clean in place in food industries” project (OzoneCIP) has been funded by the EC under the LIFE-Environment Programme (LIFE 05 ENV/E/000251). This project aims to demonstrate the environmental benefits obtained by the use of Clean In Place procedures based on ozone techniques in place of the traditional techniques. Furthermore, as a result of the achievement of environmental indicators, the classification of this technology as a BAT and its widespread knowledge and implementation within the European food processing industries is expected.

Three European R&D centres located in three different EU state members will implement the project: Ainia in Spain, Bionord in Germany and Gdansk University of Technology in Poland. The demonstration activities will focus on dairy, brewery and winery sectors. Three food companies belonging to each of the mentioned sectors will provide their contribution from an industrial point of view. Industrial partners are Domecq bodegas (wine processing), Inbev (beer processing) and Meiere-Genossenschaft e.G. Langernhorn (dairy processing).

A three-year project was started in December 2005. A prototype will be built in Ainia's facilities to enable the simulation of industrial CIP processes and assay processes based on ozone. The results should demonstrate the environmental benefits of ozone as an alternative to traditional chemicals and define environmental indicators to update BAT reference documents. Also, non-environmental factors

that can affect its feasibility at industrial level will be considered.

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