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Microbiological Aspects of Ozone Applications in Food: A Review

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ABSTRACT: Ozone is a powerful antimicrobial agent that is suitable for application in food in the gaseous and aqueous states. Molecular ozone or its decomposition products (for example, hydroxyl radical) inactivate microorganisms rapidly by reacting with intracellular enzymes, nucleic material and components of their cell envelope, spore coats, or viral capsids. Combination of ozone with appropriate initiators (for example, UV or H_2O_2) results in advanced oxidation processes (AOPs) that are potentially effective against the most resistant microorganisms; however, applications of AOPs in food are yet to be developed. When applied to food, ozone is generated on-site and it decomposes quickly, leaving no residues. Ozone is suitable for decontaminating produce, equipment, food-contact surfaces, and processing environment.

Keywords: ozone, mechanism, kinetics, reactivity, efficacy

Introduction

THE FOOD INDUSTRY IS CURRENTLY IN NEED OF INNOvative processing technologies in order to meet consumers' demand of fresher and safer ready-to-eat products. High pressure processing, pulsed electric field, and high intensity pulsed light are some of these emerging technologies. Attention is now focused on ozone as a powerful sanitizer that may meet expectations of the industry, approval of the regulatory agencies, and acceptance of the consumer. Regulatory agencies in the United States have been hesitant in the past to approve the use of ozone for treatment of drinking water and direct food applications. Currently, there are more than 3000 ozone-based water treatment installations all over the world and more than 300 potable water treatment plants in the United States (Rice and others 2000). This widespread application is a clear indication of the efficacy and usefulness of ozone. A petition submitted in August 2000 to the Food and Drug Administration (FDA) for approval of ozone as a direct food additive for the treatment, storage, and processing of foods in gas and aqueous phases has been recently accepted (Federal Register 2001).

Major advantages of ozone made it one of a few top candidate technologies attracting the attention of the food industry. Ozone is one of the most potent sanitizers known. Excess ozone auto-decomposes rapidly to produce oxygen, and thus it leaves no residues in food. The sanitizer is active against all forms of microorganisms at relatively low concentrations. The rapid developments in this field—appearance of a new body of knowledge and potential approval of ozone as a direct food additive by the U.S. government—justify the present review of various aspects of ozone-microorganisms interactions. Food processors who are introducing ozone in their facility and researchers who are exploring the feasibility of ozone use in food processing are in need of relevant and concise information about this sanitizer. This review article should address these needs.

Physicochemical properties of ozone

Ozone (O_3) results from the rearrangement of atoms when oxygen molecules are subjected to high-voltage electric discharge. The product is a bluish gas with pungent odor and strong oxidizing properties (Horvath and others 1985). Physicochemical properties of ozone are closely related to its efficacy, and thus these properties will be discussed.

Solubility of ozone in water

The gas does not appreciably react with water; therefore it forms a true physical solution (Horvath and others 1985). Dissolution of gasses that are partially soluble in water (for example, ozone) follows Henry's law which states that the amount of gas in solution, at a given temperature, is linearly proportional to the partial pressure of the gas. Consequently, saturation concentration (C_s) of a dissolved ozone in water under thermodynamic ideal conditions follows this equation (Bablon and others 1991a).

$$C_s = \beta M \times P_\gamma$$

where C_s: kg O_3/m^3 water; β (absorption coefficient): volume of ozone (expressed at NTP) dissolved per unit volume of water (at a given temperature) in the presence of equilibrating ozone at 1-atm pressure; M: mass volume of ozone, kg/m^3, at NTP (2.14 kg/m^3); P_γ: partial pressure of ozone in the gas phase

Solubility of gasses can be compared if their â values are known. Solubility in water is greater for ozone than for nitrogen and oxygen; â values are 0.64, 0.0235, and 0.049, respectively. Ozone, however, is less soluble in water than are carbon dioxide (β = 1.71) and chlorine (β = 4.54).

Dissolution of ozone in water also can be expressed in a more practical term, the solubility ratio (S_r).

$$S_r = \frac{\text{mg/L } O_3 \text{ in water}}{\text{mg/L } O_3 \text{ in the gas phase}}$$

Solubility ratio for ozone increases as the temperature of water decreases (Bablon and others, 1991a). These authors showed a negative logarithmic relationship between S_r and water temperature in the range of 0.5 °C to 43 °C.

In addition to pressure and temperature, which directly affect the solubility, other parameters practically influence

the dissolution of ozone in water. When a solution is prepared by bubbling ozone in water, smaller bubble sizes result in larger surface area of contact which increases the solubility (Katzenelson and others 1974). According to these authors, an optimum dissolution of ozone in water occurs when bubbles are 1 to 3 mm in dia. The flow rate of ozone and contact time affect the transfer of the gas to water. Appropriate mixing or turbulence increases bubble contact and solubilization in water (Katzenelson and others 1974). Design of ozone-water contactors, in general, greatly affects the rate of solubilization (Schulz and Bellamy 2000).

Purity and pH of water greatly affect the rate of ozone solubilization. J-G Kim (1998) bubbled gaseous ozone (1 mM) into double distilled, deionized or tap (from two sources) water. Ozone gas dissolved faster in deionized and distilled water than in tap water. Higher maximum ozone concentration was also obtained in the water from the former two sources. The pH values, measured before ozonation, were 5.6 and 5.9 for deionized and distilled water, respectively, and 8.23 and 8.39 for tap water from the two sources. The high pH of tap water may have destabilized ozone, and thus the apparent rate of solubilization decreased. In addition, tap water may contain organic matter that consumes ozone. Presence of minerals in water may also catalyze ozone decomposition (Hoigné and Bader 1985). Therefore, solubility of ozone increases when purity of water increases.

Stability of ozone

Ozone is relatively unstable in aqueous solutions. It decomposes continuously, but slowly, to oxygen according to a pseudo first-order reaction (Tomiyasu and others 1985). The half-life of ozone in distilled water at 20 °C is generally considered to be 20 to 30 min. However, Wynn and others (1973) found that ozone has a half-life of 165 min in distilled water at 20 °C and Wickramanayake (1984) reported a shorter half-life (2 to 4 min) in aqueous solution at pH 7.0 and 25 °C. Wickramanayake (1984) attributed this short half-life to the mechanical stirring that kept the reactor's contents completely mixed.

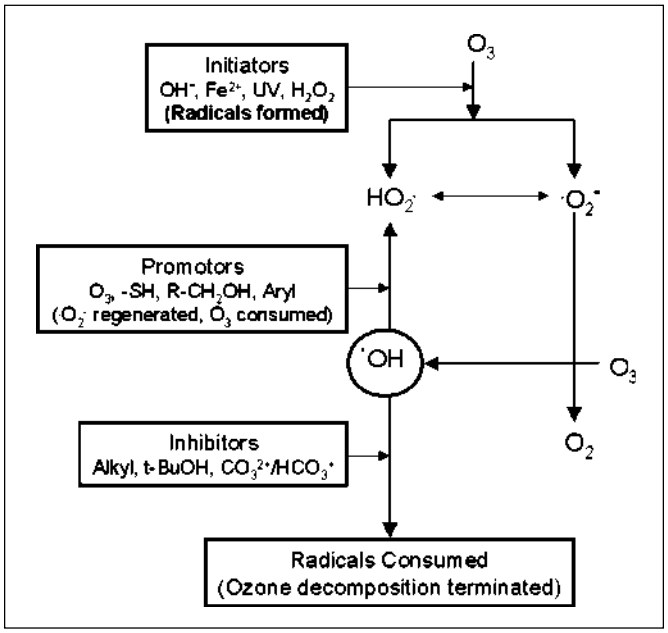

Figure 1—Ozone decomposition reactions. (O_3) ozone. ($\cdot O_2^-$) superoxide radical ion; ($HO_2\cdot$) hydroperoxide radical, ($\cdot OH$) hydroxyl radical.

The pH greatly affects the stability of ozone in aqueous solutions. J-G Kim (1998) added ozonated water, having different concentrations, into phosphate buffers (0.01 M) with pH 5.0 to 9.0, mixed for 15 s and measured the concentration of ozone using the indigo method. Stability of ozone in solution was the greatest when pH was 5.0. Ozone stability decreased as pH increased, and no ozone was detected in buffers with pH 9.0.

Decomposition of ozone follows first-order kinetics with respect to both ozone molecule and hydroxide ion.

$$-d[O_3]/dt = k[O_3][OH^-]$$

According to Staehelin and Hoigné (1985), decomposition of ozone includes initiation, promotion, and inhibition reactions (Figure 1).

(1) Initiation is the rate-limiting step which leads to formation of free radicals; these are the superoxide radical ion ($\cdot O_2^-$) and its hydrogenated form, the hydroperoxide radical ($HO_2\cdot$).

$$O_3 + OH^- \xrightarrow{k = 70\ mole^{-1}\ sec^{-1}} HO\cdot_2 + \cdot O_2^-$$

$$\cdot O_2^- + H^+ \quad [pK_a = 4.8]$$

Formation of these radicals will lead to the generation of the highly reactive hydroxyl radical ($\cdot OH$) and consumption of an ozone molecule (Figure 1). The ozonide radical ion ($\cdot O_3^-$) is formed as an intermediate reaction product. Factors that enhance this stage of ozone decomposition (initiators) include hydroxyl ions, some cations such as Fe^{2+}, organic compounds such as glyoxylic acids, and ultraviolet radiation (UV) at 253.7 nm.

(2) Promotion reactions regenerate the hydroperoxide and superoxide radicals, as shown in the following example.

$$O_3 + \cdot OH \xrightarrow{k = 2.0 \times 10^9\ mole^{-1}\ sec^{-1}} HO\cdot_4$$

$$\cdot O_2^- + H^+ \rightleftharpoons HO\cdot_2 + O_2 \quad (k = 2.8 \times 10^4\ sec^{-1})$$

Promotors include formic acid, glyoxylic acids, primary alcohols, and aryl groups.

(3) Inhibition refers to reactions leading to consumption of hydroxyl radical without regenerating the superoxide radical ion

$$\cdot OH + HCO_3^- \xrightarrow{k = 4.2 \times 10^8\ mole^{-1}\ sec^{-1}} OH^- + HCO\cdot_3$$

Inhibitors include bicarbonate, carbonate, tertiary alcohols, and alkyl groups.

In practical terms, stability of ozone in aqueous solutions depends on the source of water. Water used in food processing or drinking usually contains readily oxidizable organic and inorganic substances. These substances may react rapidly with ozone, considerably decreasing its half-life. J-G Kim (1998) bubbled ozone in distilled, deionized, HPLC-grade and tap water from two sources, and phosphate buffer (0.5M, pH 7) to attain 0.10 to 0.15 absorbance at 258 nm

(A_{258}). Ozone decomposition rate was monitored during storage at 25 °C for 8 min. Concentration of ozone decreased during storage, but rates of decrease were greater in buffer and tap water than in distilled, deionized, and HPLC-grade water. These data indicate that ozone degrades faster in buffer and tap water than in purer water. It is apparent that high pH and presence of ozone-demand materials enhance decomposition of ozone.

Reactivity of ozone

The ozone molecule acts as dipole with electrophilic and nucleophilic properties. Organic and inorganic compounds in aqueous solutions react with ozone in one of two pathways (Staehelin and Hoigné 1985):

(a) Direct reaction of organic compound (M) with molecular ozone.

$$O_3 + M \longrightarrow M_{ox}$$

(b) Decomposition of ozone in water into a radical (for example, OH) which reacts with the compound (M).

$$O_3 \xrightarrow{OH^-} OH \xrightarrow{M} M_{ox}$$

Molecular ozone reactions are selective and limited to unsaturated aromatic and aliphatic compounds. Ozone oxidizes these compounds through cycle-addition to double bonds (Bablon and others 1991a). Oxidation of sulfhydryl groups, which are abundant in microbial enzymes, may explain rapid inactivation of microorganisms and bacterial spores by ozone.

Ozone reacts with polysaccharides slowly, leading to breakage of glycosidic bonds and formation of aliphatic acids and aldehydes (Bablon and others 1991a). Reaction of ozone with primary and secondary aliphatic alcohols may lead to formation of hydroxy-hydroperoxides, precursors to hydroxyl radicals, which in turn react strongly with the hydrocarbons (Anbar and Neta 1967). Perez and others (1995) showed that N-acetyl glucosamine, a compound present in the peptidoglycan of bacterial cell walls and in viral capsids, was resistant to the action of ozone in aqueous solution at pH 3 to 7. Glucosamine reacted relatively fast with ozone, but glucose was relatively resistant to degradation. This observation may explain, at least in part, the higher resistance of gram-positive bacteria compared to gram negative ones; the former contains greater amounts of peptidoglycan in their cell walls. The action of ozone on amino acids and peptides is significant especially at neutral and basic pH. Ozone attacks the nitrogen atom or the R group or both.

Ozone reacts slowly with saturated fatty acids. Unsaturated fatty acids are readily oxidized with ozone and cycle-addition products are formed. Ozone reacts quickly with nucleobases, especially thymine, guanine, and uracil. Reaction of ozone with the nucleotides releases the carbohydrate and phosphate ions (Ishizaki and others 1981).

Factors altering reactivity and antimicrobial efficacy.

A factor such as treatment temperature affects solubility, stability, and reactivity of ozone differently. Consequently, it is difficult to predict the influence of this factor on the efficacy of ozone in real applications. Factors that affect these interrelated parameters simultaneously will be discussed.

Temperature. The rate of destruction of microorganisms by a disinfectant generally increases with increasing temperature. According to the van't Hoff-Arrhenius theory (Fair and others 1968), temperature partly determines the rate at which the disinfectant diffuses through the surfaces of microorganisms and its rate of reaction with the substrate. At constant reagent concentration, increasing the temperature by 10 °C increases the reaction rate with the substrate by a factor of 2 or 3. In the case of ozone, however, as temperature increases ozone becomes less soluble and less stable, but the ozone reaction rate with the substrate increases. As the temperature increased from 0 °C to 30 °C, the rate of inactivating *Giardia* cysts increased (Wickramanayake and others 1984). However, Kinman (1975) reported that when bacteria were treated with ozone at 0 °C to 30 °C, treatment temperature had virtually no effect on the disinfection rate. The researcher related this observation to the decrease in solubility and increase in the decomposition and reactivity of ozone as temperature increases. Achen and Yousef (2001) treated *Escherichia coli*-contaminated apples with ozone at 4, 22, and 45 °C, and observed that counts of the bacterium on the surface decreased 3.3, 3.7, and 3.4 $\log_{10}$-units, respectively. Statistical analysis showed no significant differences among the three treatments ($P > 0.05$). The residual ozone concentration was greatest at the lowest temperature (4 °C) and decreased with increasing temperature. It appears that when treatment temperature increased, the increase in ozone reactivity compensated for the decrease in its stability, and thus no appreciable change in efficacy was observed. On the contrary, J-G Kim (1998) observed that ozone reduced more microbial contaminants when it was applied at higher than the refrigeration temperatures.

pH value. Under constant residual ozone concentrations, the degree of microbial inactivation remained virtually unchanged for pH's in the range of 5.7 to 10.1 (Farooq and others 1977). However, efficacy of ozone seems to decrease at alkaline pH for rotaviruses (Vaughn and others 1987) and poliovirus type 1 (Harakeh and Butler 1985). Ozone is more stable at low than at high pH values, as indicated earlier. Inactivation of microorganisms is mostly through reaction with molecular ozone when the pH is low. Ozone decomposes at high pH values and the resulting radicals contribute to its efficacy. The relative importance of these two inactivation mechanisms may vary with the microorganism and treatment conditions (for example, presence of ozone-demanding contaminants).

Ozone-consuming compounds. Presence of organic substances with high ozone demand may compete with microorganisms for ozone. Viruses and bacteria associated with cells, cell debris, or feces are resistant to ozone, but purified viruses are readily inactivated with the sanitizer (Emerson and others 1982). Similar results have been found in our laboratory for ozone inactivation of rotavirus in suspension comparative to 1-h adsorbed virus to the MA 104 cell monolayers (Khadre and Yousef 2001c). Hence, the presence of organic matter in water intended for use in ozone-associated food processing is highly undesirable. Furthermore, unwanted by-products from ozone action on organic compounds may shorten the shelf-life, change the organoleptic quality, or jeopardize the safety of the final product.

Determination of ozone concentrations

Physical, physicochemical, and chemical methods have been used for determination of ozone. Physical methods measure direct absorption in the UV, visible, or infrared region of the spectrum. Physicochemical methods are dependent upon effects such as heat or chemiluminescence caused by the reaction. Chemical methods quantitate the products

released when ozone reacts with a chemical reagent such as potassium iodide.

The iodometric method has been approved by the International Ozone Association (Gordon and Grunwell, 1983). Ozone oxidizes iodide ion, releasing iodine; the latter is then titrated with sodium thiosulfate to a starch endpoint. This method measures not only ozone, but also all other oxidizing species resulting from ozone decomposition in solutions; for example, $^{\cdot}O_3^{-}$, $HO_2^{\cdot}$, and $^{\cdot}O_2^{-}$. Hence, measurement of residual ozone cannot be accurately done by the iodometric method.

The commonly used indigo method (Bader and Hoigné 1981) is precise, fast, and sensitive (lowest detection level is 0.005 μg/mL). The indigo reagent reacts additively with the carbon-carbon double bond of sulfonated indigo dye causing its decolorization and the resulting change in color is determined spectrophotometrically. Ozone measurement by the indigo method is not compromised by the presence of hydrogen peroxide, organic peroxides, manganous ions, and oxidized species in drinking water. Compared to the iodometric method, the indigo method is more suitable for measuring residual ozone.

Several manufacturers produce instruments that measure ozone by determining the amount of UV light absorbed. Gaseous ozone absorbs short-UV wavelengths with a maximum absorption at 253.7 nm and the gas-phase absorption coefficient of 3000 ± 30 $mole^{-1}$ cm^{-1} at 273 °K and 1 atm (Gordon and Grunwell 1983). Calorimetric methods of ozone measurement depend on the decomposition of ozone in the presence of a catalyst producing heat. Instruments using amperometric methods to measure the oxidation-reduction potential of ozone are available commercially.

Kinetics of microbial inactivation by ozone

Ozone is a strong, broad-spectrum antimicrobial agent that is active against bacteria, fungi, viruses, protozoa, and bacterial and fungal spores. There is a little agreement, however, among researchers regarding the relative sensitivity of different microorganisms to ozone. Additionally, reported sensitivity of a single microorganism varies among studies. Strain of the microorganism, age of the culture, density of the treated population, presence of ozone-demanding medium components, method of applying ozone (that is, gas bubbles, or uniform aqueous solution), accuracy of ozone measuring procedures and devices, and method of measuring antimicrobial efficacy (for example, single point determinations in contrast to systematic kinetic studies) are some of the confounding factors that make comparison among different studies unfeasible. Based on our experience, sensitivity of bacteria to aqueous ozone ideally is tested as follows; (1) grow cells to late exponential or early stationary phases, (2) separate and wash cells from the growth medium, (3) suspend washed cells uniformly in ozone demand-free medium, for example, pure water, to attain 10^7 to 10^8 CFU/mL, (4) apply a dose of ozone that kills a significant portion of the population (~2 to 3 $\log_{10}$-units) but without leaving residual ozone in the treatment mixture, (5) measure cell viability at the end of the treatment, and (6) correlate the population inactivated with the ozone dose. If this procedure is carefully executed, results may be used to estimate the number of ozone molecules sufficient to inactivate a single bacterial cell (n_z). Relative sensitivity of different microorganisms or the same microorganism under different cultural, physiological, or experimental conditions may be reliably determined by comparing their n_z values. Using a similar approach, Kim and Yousef (2000) estimated n_z for *Leuconostoc mesenteroides* at 10^9. In an earlier study, Finch and others (1988) found that 3×10^8 molecules of ozone were used to inactivate each cell of *E. coli*.

The procedure just described can be modified to estimate inactivation rate $\{\Delta(\log_{10}$ CFU/mL$)/(\Delta$ time$)\}$ in response to a given ozone concentration. The modification replaces steps (v) and (vi) as follows: (v) at suitable treatment time intervals, quench the reaction and measure cell viability, and (vi) construct the survivor's plot. The rate of microbial inactivation $\{\Delta(\log_{10}$ CFU/mL$)/(\Delta$ time$)\}$ is calculated using the linear plot or the steepest slope on the survivor curve. The negative reciprocal of this inactivation rate, known as decimal reduction time or D-value, is a useful term in comparing resistance to ozone of different microorganisms or of the same microorganism under different conditions. Microbial inactivation by ozone does not seem to produce linear survivor plots (Figure 2). Finch and others (1988), Kim and Yousef (2000) and many other researchers observed a tailing in these plots. Tailing of heat inactivation survivor plots are normally attributed to poorly designed experiments or to inaccuracies in measurements, but these causes do not necessarily explain the tailing in ozone survivor plots. While heat and other physical factors are applied constantly during the course of the treatment, ozone is commonly applied as a single dose at the beginning of the treatment; therefore, it may be reasonable to predict the nonlinearity in the latter case. These nonlinear plots, nevertheless, may be used to measure initial inactivation rates and calculate the corresponding D-values. Kim and Yousef (2000) applied ozone to bacterial cell suspensions in a continuous, rather than a batch, mode and obtained survivor plots that are linear for 5 to 20 s of the treatment. This study proved that ozone reacts with microorganisms rapidly, and a nonlethal threshold concentration

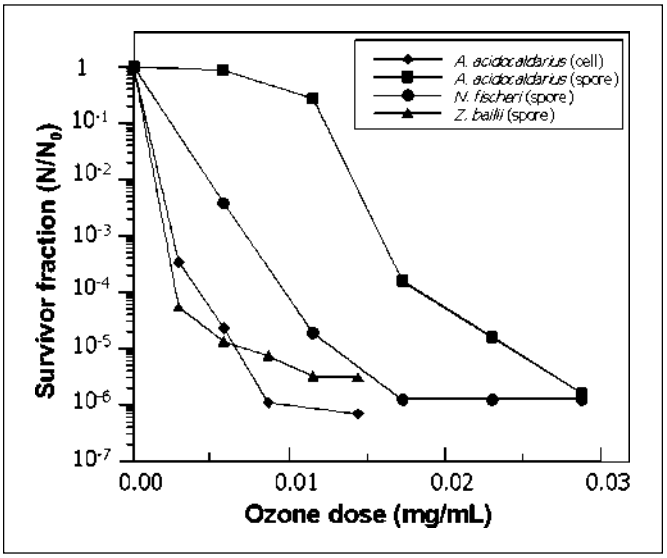

Figure 2—Inactivation of bacterial vegetative cells and endospores (*Alicyclobacillus acidocaldarius*) and mold (*Neosartorya fischeri*) and yeast (*Zygosaccharomyces bailii*) ascospores when aqueous suspensions containing 6.4 × 10^6 to 1.5 × 10^7 CFU/mL, initially, were treated with ozone in a continuous reactor (Kim and others 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). N_o: CFU/mL in untreated sample; N: CFU/mL in treated sample.

is reached quickly in a batch treatment. Continuous treatments, coupled with rapid sampling techniques, allow a relatively accurate determination of D-values.

Determination of microbial inactivation kinetics in a continuous treatment system may be simplified by measuring ozone dose as C.T value. Based on this concept, C.T is a measure of disinfectant concentration (C) multiplied by the time (T) required to achieve a given inactivation level of a microorganism. It was originally introduced by Watson (1908) as a solution for the occasional absence of a straight line in disinfectant log plots. However, Watson emphasized the importance of a constant disinfectant concentration during the time of contact. Although the C.T concept provides an excellent measure of ozone doses, accurate determination of C.T value is difficult in the case of ozone due to its instability and short half-life. To overcome this problem, some authors used residual ozone concentration at the end of the contact period as an estimate of "C" in the "C.T" term; this approach obviously results in inaccurate dose measurement. Gyurek and others (1997) questioned the validity of the C.T concept. They stipulated that extrapolation of a C.T product at a high concentration for chlorine to low concentration conditions is inappropriate because of the modeling discontinuity that may exist between high and low concentrations.

Mechanism of microbicidal action of ozone

Inactivation of bacteria by ozone is a complex process because ozone attacks numerous cellular constituents including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids. Some authors concluded that molecular ozone is the main inactivator of microorganisms, while others emphasize the antimicrobial activity of the reactive by-products of ozone decomposition such as $^{\cdot}OH$, $^{\cdot}O_2^{-}$, and $HO^{\cdot}_3$ (Chang 1971; Harakeh and Butler 1985; Glaze and Kang 1989; Bablon and others 1991b; Hunt and Marinas 1997).

Cell envelopes. Ozone may oxidize various components of cell envelope including polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins and glycolipids leading to leakage of cell contents and eventually causing lysis (Scott and Lesher 1963; Murray and others 1965). When the double bonds of unsaturated lipids and the sulfhydryl groups of enzymes are oxidized by ozone, disruption of normal cellular activity including cell permeability and rapid death ensues. In our laboratory, Dave (1999) found that treatment of *Salmonella enteritidis* with aqueous ozone disrupted the cell membranes as seen in transmission electron micrographs (Figure 3). However, Komanapalli and Lau (1996) found that short-term exposures of *E. coli* K-12 to ozone gas compromised the membrane permeability but did not affect viability, which progressively decreased with longer exposure.

Bacterial spore coats. Foegeding (1985) found that *Bacillus cereus* spores with coat proteins removed were rapidly inactivated by ozone, compared to intact spores. The researcher concluded that the spore coat is a primary protective barrier against ozone. Recently, Khadre and Yousef (2001b) found that spores of *Bacillus subtilis* treated with aqueous ozone showed heavily disrupted outer spore coats (Figure 3).

Enzymes. Several authors referred to enzyme inactivation as an important mechanism by which ozone kills cells. Sykes (1965) reported that chlorine selectively destroyed certain enzymes, whereas ozone acted as a general protoplasmic oxidant. Ingram and Haines (1949), in view of their finding general destruction of the dehydrogenating enzyme systems in the cell, proposed that ozone kills *E. coli* by interfering with the respiratory system. Takamoto and others (1992) observed that ozone decreased enzyme activity in *E. coli* at a greater degree in case of cytoplasmic â-galactosidase than in case of the periplasmic alkaline phosphatase. Inactivation of enzymes by ozone is probably due to oxidation of sulfhydryl groups in Cysteine residues (Chang 1971).

Nucleic material. Reaction of aqueous ozone with nucleic acids *in vitro* supports the notion that it may damage nucleic material inside the cell. Ozone modified nucleic acids *in vitro* , with thymine being more sensitive than cytosine and uracil (Scott 1975; Ishizaki and others 1981). In another study, ozone opened the circular plasmid DNA and reduced its transforming ability, produced single- and double-strand breaks in plasmid DNA (Hamelin 1985), and decreased transcription activity (Mura and Chung 1990). Studying *E. coli,* l'Herault and Chung (1984) found that ozone may induce mutations. However, other investigators did not detect any mutagenic effect of ozone on *Salmonella* spp. (Victorin and Stahlberg 1988). Compared to other known mutagens, ozone was found to be a weak mutagen on *Saccharomyces cerevisi-*

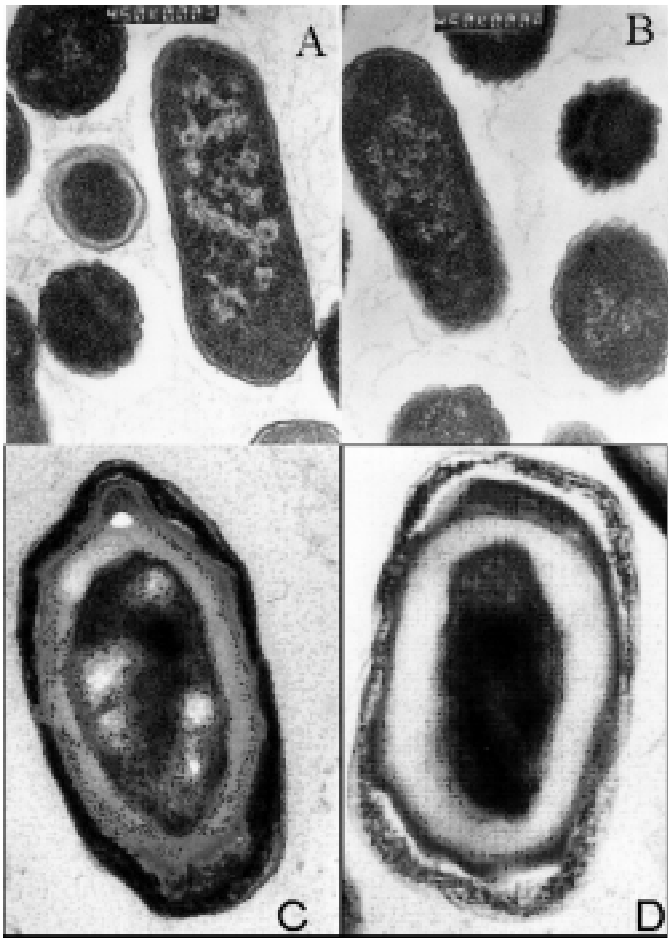

Figure 3—Disruption of *Salmonella enteritidis* outer membrane (Dave 1999) and the outer coat of *Bacillus subtilis* spores (Khadre and Yousef 2001b) after treatment with sublethal levels of ozone as seen by the transmission electron microscope. (A and B) *S. enteritidis*; (C and D) *B. subtilis*, before and after ozone treatment, respectively.

Table1—Inactivation of gram-positive bacteria by ozone in ozone demand-free water

	Treatment Conditions					
Bacterium	Ozone (μg/mL)	Time (min)	pH	Temp. (°C)	Log_{10}-units decreased	References
Bacillus megaterium	0.19	5		28	> 2.0	Broadwater and others 1973
B. cereus	0.12	5		28	> 2.0	Broadwater and others 1973
Leuconostoc Mesenteroides	0.3 to 3.8	0.5	5.9	25	1.3 to ~7	Kim and Yousef 2000
Listeria monocytogenes	0.2 to 1.8	0.5	5.9	25	0.7 to ~7	Kim and Yousef 2000
L. monocytogenes	0.1[a]	10	7.2	25	60 to 70%[b]	Lee and others 1998
Mycobacterium fortuitum	0.23 to 0.26	1.67	7.0	24	1.0	Farooq and Akhlaque 1983
S. aureus	0.3 to 1.97	10			4 to 6	Lezcano and others 1998
S. aureus		0.25	7.0	25	> 2.0	Burleson and others 1975

[a]Phosphate buffer
[b]Per cent injured cells

ae (Dubeau and Chung 1982). The effect of ozone on viral nucleic acids is discussed in a later section.

Viruses. Sproul and Kim (1980) and CK Kim and others (1980) found that aqueous ozone inactivated both f2 and T4 bacteriophages by attacking capsid protein, with liberation and inactivation of the nucleic acid. The RNA from f2 bacteriophage was partially inactivated prior to release from the capsid. They suggested that ozone breaks the protein capsid into subunits liberating RNA and disrupting virus adsorption to the host pili, and that the RNA may be secondarily inactivated. The DNA released from T4 bacteriophage was rapidly inactivated by ozone at about the same rate as that in the intact phage. CK Kim and others (1984) confirmed the results of Sproul and Kim (1980) about bacteriophage T4; they found that ozone randomly destroyed the head, collar, contractile sheath, end plate, and tail fibers and liberated the DNA from the head.

Yoshizaki and others (1988) found that aqueous ozone caused the coat proteins subunits of tobacco mosaic virus (TMV) to aggregate with each other and cross-link with the viral RNA. Despite their observation of a good correlation between loss of infectivity and decrease of recovery of viral RNA, Yoshizaki and others (1988) and Shriniki and others (1988) concluded that the major cause of TMV inactivation by ozone was the inability of the treated virus to uncoat. Roy and others (1981) found that ozone altered two of the four polypeptide chains in the poliovirus protein coat. They, however, attributed the inactivation of the virus to the damage in its RNA by ozone. The observation by Herbold and others (1989) that 0.38 μg/mL aqueous ozone was needed for complete inactivation of hepatitis A virus (HAV) and only 0.13 μg/mL for complete inactivation of poliovirus may support the hypothesis that damage to viral envelopes is the main cause of inactivation of viruses by ozone. Enveloped viruses such as HAV are expected to be much more resistant to ozone compared to nonenveloped viruses such as poliomyelitis.

Efficacy of ozone

Efficacy of ozone is demonstrated more readily when targeted microorganisms are suspended and treated in pure water or simple buffers (with low ozone demand) than in complex systems such as food. The simplicity of low-ozone-demand aqueous environment makes it possible to compare ozone efficacy against microorganisms within the same study, and occasionally among different studies. Ozone also may be compared with other sanitizers when experiments are done in the simple treatment environments just indicated, but differences in experimental designs, treatment conditions, and microbial strains tested should be considered. Therefore, in the following discussion we will compare efficacies with consideration to the factors just indicated. This discussion will be limited to bacteria and viruses since they were more extensively investigated than other groups of microorganisms.

Inactivation spectrum

Bacteria. Studies summarized in Table1 show that 0.12 to 3.8 μg/mL aqueous ozone inactivated gram-positive bacteria by 1 to 7 log_{10} CFU/mL. When gram-negative bacteria were treated with 0.004 to 6.5 μg/mL aqueous ozone, their populations decreased 0.5 to 6.5 log_{10} CFU/mL (Table 2). It may not be possible to compare ozone sensitivity of gram-positive and gram-negative bacteria using summaries of data in Tables 1 and 2; therefore, studies that directly compare these two categories will be presented. Sobsey (1989) reviewed studies to inactivate health-related microorganisms in water by several disinfectants and concluded that gram-positive bacteria, including *S. aureus* and *Bacillus* spp., and the *Mycobacteria* were more resistant than were gram-negatives. Lee and Deniniger (2000) observed the dominance of gram-positive bacteria among the surviving microorganisms in ozonated drinking water. When gram-positive and gram-negative bacteria were compared in side-by-side experiments, however, variable results were obtained. Restaino and others (1995) studying a group of food-related microorganisms, observed that gram-negative bacteria were substantially more sensitive to ozone in pure water than were the gram-positive ones including *L. monocytogenes*. Kim and Yousef (2000) and J-G Kim and others (1999b) treated foodborne spoilage and pathogenic bacteria with ozone under identical conditions and found results inconsistent with the previous conclusion. Resistance of bacteria tested in this study followed this descending order: *Escherichia coli* O157:H7, *Pseudomonas fluorescens*, *Leuconostoc mesenteroides*, and *Listeria monocytogenes*.

Ozone is generally more effective against vegetative bacterial cells than bacterial and fungal spores. In our laboratory, J-G Kim and others (2001) studied inactivation kinetics of different microorganisms that commonly spoil fruit juices (Figure 2). Results of this study show that *Alicyclobacillus acidocaldarius* vegetative cells and *Zygosaccharomyces bailii* ascospores were inactivated rapidly with aqueous ozone. Spores of *A. acidocaldarius* were the most resistant to ozone, and survivor's plot exhibited both a shoulder and a tail. Mold spores (*Neosartorya fischeri*) were intermediate in resistance to ozone, and tailing of survivor plots was apparent. Khadre and Yousef (2001b) measured ozone efficacy

Table2—Inactivation of gram-negative bacteria by ozone in water

	Treatment Conditions					
Bacterium	Ozone (μg/mL)	Time (min)	pH	Temp. (°C)	Log_{10}-units decreased	References
Escherichia coli	0.065[a]	0.5			3.5	Katzenelson and others 1974
E. coli	0.004 to 0.8[b]	0.5 to 2.0	6.9		0.5 to 6.5	Finch and others 1988
E. coli	0.19[a]	5	28		> 2.0	Broadwater and others 1973
E. coli	0.23 to 0.26[a]	1.67	7.0	24	4.0	Farooq and Akhlaque 1983
E. coli	0.53[b]	0.1	6.8	1	2.0	Fetner and Ingols 1956
E. coli O157:H7	0.3-1.0[a]	< 0.5	5.9	25	1.3-3.8	Kim and Yousef 2000
Legionella	0.32[a]	20	7.0	24	> 4.5	Edelstein and others 1982
peumophila	0.47	20	7.0	24	>5.0	
L. pneumophila	0.21	5			> 2.0	Domingue and others 1988
Salmonella enteritidis	0.5 to 6.5	0.5		25	0.6 to ~4	Dave 1999
S. typhimurium	0.23 to 0.26[a]	1.67	7.0	24	4.3	Farooq and Akhlaque 1983
Pseudomonas fluorescens	0.2 to 1.2[a]	< 0.5	5.9	25	0.9 to 5	Kim and Yousef 2000

[a]O_3 demand-free water
[b]Phosphate buffer

Table3—Inactivation of viruses by ozone in water

	Treatment Conditions					
Virus	Ozone (μg/mL)	Time (min)	pH	Temp. (°C)	Log_{10}-units decreased	References
Bacteriophage f2	0.09 to 0.8[a]	0.08	7.0	25	5.0 to 7.0	Kim and others 1980
Bacteriophage MS2	0.6[b]	0.3	6.9	22	2.96	Finch and Fairbairn 1991
Bacteriophage MS2	0.3 to 0.4[b]	0.08	6 to 10	3 to 10	6.0	Hall and Sobsey 1993
Hepatitia A virus	0.3 to 0.4[b]	0.08	6 to 10	3 to 10	3.9	Hall and Sobsey 1993
Hepatitia A virus	0.25[b]	0.02	7.2	20	2.7	Herbold and others 1989
Hepatitis A virus	1.0[b]		6 to 8	4	5.0	Novotny and Strout 1990
Poliomyelitis virus	0.3 residual.	4.0			4.0	Coin and others 1967
Poliovirus type 1	1 to 10 (initial)[a] 0.6 (residual)	5			4.0	Mitsumi 1989
Poliovirus type 1	0.3 residual[a]	< 0.14			2.0	Katzenelson and others 1974
Polioviris type 1	0.23 to 0.26[a] residual	0.5			2.0	Farooq and Akhlaque 1983
Poliovirus type 1 (Mahoney)	0.23 to 0.26[a]	1.67	7.0	24	2.5 to 3.0	Farooq and Akhlaque 1983
Poliovirus type 3	0.6[b]	0.3	6.9	22	1.63	Finch and Fairbairn 1991
Rotavirus human	0.1 to 0.3[b]	6.0	6-8	4	3.0	Vaughn and others 1987
Rotavirus SA 11 simian	0.1 to 0.25[b]	6-8	6-8	4	3.0	Vaughn and others 1987
Rotavirus Wa human ATCC	2.1 to 4.2	1.0		22	0.0 to 1.0	Khadre and Yousef (2001c)
Rotavirus Wa human Wooster	1.9 to 15.9	1.0		22	1.0 to 5.0	Khadre and Yousef (2001c)
Vesicular stomatitis virus		0.25	7.0	25	> 2.0	Burleson and others 1975

[a]O_3 demand-free water
[b]Phosphate buffer

against spores of 8 *Bacillus* spp. *B. stearothermophilus,* which is known for high resistance to heat, also possessed the highest resistance to ozone among the species tested.

Viruses. A limited number of studies on inactivation of viruses with ozone have been published. Researchers (Table3) tested ozone concentrations in the range of 0.1 to 15.9 μg/mL against 8 different viruses; the treatment caused destruction of 0 to 7 log_{10}-units. This may indicate that viruses are comparable to bacteria in sensitivity to ozone. Sobsey (1989), however, concluded that viruses are generally more resistant than vegetative bacteria and that bacteriophages are the most sensitive to ozone among the viruses tested. Other researchers (CK Kim and others 1980; Hall and Sobsey 1993) also reported the sensitivity of the bacteriophages MS2, and f2 to ozone. Based on the limited studies in Table3, it may be concluded that bacteriophages are the least resistant to ozone, followed by polioviruses, whereas human rotavirus was the most resistant to the sanitizer. This conclusion is in agreement with those reports by Herbold and others (1989) and Hall and Sobsey (1993).

Combination treatments for increased efficacy

Advanced oxidation processes. Advanced oxidation processes (AOPs) are processes designed to generate highly reactive intermediates, particularly the hydroxyl radical (·OH), for treatment of recalcitrant organic compounds in water. Among the AOPs are ozonation at high pH, H_2O_2/O_3 processes and UV photolysis of H_2O_2 (Arselan and others 1999). Hydrogen peroxide in aqueous solutions partially dissociates to hydroperoxide anion (HO_2^-) which is highly reactive with ozone (Taube and Bray 1940).

$$H_2O_2 + H_2O \leftrightarrow HO_2^- + H_3O^+$$

The hydroperoxide ions consumed by ozone are quickly replaced by shifting the equilibrium in the above reaction to the right. Hence, very small concentrations of H_2O_2 should be effective in initiating ozone decomposition.

Different AOPs vary in efficacy. Arselan and others (1999) found that ozone at pH 11.5 was more effective than a combination of H_2O_2/O_3 at pH 7.5 for decreasing color in dyehouse wastewater and removing dissolved organic compounds. Cortes and others (2000) found that O_3/catalyst (Fe^{2+}, Fe^{3+} and Mn^{2+}) combination was more effective than O_3/high pH for the elimination of chlorobenzenes, which are stable nonbiodegradable and toxic substances, in industrial wastewater. Other researchers disputed the efficacy of AOPs. Rajala-Mustonen and Heinoen-Tanski (1995) reported that ozone alone in tap water was much more effective in inactivation of coliphages than were AOPs using UV light with hydrogen peroxide. Harakeh and Butler (1985) found that 0.2 ppm ozone at pH 4 gave significantly higher reduction of poliovirus than at pH 7.2 or 9. In the presence of 0.5 M sodium bicarbonate, an ozone decomposition inhibitor, viral sensitivity to ozone increased about 10-fold at each pH value tested. Hence, enhancing ozone efficacy through generation of AOPs seems theoretically feasible but still lacks sufficient proof for practical application in foods.

Ozone-Chlorine. Ozone seems to possess an activity that is lacking in chlorine; it alters membrane permeability. This is evident from the work of Gyurek and others (1996), who found that free chlorine is relatively ineffective against *Cryptosporidium parvum* oocysts unless it is preceded by a small dose of ozone. They assumed that preozonation alters the permeability of the oocyst membranes, thus allowing free chlorine to penetrate and cause a significant inactivation of the oocysts.

Ozone-pulsed electric field. Unal and others (2001) studied inactivation of *E. coli* O157:H7, *Listeria monocytogenes*, and *Lactobacillus leichmannii* by combinations of ozone and pulsed electric field (PEF). Cells were treated with 0.25 to 1.00 μg ozone/mL cell suspension, PEF at 10 to 30 kV/cm, or selected combinations of ozone and PEF. Treatment of *L. leichmannii* with PEF (20 kV/cm), after exposure to 0.75 and 1.00 μg/mL ozone, inactivated 7.1, and 7.2 $\log_{10}$ CFU/mL, respectively; however, ozone at 0.75 and 1.00 μg/mL and PEF at 20 kV/cm inactivated 2.2, 3.6, and 1.3 $\log_{10}$ CFU/mL, respectively. When *E. coli* O157:H7 and *L. monocytogenes* were treated with ozone and PEF, less pronounced synergistic bactericidal effects were observed. Ohshima and others (1997) also reported a synergistic effect of the simultaneous application of ozone and PEF on *E. coli*. Inspecting the data of Ohshima and others (1997), however, we found that ozone and PEF combinations, as tested in this study, had an additive rather than a synergistic action.

Ozone application in food processing

Ozone is one of the most effective sanitizers known, yet it leaves no hazardous residues on food or food-contact surfaces. The precursors for industrial production of ozone (that is, O_2 or H_2O) are abundant and inexhaustible. Ozone treatment requires no heat and hence saves energy. Ozone must be produced on-site; this leads to considerable savings in the costs of transporting and storing sanitizers. The initial cost of ozone generators may be of concern to small processors; however, long-term application may justify these costs. The economics of ozone application is beyond the scope of this review; but the fact that ozone has been and is still being used in Europe and some places in the United States suggests that it is reasonably economical.

Products tested

Several investigators demonstrated the microbicidal effects of ozone gas injection or sparging in reconditioning poultry chiller water (Waldroup and others 1993; Diaz and Law 1999). Effective prefiltration of chiller water prior to ozone treatment is recommended for optimum reduction of microbiological levels and efficient use of ozone (Sheldon 1986). Aqueous ozone also was used to decontaminate beef and beef brisket fat (Gorman and others 1997), poultry meat (Dave 1999), salmon (Goche and Cox 1999), apples (Achen and Yousef 2001; McLoughlin 2000), strawberries (Lyons-Magnus 1999), lettuce (J-G Kim and others 1999a) and broccoflower (Hampson and Fiori 1997). Microbial studies typically show 2-logs reduction of total counts and significant reduction of spoilage and potentially pathogenic species most commonly associated with fruit and vegetable products.

Some researchers treated raw ingredients with ozone before processing of food. M-J Kim and others (1993) treated various spices, used to prepare kimchi, with gaseous ozone and improved the fermentation of the final products. In our laboratory, K-G Kim and others (2001) used gaseous ozone injection to decontaminate the ingredients of fruit juices such as high-fructose corn syrup. The researchers speculated that ozone treatment of ingredients rather than final juice products can reduce ozone usage and minimize the damage to the sensory quality of the final product. Naitoh and others (1989) reported that the treatment of wheat flour with gaseous ozone inhibited microbial growth in namamen products and increased their storage life.

Gaseous ozone can be used during storage of foods. Ozone was tested to prevent the growth of surface contaminants on meat (Greer and Jones 1989), grapes (Sarig and others 1996), and broccoli florets (Zhuang and others 1996). Low concentration (< 1 ppm) and long contact time (several days) were needed to inhibit microbial growth during storage. Aqueous ozone was also used to treat packaging and food-contact materials (Khadre and Yousef 2001a). Combinations of ozone with other oxidants such as hydrogen peroxide were also used to sanitize packaging films (Gardner and Sharma 1998), a confectionery plant (Naitoh 1989), and hatchery equipments (Whistler and Sheldon 1989). Ozone decreased surface flora by ~ 3 $\log_{10}$-units when tested in wineries for barrel cleaning, tank sanitation, and clean-in-place processes (Hampson 2000).

In spite of its efficacy against microorganisms both in the vegetative and spore forms, ozone is unlikely to be used directly in foods containing high-ozone-demand materials, such as meat products. Applying ozone at doses that are large enough for effective decontamination may change the sensory qualities of these products. Additionally, microorganisms embedded in product surfaces are more resistant to ozone than those readily exposed to the sanitizer. Application of aqueous ozone on products having smooth intact surfaces with low ozone demand (for example, fruits and vegetables) produced promising results (Achen and Yousef, 2001; Kim and others 1999a). Application methods, however, must assure direct contact of ozone with the target microbial cells. A variety of methods have been used to accomplish this, including stirring, pumping, flumming, bubbling, sonication, abrasion, and pressure washing.

Microorganisms for measuring ozone efficacy

The efficacy of a sanitizer in food processing is ideally tested by inoculating targeted microorganisms (spoilage or pathogenic) on the surface of food, equipment, or food-contact surface, and treating these surfaces with the sanitizer at

conditions that simulate normal processing. Alternatively, an indicator (surrogate) microorganism with resistance to the sanitizer that is similar or greater than that of the targeted microorganism may be used. The indicator is ideally similar biologically to the targeted microorganism, but it should not be pathogenic if the study is carried out in the processing facilities. Since sanitization commonly targets a variety of microorganisms, an indicator with the greatest resistance to the sanitizer is preferable in these challenge studies.

Clostridium sporogenes PA 3679 has been effectively used as a surrogate to *C. botulinum* in heat inactivation studies, but *Bacillus stearothermophilus* is also used (Russell 1982). *L. innocua* has been used to study treatments that target *L. monocytogenes* (Gervilla and others 1997). Selected *B. subtilis* strains are used in determining the efficacy of H_2O_2 and heat in aseptic fillers (Anonymous 1995 and 1999). Very little research has been done in the quest for the ideal microorganism to use in measuring ozone efficacy.

In a comparative study, Khadre and Yousef (2001b) found that resistance of *Bacillus* spp. spores to ozone was highest for *B. stearothermophilus* and lowest for *B. cereus*. Spores of *B. subtilis* var niger ATCC 9372 are used as indicators in dry heat and ethylene oxide sterilizations (Anonymous 1995 and 1999), but in our study these spores were sensitive to ozone. Hence, we suggest using *B. stearothermophilus* spores in testing the efficiency of sanitization by ozone.

Residual ozone and process efficacy.

During treatment of food, ozone may desolubilize, decompose, or react with food constituents and targeted microorganisms. The rapid reaction and degradation of ozone diminish the residuals of this sanitizer during processing. The lack of residuals may limit the processor's ability for in-line testing of efficacy; this is an often-cited disadvantage of using ozone as a disinfectant. Stalder and Klosterkoetter (1976) clearly illustrated this problem—they observed that 1.5 μg/mL ozone treatment kept water sterile for greater than 1 mo with no detectable residuals. However, passage of this water through a 12 m-long pipeline led to recontamination and considerable growth of microorganisms. Lack of residual ozone in the water led to this recontamination problem. Food is packaged after processing; therefore, product recontamination is less likely in food than in drinking water. Lack of residues, however, minimizes a processor's ability to monitor ozone level in wash water as an important critical control point within a hazard analysis and critical control point (HACCP) plan.

Validation

Process validation is a practice that accompanies introduction of a new processing technology or unit operation. Results of validating ozone use in drinking water at the Neuilly-sur-Marne plant in France has been published (Bablon and others 1991b). This plant produces 600,000 m^3/d of water from the Marne River near Paris. The filtered water is disinfected with ozone at an average dose of 1.5 μg/mL for an average contact time of 12 min. Ozone is diffused through porous plates to contact chambers. The residual ozone concentration at the end of the contactors is 0.4 μg/mL. A post-disinfection dose of chlorine is added to give a residual chlorine concentration of 0.4 μg/mL in the water leaving the plant and entering the distribution system. The bacteriological results of samples taken during 1988 to monitor bacterial levels before and after ozonation indicate a substantial reduction in microbial population. Fecal streptococci were not detected in water samples (100 mL), total and fecal coliform bacteria decreased > 4 and 3 $\log_{10}$-units, respectively, and heterotrophic plate count bacteria were reduced 2 to 3 $\log_{10}$-units.

Sheldon and others (1985) tested the effects of ozone on the microbiological characteristics of spent poultry prechiller water (95 L obtained from a poultry plant and tested in a pilot plant-size ozone contactor). Ozone was generated at a rate of 292 ppm per min for 60 min. After ozonation, the total aerobic population decreased ~7 $\log_{10}$-units, the coliform count decreased > 3 $\log_{10}$-units, and the fecal coliforms, *E. coli* and *Salmonella*, were not detected. These authors concluded that ozone qualifies for recycling poultry chiller water under the USDA's guidelines. In 1993, Waldroup and others reported their evaluation of a prototype water recycling ozonation system installed in a commercial turkey poultry processing facility over a 4-mo period. They found similar results like those of Sheldon and others (1985) and were able to obtain USDA approval for this system for recycling poultry chiller water in 1991. Tests for validation of the use of ozone for red meat processing (Greer and Jones 1989; Gorman and others 1997) have given modest results, and more research is needed in this area probably involving ozone in combination with other factors such as hot water or hydrogen peroxide.

Monitoring ozone in work environment and personnel safety

Ozone toxicity. Low concentrations of ozone (~0.1 mg/L) cause irritation to the nose, throat, and eyes (Witheridge and Yaglou 1939). Thorp (1950) indicated that an hour exposure to ozone concentrations of 2, 4, 15, and 95 mg/L induces symptomatic, irritant, toxic, and irreversible lethal effects, respectively, in humans. The human lung is the primary target of ozone gas. Initially, there is pulmonary edema accompanied by capillary hemorrhage and inflammation of the respiratory tract. On prolonged exposure, ozone may cross the alveoli, causing damage to blood cells and serum proteins (Buckley and others 1975). Ozone appears to react with substances in the water supply, such as humic acids, to form numerous disinfection by-products which cause minor toxicological reactions, if any (Bablon and others 1991a).

Personnel safety. Safety-of-use is of prime importance for the practical application of ozone in food processing. Systems for ozone detection and destruction in addition to respirators are essential for the safety of workers in food processing facilities. An ultraviolet analyzer equipped with a large measuring cell adapted to a range of 0.01 to 100 ppm by volume (0.02 to 200 mg/m^3 NTP) must be installed in ozonation rooms at intervals covering the ozone gas distribution pipes, contactor access galleries, and at the ozone destruction point. The analyzer must trigger both a displayed and acoustic warning signal as soon as the ozone content in the ambient air exceeds 0.1 ppm (0.2 mg/m^3 NTP) (Damez and others 1991).

The off-gas from the plant must pass through a thermal or catalytic ozone destructor. A continuous ozone analyzer that functions within a range of the standard of 0.1 ppm by volume (0.2 mg/m^3 NTP) must be fitted to the air line leaving the destructor. Any overshooting of this value will trigger a general alarm (Damez and others 1991). The reason for ozone destruction is to protect personnel, equipment, structural components, and the general environment from exposure to high levels of ozone.

According to U.S. regulations (CFR 1997), an individual must not be exposed to a concentration of ozone higher

than: (a) 0.1 ppm by volume (0.2 mg/m^3 NTP), on an 8-h/d basis, of a 40-h work wk; and (b) 0.2 ppm by volume (0.4 mg/m^3 NTP), as a limit for an exposure time of 10 min. Furthermore, protective canister-type respirators must be kept available. There should be plans for remedial action in case of accidents, and response procedures for accidental ozone inhalation and training of personnel covering the nature and dangers of ozone, precautions, and first aid for ozone inhalation.

Conclusion

OZONE IS A POTENT SANITIZER WITH PROMISING APPLICATIONS in the modern food industry. The sanitizer is effective against a wide spectrum of microorganisms, and it can be used in an environment-friendly manner. Stability and efficacy of ozone at chilling temperatures constitute attractive savings to the industry which is already burdened by rising energy costs.

Chlorine and hydrogen peroxide are probably the most commonly used sanitizers in the food industry. These sanitizers have been used successfully to decontaminate processing environment, equipment surfaces, and occasionally the surfaces of solid foods. Their drawbacks, however, have prompted the quest for more effective and economical sanitizers. Currently, ozone is the most likely alternative to chlorine and hydrogen peroxide in food applications. Transition from traditional sanitizers to ozone requires a great understanding of its benefits and limitations and realistic expectations from the alternative sanitizer. Further research is still needed to explore new applications for ozone and to best utilize the unique features of this sanitizer.

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