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# 2. Ozonating: An Eco-Friendly Technique for Cleaning and Disinfection Activities in Dairy Industry

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***Abstract:***

*Cleaning and disinfection operations are of great importance within the dairy and food industries for safety reasons. Most care areas in dairy industry rely on a range of chemicals such as chlorine, quaternary ammonium compounds, bromine or iodine based products to maintain an acceptable hygiene regime. Some of the most important cleaning tasks are those related to the washing of vessels, storage tanks and pipes where cleaning in place systems (CIPs) are of common use. CIPs are characterized by automatic cleaning programs based on a succession of several solutions of water, cleaning chemicals and disinfection agents that are discharged into sewer systems together with large amounts of water necessary to rinse out residual chemicals. When chlorine based compounds combine with organic residues, the results could potentially be extremely harmful to people. Thus, health and environmental concerns with chemical use on dairy & food products or food contact facilities are supporting the need for alternative sanitation technologies.*

*In this sense, the potential utility of ozone lies in the fact that ozone is a stronger oxidant than chlorine and it has been shown to be effective over a wider spectrum of microorganisms. But, unlike other disinfectants, it leaves no chemical residuals and degrades to molecular oxygen upon reaction or natural degradation. In comparison to conventional, ozone based CIP operations requires less water and energy consumption, offers effective cleaning and lesser amount of pollutant in waste water. The different applications of ozone have been reported at experimental and industrial scale with the objective of improving at least one of the following factors: food safety, prevention of cross contamination, extension of shelf life of produce and produce surface sanitation. In dairy industry, it has successfully been used for the removal of milk residues and biofilm-forming bacteria from stainless steel surfaces; for inactivate airborne moulds in cheese ripening and storage facilities; for production of extended shelf life fluid milk, for reducing the concentrations of pollutants in dairy wastewaters etc. This article highlights the application of ozone and its important implications in dairy industry.*

***Keywords:***

*Ozone, CIP, Milk, Hygiene, Dairy Industry.*

## 2.1 Introduction:

Cleaning and disinfection are essential to maintain hygienic conditions in dairy processing plants. Cleaning and sanitization of the all equipment's is the last and first step of the production processes. Cleaning is one of the obligatory steps in dairy and food processing, which can be performed manually by the least experienced employees in the plants. Before the development of recirculation cleaning, manual cleaning was the state of the art for all food contact and non-food contact surfaces. In the circulating cleaning method or Cleaning-in-Place (CIP) method, cleaning solutions which include various types of detergents, sanitizers or disinfectants are circulated within the system (Tamime, 2008).

The commonly used alkali detergents are sodium hydroxide (caustic soda), potassium hydroxide, sodium carbonate etc. and the acid detergents include hydrochloric acid, nitric acid, phosphoric acid, citric acid etc. Typically, 0.5 – 2 % caustic soda has been commonly used in dairy industry at temperatures of up to $85^0$C to saponify fat and convert the fat to soap, which can be removed with water (Walton, 2009).

The hard deposits, which difficult to remove by alkali detergents are generally cleaned by using acid detergents. The most common acid detergent is nitric acid, which is generally used at a concentration of 0.5–1.0 per cent under either ambient or heated conditions (55–$80^0$C) for 5 to 20 minutes (Bremer and Seale, 2010). In order to ensure hygienic conditions required to provide food safety, sanitization is a compulsory step for the entire cleaning process (Amitha and Sathian, 2014).

Sanitization step usually follows alkali and acid cleaning in food processing facilities. The commonly used dairy sanitizers are: steam, hot water, hot air, and chemicals (chlorine compounds, iodophor, and quaternary ammonium compounds).

## 2.2 OZONE as Alternative sanitizer:

One of the biggest issues currently facing the dairy industry is that most high care areas continue to rely on a range of chemicals to maintain an acceptable hygiene regime. Thus, health and environmental concerns with chemical use on food products or food contact facilities are supporting the need for alternative sanitation technologies. 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 and absence of by-products that are detrimental to health (Graham, 1997).

Ozone is a triatomic oxygen molecule found in liquid form above 80 K and gaseous form above 161 K. Since ozone has 2.7 V of reduction potential, it is a powerful oxidizing agent. Although ozone has 12 hours half-life in the gaseous phase. It is reported that the half-life of the aqueous ozone is 20-30 minutes only, depending on water purity, temperature, and other factors (Rice *et al.,* 2002).

## 2.3 Ozone and CIP Application:

Ozone-CIP system has been developed by integrating an ozone generator into a conventional CIP system. Ozone-CIP was developed in the European research project in 2005 by considering its environmental and antimicrobial properties over other conventional chemical cleaning compounds (Arranz and Schories 2007).

Canut and Pascual (2007) have introduced an ozone-based CIP system, at the International Ozone Association Conference in 2007, as the best available technology (BAT) considering the antimicrobial efficiency and environmental advantages.

Ozone has been tested as a sanitizer in a laboratory-scale CIP system. Lagrange et al. (2004) has proved that aqueous ozone had an excellent antimicrobial effect when it is applied in the CIP system provided that the organic matter placed on the surface of the equipment was removed by previous cleaning.

As per one report the use of ozone in CIP systems allowed a reduction of the water consumption needed to perform cleaning and disinfection operations and reducing at least by 50% the organic load in the cleaning waste waters produced compared to conventional CIP protocols keeping, at least, the same disinfection and cleanliness efficiency.

## 2.4 Ozone Production:

Ozone requires on-site generation because of it high reactivity and rapid decomposition (Kim *et al.*, 2003). There are different methods to generate ozone. Corona discharge is the most commonly used method that generates relatively high concentration of ozone.

Two electrodes separated by a discharge opening are used to create a high-energy discharge, which can separate the oxygen molecules into its atomic form. Oxygen atoms come together naturally to form its triatomic form. Dry oxygen or air is used as inlet gas in corona discharge method. Other methods to produce ozone include UV light and electrolysis of water, which produce relatively low ozone concentrations. Ozone can be generated in two phases: (i) gaseous ozone, (ii) aqueous ozone which is formed by infusion of gaseous ozone in water.

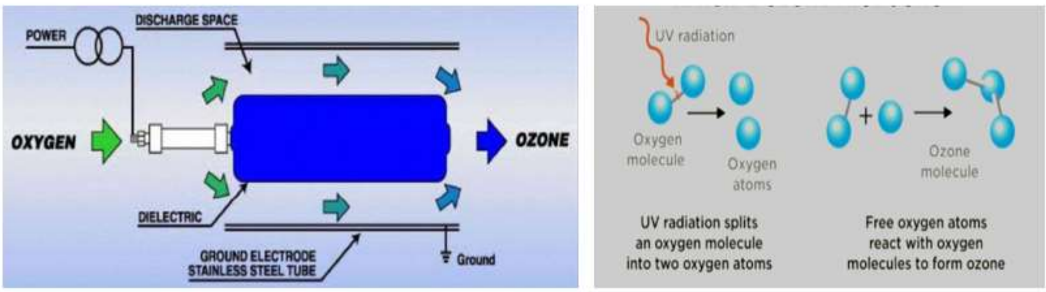


**Figure 3.1: (a) Corona discharge ozone generator (b) Natural ozone production**

## 2.5 Ozone Decomposition:

When ozone decomposes, it forms many oxidative radicals such as superoxide anion radical and hydroperoxide radicals. Those are the main source of the hydroxyl radicals, which are considered main radicals for the microbial inactivation by ozone. Hydroxyl radicals interact with cell components and inactivate the cells (Ligimol *et al.,* 2002).

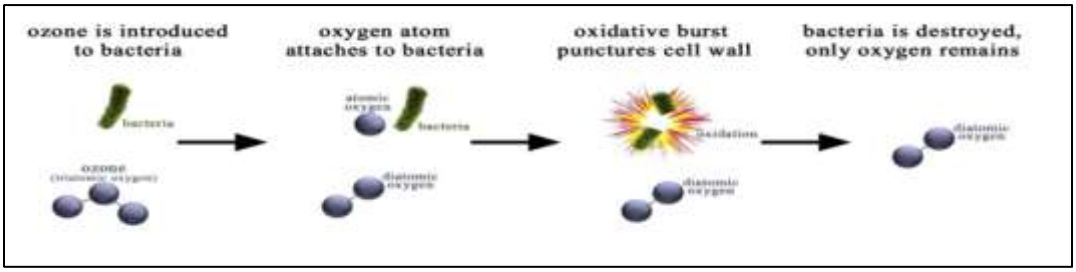


**Figure 2.2: Ozone Decomposition**

## 2.6 Application of Ozone in Dairy Industry:

Ozone was firstly used as an antimicrobial to treat drinking water. Ozone had gained the generally recognized as safe (GRAS) status in 1982 to be used in the bottled water treatments.

In the dairy industry, ozone can be applied both as a gas and in ozonated water (Laszlo and Sziget, 2016). Some of its applications in dairy industry is highlighted as below:

### A. Ozone as a Sanitizer in Dairy Farm:

The production of quality milk begins with good hygienic practices. Dirty cows and soiled equipment can lead to elevated bacterial levels in the bulk tank. Ozone as a sanitizing agent are used alternatively in dairy farm.

The entire udder and the rear legs of milking animals are washed off with ozonated water (0.04 to 1.5 ppm ozone per litre of water) prior to milking in order to destroy bacteria, pathogens and viruses present (Heacox, 2013). Ozonated water can also be used for rinsing the milking equipment, sanitizing it, thus reducing the chances of milk contamination. All equipment that is used to provide feed for the cows may be washed out with ozonated water on a regular basis to kill mold, mildew, and bacteria (Ozone Systems, 2014).

### B. Ozone Treatment of Fluid Milk:

Raw milk is traditionally treated with thermal processes in order to be safe for human consumption. Heating, however, may negatively influence both the nutritional value and the sensory properties of milk. Various authors reported ozone as an alternative to reduce the microbial load and to increase the shelf-life of fluid milk (Table 2.1).

**Table 2.1: The summary of ozonating in fluid milk**

| Treatment | Target | Result | Reference |
|---|---|---|---|
| 'Mild' ozone treatment (parameters unknown) | Milk and fluid milk products | Minimised deterioration of product quality | Sander (1985) |
| Pressurised ozone (5–35 mg/L for 5–25 min) | Microbial population of skim milk | 2.4 $\log_{10}$ cfu/mL reduction in psychrotrophic counts | Rojek *et al*. (1995) |
| Gaseous ozone (generation rate: 0.2 g/h) | *Listeria monocytogenes* in commercial raw and branded milk samples (mean viable counts: 5.5 and 5.7 $\log_{10}$ cfu/mL, respectively) | Complete elimination of *Listeria monocytogenes* after 15 min | Sheelamary and Muthukumar (2011) |
| Gaseous ozone (1.5 mg/L for 5–15 min) | Microbiota of raw milk (mean total plate count: 4.18 $\log_{10}$ cfu/mL) | Up to 1 $\log_{10}$ cfu/mL reductions in bacterial and fungal counts after 15 min | Cavalcante *et al*. (2013a) |
| Gaseous ozone pre-treatment (concentration and duration unspecified) followed by pasteurisation | Raw milk | Shelf life extension without excessive lipid or protein oxidation in final product (commercial fluid milk) | Pastair (2014) |

## C. Ozone in Powdered Milk Products:

The application of ozone and its effect on quality and functionality of powdered milk products have been reported by the various authors (Table 2.2).

**Table 2.2: Ozonating in dried milk products**

| Treatment | Target | Result | Reference |
|---|---|---|---|
| Gaseous ozone (2.8 mg/L or 5.3 mg/L for 0.5–2 h) | *Cronobacter sakazakii* ATCC 51329 in skim milk powder (SMP) and whole milk powder (WMP) at 5.92 $\log_{10}$ cfu/g | $\text{Log}_{10}$ cfu/g reductions of approximately 3 (SMP) and 1.4 (WMP) in *Cronobacter* counts after 2 h | Torlak and Sert (2013) |

| **Treatment** | **Target** | **Result** | **Reference** |
|---|---|---|---|
| Gaseous ozone (2 ppb or 32 ppb during manufacture of milk powders) | SMP and WMP | Decreasing sensory scores with increasing background ozone levels and fat contents | Kurtz *et al.* (1969) |
| Gaseous ozone (treatment parameters unknown) | WMP | Negative effect on organoleptic properties due to lipid oxidation | Ipsen (1989) |
| Gaseous (60 g/h) or aqueous (4.5 ppm) ozone for up to 15 min | Whey protein isolates (WPI) | Enhanced foam formation and foam stability, whereas reduced solubility and emulsion stability of WPI | Uzun *et al.* (2012) |
| Gaseous ozone (approximately 20 mg/L for 30–480 min) | WPI | Improved foaming capacity and foam stability, whereas reduced solubility of WPI | Segat *et al.* (2014b) |

## D. Cheese and indoor atmosphere in cheese ripening and storage rooms:

Ozone was used in cheese-storage facilities first in the USA as early as in the 1940s. Some years later, the application of ozone at low levels to prevent mould growth on cheese during ripening was recommended by various authors.

**Table 2.3: Ozone treatment in cheese.**

| **Treatment** | **Target** | **Result** | **Reference** |
|---|---|---|---|
| Gaseous ozone (3–10 ppm for up to 30 d) | Heavy mould growth on Cheddar cheese | Mould growth inhibition without mould destruction (fungistatic effect); and 94% reduction in mould spore counts in the air of storage room | Gibson *et al.* (1960) |
| Gaseous ozone (0.2–0.3 ppm for up to 63 d) | Mould growth on Cheddar cheese | Mould growth inhibition on the sides of cheese; and 88% reduction in mould spore counts in the air of storage room | Gibson *et al.* (1960) |
| Gaseous ozone (2.5–3.5 ppm for 4 h at 2- to 3-day intervals) | Russian- and Swiss-type cheeses | Mould growth inhibition on cheeses and packaging materials up to 4 months of refrigerated storage | Gabrielyants *et al.* (1980) |
| Gaseous ozone | Italian cheeses spiked with *Listeria monocytogenes* (up to | Complete elimination of *L. monocytogenes* only from cheeses | Morandi *et al.* (2009) |

| Treatment | Target | Result | Reference |
|---|---|---|---|
| (4 ppm for 8 min) | 3 $\log_{10}$ cfu/g) at different stages of ripening | contaminated during the first week of ripening | |
| Ozonated water (2 mg/L for 1–2 min) | Microbiota of Minas Frescal cheese | Approximately 2 $\log_{10}$ cfu/g reduction in initial bacterial and fungal counts ($P < 0.05$) | Cavalcante *et al*. (2013b) |
| Pre-ozonated (2 mg/L) cooling water (15 °C) | Microbiota of high-moisture mozzarella cheese | By 3.58 and 6.09 $\log_{10}$ cfu/g lower total plate counts and *Pseudomonas*spp. counts, respectively, than in control mozzarella samples cooled with nonozonated water, following 21 d of storage | Segat *et al*. (2014a) |
| Gaseous ozone (up to 5 ppm) | Air of cheese ripening room | Up to 99% decrease in viable counts of airborne moulds | Shiler *et al*. (1978) |
| Gaseous ozone (generation rate: 4–8 g/h) for 20 week | Air of cheese ripening room | Tenfold reduction in viable airborne mould load (to < 50 MPN/$m^3$), with majority of isolates belonging to *Penicillium* spp. | Serra *et al*. (2003) |
| Gaseous ozone (0.24 ppm for 40 d) | Parmesan-type cheese surfaces, shelf surfaces and air of cheese ripening room | 0.74, 0.93 and 2.07 $\log_{10}$ reductions, respectively, in fungal viable counts ($P < 0.05$) | Pinto *et al*. (2007) |
| Gaseous ozone (0.38 ppm for 60 d) | Air of cheese ripening room | 63% decrease in viable counts of airborne yeasts and moulds | Lanita and da Silva (2008) |

## E. Cleaning & disinfection of dairy process equipment's:

Several studies have examined its efficacy by testing different treatments on various surfaces and micro-organisms, some of these are listed in Table 2.4.

**Table 2.4: Summary of studies of surface disinfection using ozone**

| Treatment | Target | Result | Reference |
|---|---|---|---|
| Ozonated cold water (10 °C) for 15 min | Heated dairy soil (reconstituted non-fat dry milk, 20% total solids) on metal plates | 84% of dairy soil removed from plates | Guzel-Seydim *et al.* (2000) |

| **Treatment** | **Target** | **Result** | **Reference** |
|---|---|---|---|
| Ozonated water (40 NL/h, 80 g/$Nm^3$) in a bath-substrate-flow device for up to 30 min | Heat-denatured whey protein concentrate (WPC) on stainless steel coupons | Increased WPC removal rates within 10 min compared to treatment with 0.5% (w/w) NaOH solution | Jurado-Alameda *et al.* (2014) |
| Gaseous ozone pre-treatment (0.1–0.5%, v/v) | Heat-treated bovine serum albumin (BSA) on stainless steel particles | Increased BSA desorption rates during subsequent caustic alkali cleaning | Fukuzaki (2006) |
| Ozonated deionised water (0.5 ppm for 10 min) | *Pseudomonas fluorescens* ATCC 949 and *Alcaligenes faecalis* ATCC 337 in UHT milk biofilm on stainless steel plates | 5.6 and 4.4 $log_{10}$ cfu/$cm^2$ reductions, respectively | Greene *et al.* (1993) |
| Ozonated phosphate-buffered saline (0.6 ppm for 10 min) | *Pseudomonas* spp. in UHT milk biofilm on stainless steel coupons | Approximately 3–4 $log_{10}$ cfu/$in^2$reductions in *Pseudomonas* populations | Dosti *et al.* (2005) |
| Gaseous ozone (2 ppm for 4 h) | *Escherichia coli* ATCC 25922, *Listeria innocua*, *Serratia liquefaciens*, *Staphylococcus aureus* and *Rhodotorula rubra*in UHT milk biofilm on stainless steel squares | Up to 5.64 $log_{10}$ cfu/$cm^2$ reductions in microbial viability | Moore *et al.* (2000) |
| Distilled, deionised water treated with pulsed ozone (0.4–0.5 ppm) applied for 20 min/d for 7 d at 21–23 °C | Metal materials (plates) used as food contact surfaces in dairy processing | Significant weight loss of carbon steel plates ($P < 0.05$) | Greene *et al.* (1999) |

## F. Waste Water Treatment in Dairy Processing:

In dairy industry, cleaning and disinfection generated large quantities of wastewater. These wastewaters are conventionally purified with physicochemical and biological methods. Over the recent years, however, several researchers have tested ozonation, using it either alone or in combination with other technologies, in order to reuse, at least in part, the wastewater produced by the dairy sector.

**Table 2.5: Ozone for waste water treatment in dairy processing**

| Treatment | Target | Result | Reference |
|---|---|---|---|
| Gaseous ozone (treatment parameters unknown) | Dairy effluent with 80–230 mg/L of fat content | 96–98% decrease in fat content | Loorits *et al.* (1975) |
| Gaseous ozone pretreatment (150 mg/L/h for 60 min) | Dairy wastewater with 6100 mg/L of chemical oxygen demand (COD) | Enhanced COD removal and increased biodegradability of retentate during subsequent nanofiltration (4.0 MPa at 20 °C) | László *et al*. (2007) |
| Gaseous ozone treatment (30 mg/L for 5–20 min) | Model dairy wastewater with 4000 mg/L of COD | Up to 25% decrease in COD | László *et al*. (2009) |
| Gaseous ozone pretreatment (30 mg/L for 5–20 min) | Model dairy wastewater with 4000 mg/L of COD | Increased flux and decreased membrane fouling during subsequent nanofiltration (3.0 MPa at 25 °C) | László *et al*. (2009) |
| Gaseous ozone pretreatment (2 g/h for 240 min at pH 7–12) | Dairy wastewater with 6300 mg/L of COD | High COD removal efficiency (71%) at pH 12 | Sivrioğlu and Yonar (2015) |
| Ultrasonication (76.4 kJ/kg TS of specific energy) followed by gaseous ozone pretreatment (0.0011 mg $O_3$/mg SS) | Dairy waste activated sludge with soluble COD, suspended solids (SS) and total solids (TS) levels of 400, 7000 and 12 560 mg/L, respectively | Enhanced COD solubilisation, SS reduction and anaerobic biodegradability (compared to single pre-ozonation) | Packyam *et al.* (2015) |
| Gaseous ozone (10 g/$Nm^3$ for up to 7 h at pH 2–10) | Biologically pretreated cheese whey wastewater with 520 mg/L of COD | Substantial decrease in COD, especially when ozonation was combined with application of 16.5–33.0 mM of $H_2O_2$ | Martins and Quinta-Ferreira (2010) |

## 2.7 Advantages of Ozonating Over Conventional Cleaning and Disinfection:

Water consumption in dairies is mainly associated to cleaning operations. The wastewater is the main environmental issue in the dairy sector. The pollution load on the wastewater is high due to residual milk fat and proteins as well as cleaning chemicals. Adopting ozone in cleaning and disinfection processes can bring various advantages over commonly employed disinfectants in the dairy industry. 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 (Canut and Pascual, 2007). 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 (Canut and Pascual, 2008). 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 reozonation 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 the spot’’, ozone removes the need to store hazardous substances which could give rise to accidents that endanger human and environmental health and safety Pascual et al., (2007).

## 2.8 Conclusions:

The dairy industry continues to apply more focus on the importance of reducing water cost and the recovery of valuable raw materials. Water consumption in this industry is mainly associated with cleaning operations, cooling water and process water. The concerns for health and environmental challenges have given rise to alternative solutions for disinfection. Ozone is at minimum as effective as common sanitizing agents used in wash waters in the dairy & food industry. It has been reported that the ozone CIP system allows a reduction of the water consumption needed to perform cleaning and disinfection operations of closed equipment’s in the dairy sectors. Compared to conventional CIP at least, the same disinfection and cleanliness efficiency and reducing at least by 50% the organic load in the cleaning waste waters produced. The equipment necessary is available at the market, the investment cost is somewhat higher than current CIP systems and running costs lower. Other considerations such as hazards and material compatibility are well identified and documented. Thus, ozone can be considered a complimentary sanitizing regime to help maintain the overall cleanliness and sanitation of any food processing facility.

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