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The use of ozone in whole room disinfection

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1. BACKGROUND

Food can be exposed to microbiological cross-contamination from food contact surfaces via direct contact, and from non food contact surfaces via vectors such as the air, people etc, which may give rise to food spoilage and safety issues. The traditional approach to controlling such contamination has been to target specific sites within the manufacturing environment with cleaning and disinfection regimes. The primary focus is typically on food production equipment and drains. The remaining food production environment/processing area, whilst cleaned, may not be routinely disinfected. This targeted approach may have been sufficient to maintain day-to-day control of contamination, but does not eliminate all of the organisms within a production environment and, in some instances, microbial strains have become persistent in food factories, surviving for several years (Holah *et al* 2002, Holah *et al* 2004). Clearly, these organisms present a cross contamination risk and if there was any loss of hygiene control in these factories, these organisms could present a risk to product safety.

In high risk food processing areas, thorough disinfection of surfaces is required in order to reduce the numbers of microorganisms and to prevent transmission of these contaminants. Through the regular use of various disinfection techniques it is believed that the “whole room” can decontaminated. This will reduce the number of environmental microorganisms in the production areas (bioburden), and may also help reduce the incidence of persistent strains; thus reducing the risk that these organisms would contaminate product and improving the quality and safety of the food being produced, thereby reducing wastage and increasing profitability.

One of the systems investigated at Campden BRI and successfully used in factories is the application of ozone gas in a non-condensing humid environment (Holah *et al* 2002, Holah *et al* 2004, Middleton 2010).

2. INTRODUCTION

There is now a growing desire to supplement traditional, targeted chemical disinfection with alternative approaches which will control micro-organisms in the greater food processing environment, be it a wet, high care or dry production environment. This technique is termed "whole room disinfection". Novel disinfection techniques that are able to disinfect whole areas have been implemented in the pharmaceutical, clinical and now food sectors; one of those techniques is the use of ozone gas within a non condensing humidified atmosphere.

2.1 Ozone: what is it?

Ozone is the triatomic form of oxygen and is found in our atmosphere (the majority of this is in the "ozone layer"). It is unstable, naturally breaking down into molecular oxygen. The rate of break down is dependent upon environmental conditions such as temperature, humidity, and pollution but its approximate half life is 20 minutes in air and 30 minutes in water.

Ozone is a strong oxidiser and highly reactive. This, combined with penetrability and spontaneous decomposition into a non-toxic product, make ozone a viable disinfectant for use in food production areas.

2.2 Health and safety

Ozone is a toxic gas and worker exposure should be controlled. Table 1 gives examples of worker exposure limits for ozone in the US and UK. Many people can detect ozone in the air via smell at around 0.03 ppm, far below the recommended exposure limits, and at higher concentrations it can lead to headaches along with irritation to the eyes and respiratory tract.

Whilst ozone is a toxic gas, it is considered environmentally friendly as it readily breaks down to molecular oxygen (half life of approx. 20 minutes) and leaves no chemical residues.

Table 1: Ozone exposure (limits in US and UK)

USA	Occupational Safety and Health Administration (OSHA)	PEL is 0.1 ppm	OSHA 29 CFR 1910.1000(a)(2) Table Z-1
	Occupational Safety and Health Administration (OSHA)	STEL 0.3ppm	OSHA PEL Project Documentation 1988 TABLE AC-1 PERMISSIBLE EXPOSURE LIMITS FOR CHEMICAL CONTAMINANTS
	National Institute for Occupational Safety and Health (NIOSH)	Immediately Dangerous to Life or Health Concentration (IDLH) = Ozone 5 ppm Recommended STEL = 0.1 ppm ceiling	NTIS Pub PB-94-195047 (1995)
	American Conference of Governmental Industrial Hygienists	Heavy work: 0.05 ppm 8-hour TWA Moderate work: 0.08 ppm 8-hour TWA Light work:0.1 ppm 8-hour TWA All workloads: 0.2 ppm 2-hour TWA	American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value (TLV) (2001)
UK	HSE	STEL 0.2ppm LTEL (none given)	EH40/2005 Workplace exposure limits document
	UK National Air Quality Standards website*	air quality objective of 50ppb (0.05ppm) as the 8-hour mean	: www.airquality.co.uk/archive/standards.php#band

Legend

- *PEL Permissible Exposure Limit. An employee's exposure to any substance in OSHA Table z-1 shall not exceed the 8-hour Time Weighted Average given for that substance in any 8 hour work shift for a 40-hour work week.*
- *STEL Short Term Exposure Limit. An employee's exposure shall not exceed this Time Weighted Average over 15 minutes.*
- *TWA Time Weighted Average*
- *LTEL Long Term Exposure Limit*
- *ppm Parts per million*
- *ppb Parts per billion*
- ** This is an objective for "natural" ozone in the air and should not be used as an LTEL*

2.3 How is ozone made and applied?

Due to its reactive, unstable nature, ozone is produced at the point of use. Ozone generators effectively pass air or oxygen through a high-energy source within the equipment and the resulting physicochemical reaction leads to the formation of ozone that can be used for area or surface decontamination. Widely used high-energy sources include UV light (produce ≤0.5% ozone used in spas and swimming pool water) and electrochemical cells or corona discharge ozone generation (3-6% ozone). A corona is formed by an electrical discharge around a gas (often air but oxygen can also be used), which causes ionisation of the gas and consequently the formation of ozone. The production of ozone is most effective in a temperature-controlled environment, since the stability of ozone decreases as the temperature increases.

Its use typically involves humidification (70%-90% RH) of the environment followed by the application of ozone in the humidified environment, which is maintained for the required contact time, and finally reduction of ozone/humidification to normal levels, via either air replacement, natural breakdown or use of a catalyst system to actively break down ozone.

2.4 Anti microbial uses and microbial susceptibility

Surfaces can be treated using ozone dissolved in water, or as a gas in a humid atmosphere. Microorganisms inherently vary in their sensitivity to ozone, with factors such as temperature, humidity, the presence of chemicals, and the amount of organic matter surrounding the cell greatly affecting the degree of inactivation. At the concentrations typically used, ozone is an effective bactericide and virucide (Maillard *et al.* 2012, Hudson *et al.* 2007) whilst mycobacteria and bacterial spores have been shown to be less susceptible. Effective sporicidal activity is only seen at high relative humidity (75 to 95%) and high concentrations with long contact times (Dusseau *et al.* 2012). Yeasts and moulds have been reported to have a wide range of resistance profiles; however, ozone has been demonstrated to control post harvest spoilage of cereals, grains and fruit as well as reducing mould and yeast contamination of cheese during ripening (Boisrobert 2002, Siqueria and Botelho da Silva, 2008*).* However, mould spores are reportedly less resistant than bacterial spores to ozone and disinfectants generally.

A review of papers assessing the use of ozone applied as a gas in humid atmospheres for different applications is shown below (Table 2).

Table 2
Summary of literature search results looking at ozone applied via gas for surface disinfection in food industry (2000 – 2013)

Title	Author	Source	Notes	Year
Bactericidal properties of ozone and its potential application as a terminal disinfectant	Moore, G., Griffiths, C. and Peters, A.	Journal of Food Protection 63(8),1100-1106	2ppm ozone, 20ºC, 77%RH produced 2 - 7 log reduction against a range of bacteria in the presence and absence of UHT milk	2000
The evaluation of ozone on airborne and surface disinfection	Taylor, J. and Chana, D.	R&D report No 109 Campden BRI	Demonstrated efficacy against *P. aeruginosa* in the air and on stainless steel surfaces	2000
Inactivation kinetics of foodborne spoilage and pathogenic bacteria by ozone.	Kim, J.-G. & and Yousef, A.E.	Journal of Food Science, 65(3), 521-528.	Ozone was tested against .*P. fluorescens, E. coli* O157:H7, *L. mesenteroides* and *L. monocytogenes.* Survivor plots in the continuous system were linear initially, followed by a concave downward pattern. Exposure of bacteria to ozone at 2.5 ppm for 40 s caused a 5 to 6 log decrease in count. Resistance of tested bacteria to ozone followed this descending order: *E. coli* O157:H7, *P. fluorescens, L. mesenteroides*, and *L. monocytogenes.*	2000
US regulatory review of the use of ozone in the food industry	Biosrobert, C.	Agricultural and food processing applications of ozone as an antimicrobial	Reviews use of ozone to control spoilage organisms including fungi	2002
Gaseous ozone treatment inactivates *Listeria innocua in vitro*.	Fan, L., Song, J., McRae, K.B., Walker, B.A. and Sharpe, D	Journal of Applied Microbiology, 103, 2657-2663.	Average time for a 2 log reduction of *Listeria innocua* on solid media was 1.3 hours at 20^{o}C, and 2.5 hours at 5^{o}C	2007
Whole room disinfection - potential for environmental pathogen control	Malinowska, A. and Holah, JT.	New Food (no5) 2007 22-26	Ozone efficacy against *S. aureus, P. aeruginosa, L. monocytogenes*	2007
Use of ozone in food industries for reducing the environmental impact of cleaning and disinfection activities	Pascaul, A.., Llorca, I. and Canut, A.	Trends in Food Science & Technology 18 (Suppl. 1), 2007 s29-s35	Ozone use in meat processing plant and wineries	2007

Title	Author	Source	Notes	Year
Use of ozone in industrial cold rooms to control yeast and moulds during parmesan cheese ripening	Siqueria Lanita, C. de Botelho and da Silva, S.	Brazilian Journal of Food Technology; 11(3), 182-189	0.03mg/L ozone was shown to reduce air contamination and surface spoilage of product	2008
Reduction by gaseous ozone of *Salmonella* and microbial flora associated with fresh-cut cantaloupe	Selma,M.V., Ibanez, A.M,; Cantwell, M. and Suslow, T.	Food Microbiology; 25(4) 558-565	Gaseous ozone is an effective option in risk reduction and spoilage control of fresh and fresh cut melon. Ozone treatment combined with rapid drying reduces persistence of *Salmonella* on surface and reduces risk of transference from rind to flesh during cutting	2008
The case for ozone	Brandit, J.	Food Quality (Dec/Jan) 2009	Review	2009
Whole room disinfection	Middleton, K.	Food and Beverage International; Vol 8(6), 2009, 46-47	Efficacy of whole room disinfection methods including ozone	2009
Whole room disinfection	Middleton, K.	Campden BRI R&D report 299	Efficacy of whole room disinfection methods including ozone	2010
Application of gaseous ozone to inactivate *Bacillus cereus* in processed rice.	Shah N.N.A.K. Rahman, R.A. and Chuan, L.T.	Journal of Food Process Engineering 34(6), 2220-2232	Approximately 2 log reduction at 0.4ppm 20C 50%RH 420 min. 1.63 log reduction 0.3ppm 20C 50% RH 420 min	2011
Inactivation of *Listeria, Salmonella* Typhimurium and *Escherichia coli* O157H7 on surface and stem scar areas of tomatoes using in package ozonation	Xuetong Fan, Sokoral, K. J. B., Engermann, J, Gutler, J.B. and Yanhong Liyu	Journal of food protection; 75(9), 1611-1618	Bacteria responded differently to ozonation *Listeria* susceptible ≥4 log reduction within 40s *E. coli* and *Salmonella* 2-3 log reduction after 2-3 min (1000ppm *in-situ* after 1 minute)	2012
Mould control by ozonation in ripening cheese room	Troller Pinto, A., Scmidit, V. and Aparecida Raimundo. S.	Acta Scientiae Veterinariae, 35 (3), 333-337	Control of environmental and surface fungi 0.74 log on cheese surface, 0.91 log reduction on shelf surface and 1.5 log reduction in air	2013
Disinfection of selected vegetables under non-thermal treatments: chlorine, citric acid, ultraviolet light and ozone	Bermudez-Aguirre, D. and Barbosa-Canovas, G.V.	Food Control 29 (1), 82-90	5 ppm ozone demonstrated *E. coli* log reductions of 2.2 log on tomatoes, but affected greenness of lettuce	2013

It can be seen from the data in Table 2 that the efficacy of ozone like other disinfectants is dependent upon a range of factors: concentration, contact time, temperature, presence of interfering organic matter, and the target organisms.

However, all references demonstrate an added benefit when used in an appropriate manner.

In a number of tests *L. monocytogenes* seems to be the most susceptible organism tested

2.5 General consideration of use

The critical factors to address before using techniques such as whole room disinfection via gaseous or aerosolised disinfectants include:

- identifying areas where the decontamination processes can be applied
- health and safety issues related to using the technique, e.g. staff exposure
- effects on the fabric of the equipment and the building, e.g. potential corrosion.

These can be controlled through risk assessments and the implementation of management procedures to monitor ozone concentrations and dispersal (both of the areas treated and adjacent areas), control of access to treated areas, and the monitoring of efficacy.

The techniques can be used daily, weekly, or monthly, or on an ad-hoc basis as a reaction to a particular issue. The frequency of application, concentrations of ozone applied, contact time and target organism along with the type of environmental contamination to be encountered have all been shown to affect efficacy. Therefore, it is imperative that the method is validated in some way to be sure that it is effective.

3. SUPPORTING DATA

Various laboratory and field trials are quoted above in Table 2, which also includes laboratory and field trials carried out by Campden BRI.

3.1 Laboratory trials at Campden BRI

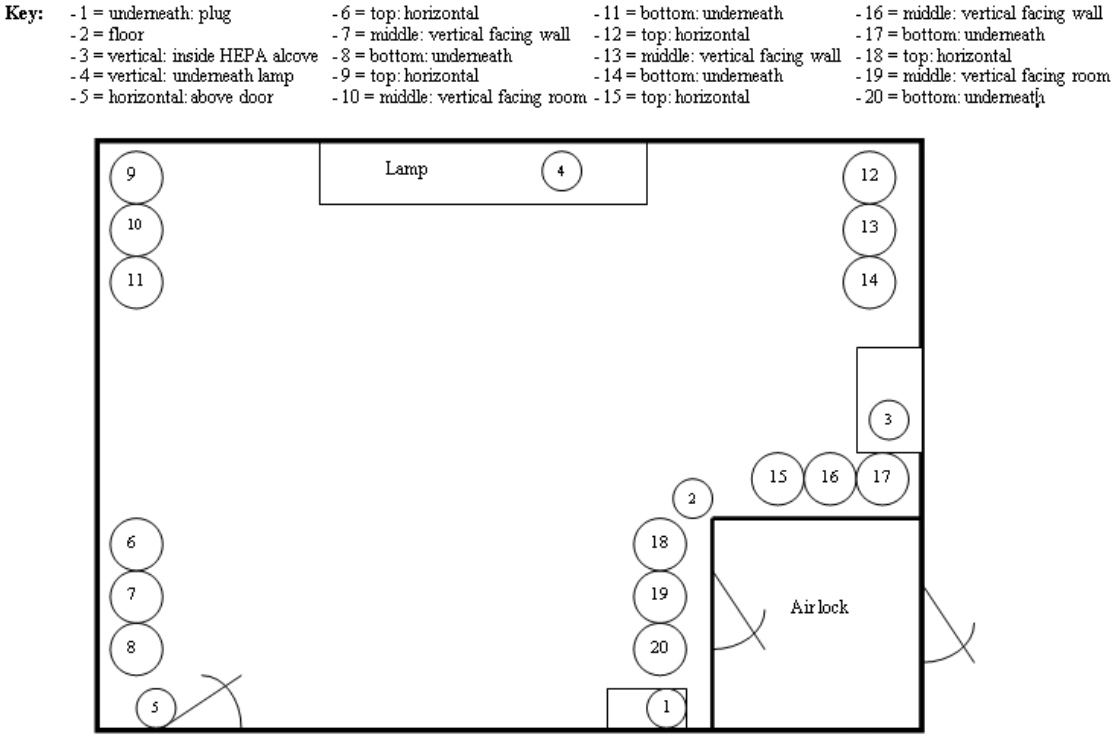
Laboratory trials (Malinowska and Holah 2007, and Middleton, 2010) looked at *Pseudomonas aeruginosa, Listeria monocytogenes,* and *Staphylococcus aureus.* The purpose of these laboratory trials was to further develop the method to examine whether the systems under assessment were able to decontaminate surfaces, irrespective of orientation, throughout the whole room, providing similar levels of log reduction, and to determine ozone efficacy. The laboratory trials protocol was based on the European Norm surface disinfectant test method BS EN 13697: 2001 - Chemical disinfectants and antiseptics - Quantitative non-porous surface test for evaluation of the bactericidal activity and/or

fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas (Anon 2001). In these experiments 0.05mL bacterial suspension (of approximately 1.0E+07 CFU/mL) was dried onto stainless steels surfaces (2cm diameter 1.4301(EN 10088-1): stainless steel discs with grade 2B finish on both sides (in accordance with EN10 0088-2, gauge 1.2 mm – 1.5mm).

The discs were placed in locations shown in Figure 1.

The ozone generator was placed in the centre of the room with the dispensing head approximately 1.2m from the floor. The ozone generator was activated to implement the required decontamination process.

Figure 1 - The arrangement of test surfaces in the aerobiology laboratory



During experiments, all ventilation systems in the laboratory were switched off and the room was effectively sealed to outside air movements. Any internal air currents during the trials were created by the operation of the technology under test.

The treated discs and untreated control discs were recovered into 10mL neutraliser broth with glass beads and the surviving CFU/mL were determined.

3.2 Field trials

Field trials of 1-3 treatment applications were conducted by Campden BRI in conjunction with a manufacturer of ozone in representative factories of the RTE, poultry and dry food industry (Malinowska and Holah 2007). The results from these trials were varied. In addition one longer term 4 week trial in a sandwich factory was done.

In these field trials the methodology was based on procedures developed by Campden BRI (TES-FH -013-PART2).

An independent factory validation trial (not undertaken by Campden BRI) was also carried out in two dough factory sites. This data was kindly provided by the factory involved for publication by Campden BRI.

3.2.1 Factory: Pizza manufacturer (Malinowska and Holah, 2007)

Test site: High care cook-house, room size 75m^3.
Trial protocol: the trial was conducted at the end of production on three occasions. The environment was cleaned with detergent and rinsed and then terminal disinfectant treatment was replaced by ozone treatment.

Ozone treatment: 8ppm for 40 minutes + quench.

A total of 15 swabs and 15 contact plates (Table 3.2.1) were taken as detailed below. The sites were sampled by Campden BRI staff, who took samples before cleaning, after cleaning and after disinfection.

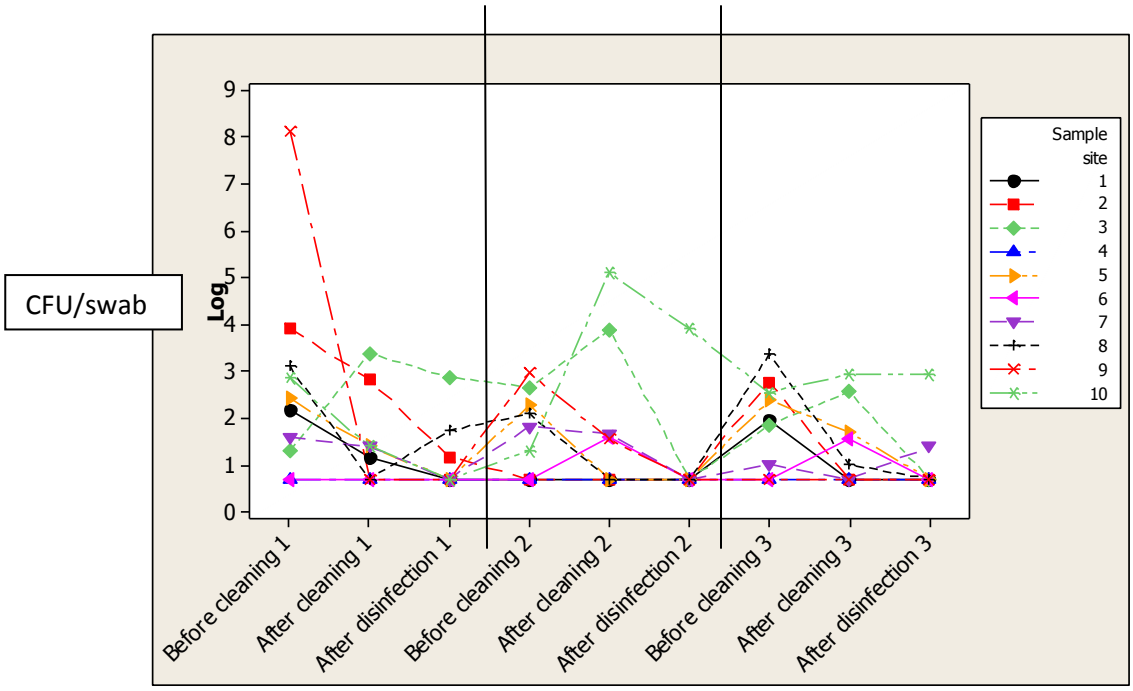


Figure 3.2.1a - Effect of gaseous O_3 treatment on microbiological log reduction of food contact surfaces within a pizza manufacture facility (Log CFU/swab recovered)

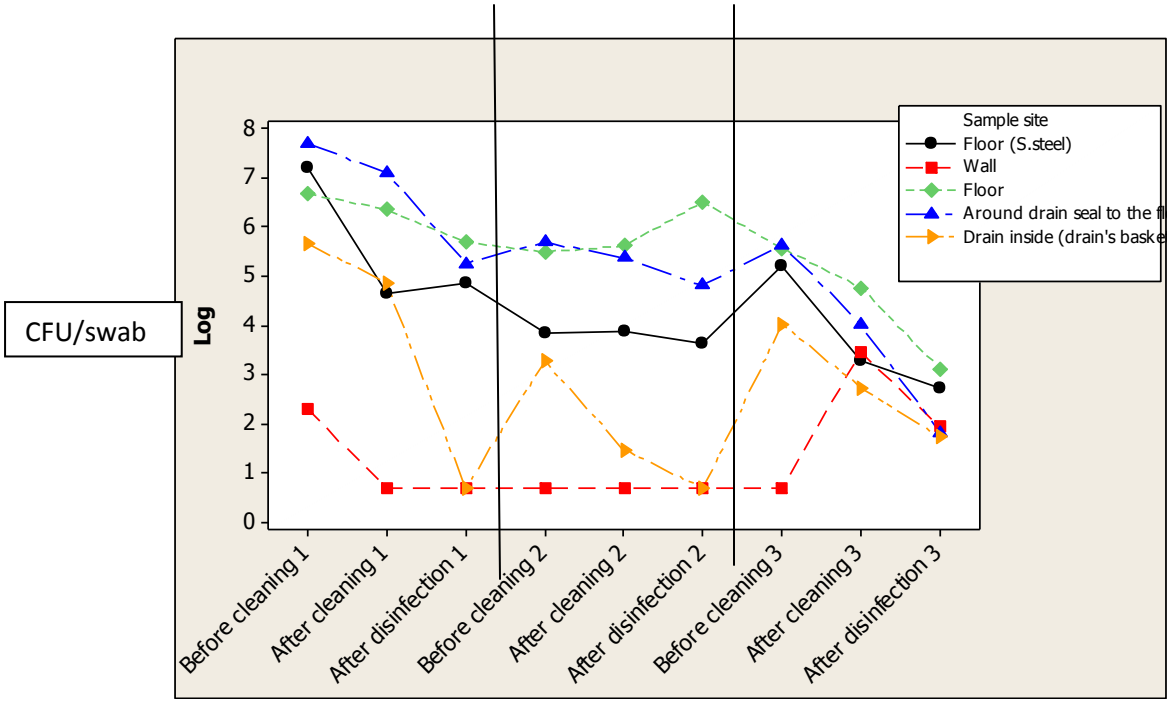


Figure 3.2.1b - Effect of gaseous O_3 treatment on microbiological log reduction of environmental surfaces within a pizza manufacture facility (log CFU/swab recovered)

3.2.3 Factory: Validation of an installed system in two dough manufacturing halls (2013)

Test site: Two manufacturing halls for two types of dough.
Trial protocol: the trial was conducted at the end of production. The environment was a dry food manufacturing one (normal relative humidity of 35%) and was appropriately cleaned prior to ozone treatment application once per week. The site considered that it had a number of persistent strains of *Listeria* spp. For the year prior to and during the trial there was no change in production volumes, methods of production or sanitisation (excluding the use of ozone) or number of staff in the areas.

Ozone treatment was 6ppm at 80% relative humidity for 4 hours with natural ozone break down (generation time 25 minutes and 4.5 hours typical natural breakdown), with a total treatment time of approximately 9.25 hours

For both halls ozone treatment was used weekly from week 1 through to week 16.

There followed a break in production between weeks 17–25 and then production was restarted in week 26. However, weekly ozone treatment was not restarted until week 35.

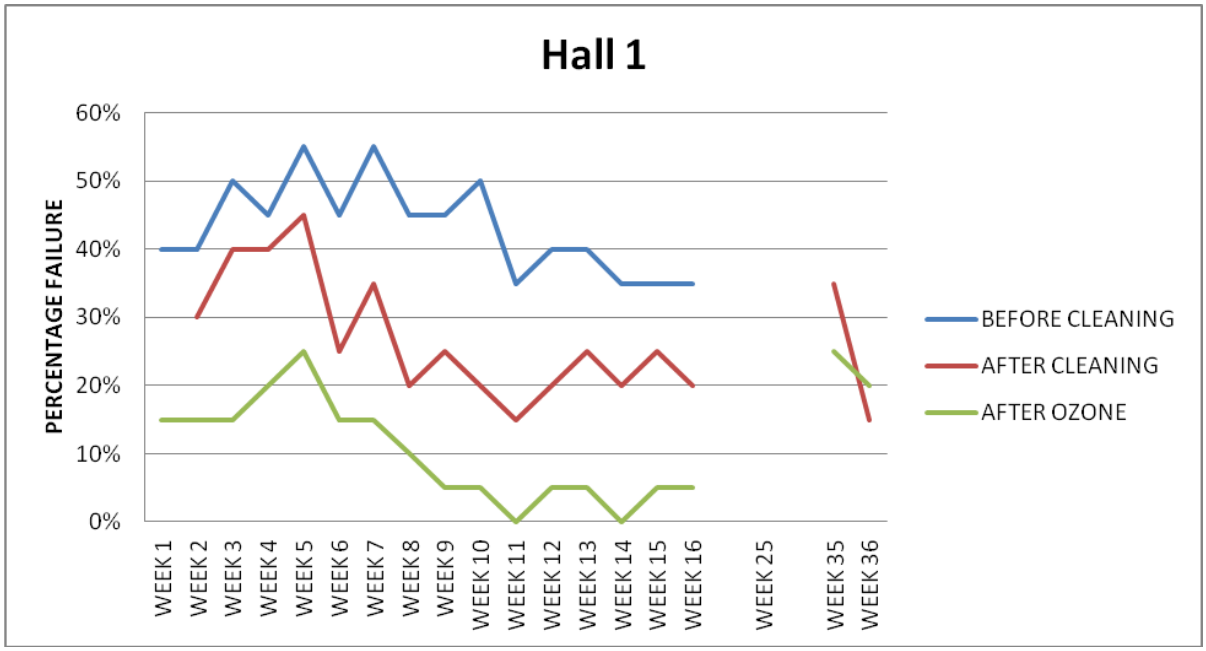
A total of 20 sites per production area (Tables 3.2.3a & b) were tested for *Listeria spp* using "3M™ Petrifilm™ Environmental Listeria Plate". The percentage positive sites found were recorded over a 15 week period and are graphically represented below (Graphs 3.2.3a and 3.2.3b).

The results over the first 16 weeks of both trials demonstrated an overall downward trend in pre and post clean positives. The post ozonation reductions demonstrated that ozone is effective in reducing *Listeria spp* in the environment compared with post cleaning, therefore reducing the risk of cross contamination.

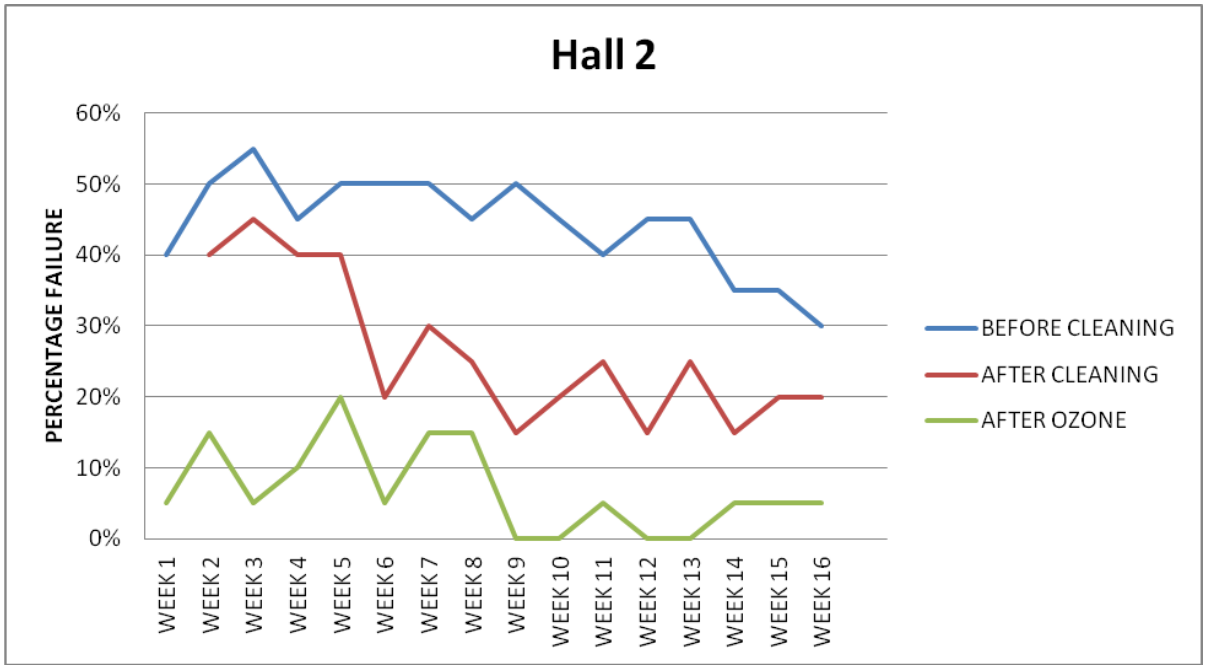
After week 16 there was an extended break in ozone treatment (week 17 – 35). This demonstrated that *Listeria* % positive sites returned to pre ozone treatment levels. However, Campden BRI has been informed that its re-instigation in one Hall "brought the *Listeria spp* positives down to levels seen prior to stopping production and ozonation, i.e. week 16".

Campden BRI had no part in the field trial and the data has kindly been released to us for publication by the validating factory. The samples were taken by trained factory personnel before and after cleaning and after ozone application. The Petrifilm results were analysed by the company in question.

Graph 3.2.3a Hall 1: % Listeria spp. positives over 16 week period



Graph 3.2.3b Hall 2: % Listeria spp. positives over 16 week period



4. CONCLUSIONS

Gaseous ozone in a high humidity atmosphere has been shown to reduce the population levels of a range of environmental and pathogenic organisms, both in laboratory trials when dried on to surfaces and in field trials carried out in various production facilities (e.g. high care sandwich making or dry food production areas such as dough manufacturing).

There is a relationship between ozone concentration, contact time, type of micro-organisms present and log reduction achieved; however, in laboratory trials there seems to be no practical difference in the reduction achieved due to test surface location or orientation, or in restricted access exposures (w-tubes – laboratory trials).

The results suggested that, for each microorganism tested, it could be possible to describe a relationship between ozone concentration and exposure time that can be described as an ozone dosage.

As an overall conclusion from the laboratory trials ozone has several advantages; it can effectively penetrate every part of a room, including sites that might prove difficult to gain access to with conventional liquids and manual disinfection procedures. The major disadvantage of using gases, such as ozone, is the potential toxicity at high concentrations, which precludes using them in areas where people are working. The technique can therefore only be used in areas that can be isolated and sealed off during the decontamination process.

The number of treatments over time has been shown to be important for food contact and environmental surfaces. Single applications or applications over two consecutive days were shown to have a limited effectiveness (Malinowska and Holah, 2007). However, studies carried out over longer periods (≥3 applications) demonstrated a downward trend after applications over 3 consecutive days use (3 applications total).

The results for the pizza factory (3.2.1) after 3 days indicated a downward trend in the numbers of microorganisms present on food contact and environmental surfaces, both before cleaning and after cleaning and disinfection, throughout. This was supported by the 4 week trial at the sandwich factory (3.2.2) and further supported by two 16-week field trials in a dry foods dough manufacturing facility (3.2.3).

Ozone generation equipment manufacturers have postulated that when ozone is first applied to a cleaned room, there is a mass of organic material that creates an ozone demand which must be satisfied by oxidation before any significant oxidation of microorganisms can occur. In essence, this is no different from the effect of organic matter on traditional oxidising chemical disinfectants, e.g. a chlorine organic break point in water treatment.

During the 4 week (3.3.2: 8 ppm 30 minutes) and 16 week (3.3.3: 6 ppm 4 hours) trials no adverse effects were observed by ozone on the structure and fabric of the building. The management of the factory have also reported no adverse effects over the time the ozonation equipment has been installed.

The results of field trials demonstrate that, to be effective in a production environment (even one that has been cleaned), ozone requires at least 3 applications; however, once it starts to be effective successive cleaning and ozone use results in a continuous downward trend in counts which carries over, reducing detectable organisms in the environment prior to cleaning/disinfection (Graphs 3.2.3a & b). If the application is stopped levels of detectable pathogens can increase (Graph 3.2.3a).

Overall, therefore, the results of available laboratory data and field trial studies demonstrate that ozone has the potential to be an effective environmental disinfectant. However, any use of ozone as an addition to normal cleaning and disinfection practices or a replacement for chemical disinfection must be appropriately validated for each factory situation.

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