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Use of Ozone in Meat Processing Premises Literature Review

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EXECUTIVE SUMMARY

- The literature reviewed the use of ozone as a disinfectant/deodouriser for water, air, surfaces, equipment and product.

- Germicidal effects of ozone are influenced by contact time, temperature, relative humidity, pH value and presence of inorganic and organic materials

- Many different ozone concentrations and contact times were investigated for the above applications. No conclusion was drawn as to the optimal ozone concentration or contact time from the literature.

- AQIS have limited ozone exposure of employees to 0.1ppm at any time. This limits the use of ozone during working hours when employees are present.

- In high concentrations, ozone is corrosive to rubber, iron and some plastics, however it does not seem to affect painted metals, vinyl, melamine laminates, concrete or coated aluminium.

- Ozone is beneficial for air decontamination in closed boning rooms, slaughter floors, chillers and transport vehicles. Glengor Pastoral Company, Gosford, operates 9 ozone generators continuously in the boning room, chillers and carton store room to a concentration of 0.1ppm. They have noticed a decrease in the microbiological condition of surfaces and in the amount of dust in the carton store. It may be possible to increase the concentration after cleandown to approximately 5ppm for overnight decontamination. A timer to switch off at least 1 hour before work commences would allow the ozone to dissipate before employees arrived.

- Ozone was reported to be an effective sanitation method for food processing equipment. There is potential for use of ozone as an alternative disinfectant to chlorine in the meat processing industry. Further research is needed, however, to determine optimal ozone contact time, the effect of ozone on different materials of construction, cost effectiveness of alternatives and safety to workers.

- Slaughterhouse effluent contains many pathogenic organisms. Ozone was found to have potential benefit for the chemical disinfection of effluent.

- Ozone application to fresh meat as a gas or in solution has benefits regarding retardation of bacterial growth, however, the concentrations required for optimal destruction of microorganisms may cause undesirable aesthetic and evaporative losses during storage, with no increase in shelf life of steaks after processing.

- Current practices of trimming and washing or washing with hot water have just as good if not better ability to reduce microbiological contamination as ozone on freshly dressed carcases.

Recommendations

1. A trial should be conducted to assess ozone as an air and surface decontaminant in closed chillers, boning rooms, slaughter floors and transport vehicles. Optimal ozone concentration and contact times should be established in such a trial.

2. A trial should be conducted to compare ozonated water and chlorinated sanitisers for plant disinfection at cleandown. Optimal ozone dosage rates would need to be determined along with any corrosive effects on surface materials examined. A cost analysis to determine the overall benefits of ozone usage would also need to be considered. Health and safety data regarding the effects of inhalation of fine aerosols of ozonated water would also need to be investigated, however, an experiment of this type may be out of the scope of projects funded by MRC.

3. As ozone is a known deodouriser, and no published literature was found evaluating its use in rendering plants, research to determine the effectiveness of ozone in this capacity should be considered.

4. Although ozone was found to have potential for the chemical disinfection of effluent, practicalities of using this technology in industry is such that benefits may be outweighed by other factors (particularly cost). It is recommended that a project of this nature be given low priority.

5. No further research is justified as to the use of ozone as a decontaminant of carcase surfaces, unless combined with the trial in Recommendation 1.

6. No further research is justified to determine the effectiveness of ozone as an alternative treatment for elimination of vermin.

1. INTRODUCTION

Ozone is a colourless to slightly bluish gas with a characteristic odour commonly experienced as the "fresh" odour accompanying thunderstorms. Ozone is formed when oxygen is exposed to UV irradiation or an electrical charge. The oxygen molecules (O_2) split to form ozone molecules (O_3). This is a very unstable arrangement and the third oxygen molecule will split off to oxidise the first pollutant with which it comes in contact. The pollutant is destroyed, and oxygen remains.

Because of its powerful oxidising ability, ozone has been recognised since the early 1900's as an effective disinfectant, deodouriser and antipollutant. It will disinfect air, destroy bad odours, toxic fumes, bacteria, algae, fungi, mould and mildew. It is more widely used commercially in the water and wastewater industries for purification and disinfection purposes than as a disinfectant in the food industry.

As an alternative to chlorine, ozone has been reported to have many beneficial characteristics that make it attractive for use in the food industry, subject to cost effectiveness. Potential uses for ozone in the meat industry include:

- Decontamination of product
- Decontamination of surfaces and equipment in vehicles, boning rooms, slaughter floors and chillers
- Disinfection of effluent
- Deodorisation of rendering areas
- Vermin treatment

The main disadvantage to the use of ozone in Australian meat processing premises is that it is toxic to humans in high concentrations and has been reported to produce undesirable qualities in meat such as discolouration and excessive carcase shrinkage.

This report aims to review the uses and potential benefits of ozone as well as the OH & S risks associated with its use. It has been funded by the Meat Research Corporation (MRC) in order to determine whether further research work is warranted for the use of ozone as a decontaminant and deodourant of product, air and equipment in Australian meat processing premises.

2. PROPERTIES OF OZONE

Ozone gas is a powerful oxidising agent that decomposes rapidly in air and in water, leaving only oxygen. The germicidal effects of ozone are affected by contact time, temperature, relative humidity, pH value, and presence of inorganic and organic materials. It has a "pleasant" odour at concentrations of less than 2ppm (ICMSF, 1980; NTP Chemical Repository, 1991) but a pungent odour at higher concentrations, which can produce respiratory symptoms and irritation of the eyes and mucous membranes (NTP Chemical Repository, 1991; CSIRO, 1997).

Ozone is more stable in air than in water. In air, decomposition is accelerated with increasing relative humidity. In water, its stability increases with low pH values and low temperatures.

Its efficacy as a bactericide is influenced by organic matter content and at low levels of organic matter, concentrations of ozone of <5ppm can destroy microorganisms. However, ozone concentrations as great as 500ppm have been reported to be ineffective at meat surfaces where microbial cells are protected (Yang and Chen, 1979$^{(1)}$). This is attributed to a possible protective effect of meat due to interactions between ozone, fat and protein.

The ozone molecule is an unstable arrangement and is readily converted to the more stable oxygen molecule. Thus, it has a short half life and does not persist in the environment. The half life of ozone has been reported to be as little as 20 minutes in water (Bott, 1991) or as long as 12 hours in air (Graham, 1997). The actual half life is influenced by the amount of organic material present in the food, water or air.

It has been reported that at high concentrations and long contact times ozone can lead to rancid flavours in edible fats (ICMSF, 1980) and cause discolouration and drying in carcases (Kaess and Weidemann, 1968; Greer and Jones, 1989).

Although use of ozone does not result in the formation of toxic byproducts, if the ozone is generated by electrical discharges, poisonous nitrous oxides are produced. Personal communication with representatives of Ozone Applications Pty Ltd and Ozonclean Air Systems has confirmed that *Ozonaire* brand ozone generator machines produce ozone by exposure to UV light. This method does not produce any toxic compounds.

3. TOXICITY OF OZONE

3.1 EFFECT OF OZONE ON HUMAN HEALTH

Ozone is irritating and injurious to humans at concentrations >1ppm (NTP Chemical Repository, 1991). On the basis of animal data, exposure at 50ppm for 60 minutes will probably be fatal to humans. (NIOSH, 1996). Inhalation causes irritation to the upper and lower respiratory tract. As concentration increases, the ability to detect odours decreases (CSIRO, 1997). Exposure to >1ppm may cause headache, upset stomach, vomiting, pain or tightness in the chest, shortness of breath and/or tiredness. High concentrations may cause severe lung damage and death may result. There are no known effects of ozone on skin or from swallowing ozonated water, but it can produce local irritation of the eyes (NTP Chemical Repository, 1991; CSIRO, 1997).

The US EPA National Ambient Air Quality Standards (NAAQS) in setting their current standard of 0.08ppm averaged over 8 hours have recognised that there is no discernible threshold below which no adverse health effects occur, i.e. no level would eliminate all risk. Therefore a zero standard for exposure to ozone is not applicable nor possible. However, 0.08ppm over 8 hours is based on judgement that at this level, public heath will be protected with an adequate margin of safety. Exposure of humans to 0.08ppm for 6.6 hours was sufficient to initiate an inflammatory reaction in the lung (Devlin et al, 1991). Hatch et al, 1994 states that greater than half of the US population live in areas that exceeded previous NAAQS levels for ozone of 0.12ppm. The (American) National Institute for Occupational Safety and Health (NIOSH) have set a limit of 5ppm as being "IDLH" (Immediate Danger to Life and Health) (NIOSH, 1996).

There is no Australian Standard as such for Ozone exposure. However, Worksafe Australia has set 0.1ppm as the acceptable maximum exposure standard adopted from the National Commission's *Adopted National Exposure Standards for Atmospheric Contaminants in the Occupational Environment* [NOHSC:1003(1995)] (Worksafe Australia, 1995). It is assumed that the standard is based on the previous American NQAAS standard of 0.12ppm not exceed for more than 1 hour more than 3 times in 3 years.

As a component of air pollution (smog), ozone has been linked to increased hospital admissions in the US for respiratory ailments such as asthma. Studies in the US and Canada have shown that ozone air pollution is associated with 10-20% of all summertime respiratory related hospital admissions. Smog is produced by the photochemical exposure of organic hydrocarbons (eg from car exhausts, industrial processing plants) which produces ozone as well as other compounds. It is unclear whether sensitivity to smog in some people with respiratory ailments is caused purely by ozone or as a combination of ozone and other compounds present in smog.

ChemWatch (1997) have stated that 0.1ppm will be tolerated by most workers including asthmatics and exposure to 0.2ppm will produce mild acute but not cumulative effects. They also provide exposure standards for different levels of work. The following table lists the different exposure standards (referenced to the American Conference of Government Industrial Hygienists [ACGIH]) stated by ChemWatch (1997):

Type of work	Exposure Standard (ppm)
Heavy	0.05
Moderate	0.08
Light	0.10

Repeated exposure to ozone can make people more susceptible to respiratory infection and lung inflammation and can aggravate preexisting respiratory diseases such as asthma. (EPA, 1997). Long term exposure to ozone can cause repeated inflammation of the lung, impairment of lung defense mechanisms and irreversible changes in lung structure, which could lead to premature aging of the lungs and/or chronic respiratory illnesses such as emphysema and chronic bronchitis.

The mechanisms of ozone toxicity appears to be related to attack on cellular membranes, resulting in lipid peroxidation and production of free radicals. It is likely that exposure to ozone results in damage to the cells and fluid components lining the lung (Devlin et al, 1991). There is considerable individual variation in response to a wide range of ozone concentrations. Some sub-populations are not affected at all (Devlin et al, 1991; Gerrity et al, 1994; Folinsbee et al, 1994). Previous studies have indicated that as much as 20% of the population does not experience significant pulmonary functional decrement even at relatively high ozone concentration (Folinsbee et al, 1994)

AQIS have approved the use of ozone in Australian meat processing premises. They have approved use based on the guarantees of ozone generator manufacturers as to the benefits and safety of ozone. They have not independently investigated these issues for use of ozone in Australian meat processing premises (Denis Clancy, AQIS Area Technical Manager, Sydney *pers comm*). As long as OH & S principles are met (ie employees are not exposed to greater than 0.1ppm ozone at any time) and as long as the generator itself is not a source of contamination (ie it does not harbour dust and vermin) then use of ozone generators is up to individual companies.

3.2 EFFECT OF OZONE ON MATERIALS OF CONSTRUCTION

In general, where an oxide layer is responsible for corrosion protection the presence of ozone is likely to be beneficial (Bott, 1991). Ozone is reported to be incompatible with oxidising agents such as chlorine and bromine (NTP Chemical Repository, 1991). The actual effects of incompatibility are thought to be simply that the stronger oxidiser (ozone) will override the effect of the weaker oxidiser (chlorine) (*pers. comm.* Graeme Price, Ozone Applications Pty Ltd). It is also incompatible with tetrafluorohydrazine, a refrigerant gas (NTP Chemical Repository, 1991) however, if refrigeration units are correctly sealed there should be no contact between the two gasses. Tetrafluorohydrazine is now obsolete as a refrigerant gas so contact with ozone will not be a concern. Ozone is corrosive to rubber, iron and some plastics in high concentrations (Masaoka et al, 1982, *pers. comm.* Graeme Price, Ozone Air Applications Pty Ltd). It does not seem to affect stainless steel (Masaoka et al, 1982; Greene et al, 1993), glass (Masaoka et al, 1982; Bott, 1991), painted metals, vinyl, melamine laminates (Masaoka et al, 1982) coated aluminium or concrete (*pers. comm.* Graeme Price, Ozone Air Applications Pty Ltd).

As with other chemicals, the extent of corrosiveness will depend on the concentration and contact times used. In a study by Masaoka et al (1982), ozone applied at a concentration of 40ppm for 72 hours, produced marked corrosion of rubber and caused iron nails to rust. However, no change was observed in painted metals, stainless steel, vinyl, or melamine laminates. At concentrations required for use in meat processing plants for disinfection of air and surfaces, the corrosiveness of ozone is thought to be nil to slight (*pers. comm.* Donald Campbell, Ozonclean Air Systems). This argument would need to be confirmed by independent research.

3.3 EFFECT OF OZONE ON VERMIN

Vermin (eg flies, cockroaches) are repelled by ozone at concentrations of less than 1ppm (*pers. comm* .Donald Campbell of Ozonclean Air Systems). Although not killed, they are repelled by the odour of ozone. He quoted a testimonial from a client to this extent but no published literature was found to either confirm or refute this argument.

3.4 SUMMARY

The following table summarises beneficial and detrimental characteristics of ozone:

Advantages	Disadvantages
More powerful oxidiser than chlorine.	Corrosive to some materials of construction eg copper, rubber, and some plastics in high concentrations.
Does not produce toxic compounds like chlorinated compounds.	Toxic to humans in high concentrations.
Unstable, has a short half life and reverts back to oxygen.	Cannot be stored – must be generated before use (requires energy and capital expenditure).
Does not persist in the environment or in food products.	Can cause discolouration and drying of fresh meat in high concentrations.

4. OZONE IN MEAT PROCESSING

4.1 WATER DISINFECTION

Ozone has been used to disinfect, to remove colour, odour and turbidity and to reduce the organic loads of European wastewater treatment plants since 1906 (Sheldon and Brown, 1986). Recently, ozonated water has been used as a sanitiser by soft drink bottlers, and it has been approved by the USDA for use in treating poultry chilling water (Greene et al, 1993).

Many cities in France use ozone as standard practice in the purification of potable water (Graham, 1997; *pers. comm.* Donald Campbell, Ozoneclean Air Systems). Numerous other cities in European countries such as the Netherlands, Germany, Austria, Switzerland use ozone for the same purpose. In 1987, more than 200 potable water treatment plants in the US were using ozone (Graham, 1997).

Several workers have investigated the use of ozone for the treatment of poultry chilling water. Studies have demonstrated microbial reduction as well as chiller water quality improvement. A chiller water recycling unit takes water from the poultry chiller overflow, cleans it and returns it to the chiller tank. The system is designed to improve the microbial quality of the product and save the processor energy, water, and sewer costs through eliminating a major portion of the chiller fresh water consumption (Anon, 1994). As well as poultry chilling water, Bohm (1989) has conducted research into the use of ozone for treatment of slaughterhouse effluent.

4.1.1 POULTRY CHILLING WATER

Ozone was evaluated in studies by Sheldon and Brown (1986) and Jindal et al (1995) for treatment of poultry chilling water to:

- improve microbiological quality of product
- improve shelf life of product after treatment
- improve water quality of poultry chiller water

Both studies reported greater reductions of bacterial numbers in water than on poultry meat.

Sheldon and Brown (1986) treated spent poultry chilling water with up to 15ppm ozone for 60 minutes (unknown temperature). After treatment of the water, Aerobic Plate Count (APC) was reduced from log 7-8 to log 1. Coliforms, *E. coli*, and *Salmonella sp* were reduced by 2.3 log cycles to not detectable. They also reported significant improvements in water quality. Decreases of one third in the Chemical Oxygen Demand (COD) and significant increases in % light transmission of the water were reported after treatment.

Jindal et al (1995) ozonated poultry chilling water for treatment of poultry meat with approximately 0.5ppm ozone for 45 minutes at 0-4°C. After treatment, APC of ozone treated water compared to non treated water was significantly reduced by approximately 2.3 log cycles. Coliforms, *E. coli* and *Pseudomonas aeruginosa* in the water were also significantly reduced by approximately 2 log cycles to not detectable.

Yang and Chen (1979$^{(1)}$; 1979$^{(2)}$) conducted research on destruction capability of ozone when applied to a microbial suspension. They found that the type of microorganism in the suspension, the temperature and pH of the liquid influenced the destruction capabilities of ozone. Higher ozone concentration and longer contact times were needed for destruction of microorganisms from fresh poultry meat than for spoiled poultry meat. Organisms associated with spoilt poultry were considered to be predominantly gram negative organisms (which include the *Enterobacteriaciae* and *Pseudomonas spp*). At temperatures of 2°C and 25°C, an ozone concentration of 37.7ppm for 10 minutes reduced counts by approximately 3.6 and 1.0 log respectively. At pH values of 3. 5, 7, 9 and 11, counts were reduced by 4.7, 3.2, 3.2, 3.2

and 1.2 log respectively when treated with 2.5ppm ozone for 5 minutes (Yang and Chen,1979$^{(2)}$).

Conflicting results regarding the effectiveness of ozonated chiller water for disinfecting poultry meat have been reported. Jindal et al (1995) reported ozonation of chiller water extended the shelf life of poultry meat by 1 - 2 days (approximately 0.5ppm ozone for 45minutes at 0-4°C). However, Sheldon and Brown (1986) reported that ozonation failed to extend refrigerated shelf life. These differences in shelf life extension as reported in the literature are most probably due to differing methods used in each study.

Sheldon and Brown (1986) found that significant reductions ($P<0.07$) in both spoilage and pathogenic microorganisms on carcases were observed after treatment with ozonated chiller water at 4.0 – 4.5ppm for 45 minutes at 7°C. No significant difference in skin colour, lipid oxidation, off flavours or sensory attributes resulted from ozone contact at these concentrations and contact times. However, it was found that over 11 days of storage, there was no significant difference in bacterial numbers between treatments.

Yang and Chen (1979$^{(1)}$) and Kaess and Weidemann (1968) found that after ozone treatment the lag phase of bacteria increased but growth rates of surviving organisms was not affected.

4.1.2 SLAUGHTERHOUSE EFFLUENT

Slaughterhouse effluent was found to contain many pathogenic microorganisms. Among these are *Salmonella, Yersinia enterocolitica, Listeria monocytogenes, Bacillus anthracis, Campylobacter sp, E. coli, Clostridium. perfringens, Staphylococcus sp*, parasitic protozoa, cestodes and nematodes (Bohm, 1989). The treatment and disposal of effluent should be of concern to the meat industry in general from both a public health and environmental point of view. Personal communication with meat industry workers has revealed that slaughterhouse effluent is treated in many different ways from no treatment and direct release to the environment to settling ponds, anaerobic and/or aerobic treatment before release either to the environment and/or waterways.

Bohm (1989) reviewed several methods of effluent treatment – chlorine, ozone, peracetic acid, lime, filtration, and heat. He concluded that chlorine was effective but it was difficult to know proper dose rates to give a residual compatible for release to the sewage system (if applicable) due to differing organic loads. Chlorine treatment was not recommended because of the increased danger of accidents when using concentrated chlorine and because of formation of toxic organic chlorine compounds. Disinfection with ozone was found to be much less problematical. Ozone dosage was 20ppm for at least 30 minutes. Ozone treatment also improved BOD, COD and odour and was therefore recommended for treatment of prepurified slaughterhouse effluent. No data were given for the reductions in number of pathogenic microorganisms after ozone treatment. Of the other methods investigated, peracetic acid required high dose rates, with similar risks as chlorine. Lime required long holding times and neutralisation of pH after treatment. Filtration methods for eliminating pathogenic microorganisms were not promising and only ultrafiltration was totally effective in removing all pathogenic microorganisms, which would be impractical. The use of heat for disinfection required high energy. Bohm concluded that ozone treatment offered the greatest advantages for the chemical disinfection of slaughterhouse effluent.

Associate Professor Mike Johns of the Chemical Engineering Department of the University of Queensland believes that disinfection of effluent from meat processing establishments with any chemical would be a cost burden the meat industry could not meet. Unless specific regulations were introduced where disinfection was mandatory, industry would not voluntarily disinfect effluent. If mandatory disinfection of effluent was introduced, the result would be that many meat processing establishments would be forced to close because of the cost burden (*Pers comm.* Assoc. Prof. Mike Johns).

4.1.3 SUMMARY

Ozone has been recognised since 1906 for its ability to purify water and wastewater by removal of bacteria, fungi, algae and odours. The ability of ozonated water to improve water quality of poultry chiller water and slaughterhouse effluent was described.

It can be concluded that ozone is a suitable treatment process for reducing spoilage and pathogenic microorganisms in poultry chiller water (in the range of 0.4 – 4.5ppm).

Slaughterhouse effluent was identified as an important source of pathogenic microorganisms and that effluent should be treated in some way so as to inactivate these organisms. Although no data was given as to efficacy of ozone at the concentration and contact time stated, ozone was concluded to offer the greatest advantages for the chemical disinfection of effluent. Further investigation to confirm these findings and to determine the optimum conditions necessary for the disinfection of slaughterhouse effluent would be of benefit. The practicalities, however, of applying the knowledge to industry are such that any benefits may be outweighed by other factors (particularly cost) which industry could not support at this time.

4.2 SURFACES AND EQUIPMENT DISINFECTION

Food processing equipment becomes contaminated with microorganisms due to the presence of food residue and suitable growth conditions. Sometimes, cleaning procedures cannot reach into crevices which leads to food material buildup. This provides ideal conditions for bacterial growth.

Chlorine is very effective and often the disinfectant of choice for surfaces and equipment in these situations. However, there are environmental problems associated with the use of chlorine such as the production of chlorinated organic compounds (considered to be carcinogenic) which persist in the environment. Transportation of concentrated chlorine is very hazardous. Thorough rinsing is required after disinfection to remove the chlorine residual, which may impart undesirable flavours to the food (leading to water wastage).

Greene et al (1993) ranked disinfectants in the following order of decreasing efficiency:

- Ozone
- Chlorine dioxide
- Hypochlorous acid
- Hypochlorite ion
- Dichloramine
- Monochloramine

Bott (1991) and Greene et al (1993) investigated the use of ozone as a disinfectant to remove bacterial biofilms from surfaces.

Bott (1991) investigated the potential for ozone treatment at relatively low concentrations on its potential as a disinfecting agent. Water contaminated with *Pseudomonas spp* was circulated through glass tubes to produce a biofilm (determined by weight). Ozone was applied as an aqueous solution at a concentration of 0.069 – 0.081ppm. The data demonstrated the effectiveness of the ozone treatment in removal of the biofilm, even using these relatively low dose concentrations. Virtually complete removal of biofilms up to 140*um* thick in up to 5 hours was achieved. Biofilms of the thickness developed and tested in this work are unlikely to be encountered in the food processing industry (disinfection would be required long before biofilms reached these proportions). Therefore, in a real life situation, the time required for disinfection would be reduced due to less organic material and increased ozone concentration could also reduce disinfection times. No wide-ranging testing was carried out in order to provide dosing strategies for industrial systems. It was also recognised that glass was not a practical surface in food situations and differences would occur between adhesion of microorganims between glass and other materials (such as stainless steel). It was concluded that ozone would be a valuable chemical to combat the accumulation of bacteria residing on surfaces and in crevices of food processing equipment.

Greene et al (1993) compared the effectiveness of chlorine and ozonated water against biofilms of milk spoilage bacteria on stainless steel plates. In this study, biofilms were allowed to develop on stainless steel plates. The plates were then either rinsed in sterile saline, chlorinated sanitiser (concentration of 100ppm for 2 mins), or ozonated water (concentration 0.5ppm for 10 mins). The results indicated that both chlorine and ozone destroyed or inhibited >99% of the bacteria on the stainless steel plates (an average of 4.4 log reduction for chlorinated sanitiser and 5 log reduction for ozone). The study concluded that ozone was effective in destroying surface attached bacteria, even at high cell densities and in the presence of high organic material. Advantages for the use of ozone included:

- Ozone requires no heat and therefore, uses less energy than systems that use steam or hot water
- Costs of chemical sanitisers would be reduced or possibly eliminated by using ozone as a sanitising agent
- Release of chlorinated chemical residues to the environment would be reduced
- Because ozone is extremely reactive, it would not persist so there would be minimal health risks associated with it unless inhaled in large quantities

The contact time and concentration used in the study by Greene (1993) was recommended by the manufacturer. Further research is required as to the optimal conditions necessary for complete sanitisation by ozone. Health and safety data as to the effect of inhalation of fine aerosols of ozonated water was not found and would need to be established further before this method could be recommended.

4.2.1 SUMMARY

The use of ozone as a sanitiser for the destruction of biofilms on glass and stainless steel surfaces was described in studies by Bott (1991) and Greene et al (1993).

Both articles concluded that the use of ozonated water (concentrations ranging from 0.07 – 0.5ppm) as a sanitiser for food equipment surfaces was effective in the removal of biofilms. Greene et al (1993) found that >99% of bacteria were removed in 10 minutes at a concentration of 0.5ppm. Chlorine was an effective sanitiser, but its use had several disadvantages including:

- environmental concerns such as the production of chlorinated organic compounds (considered to be carcinogenic) which persist in the environment
- OH & S concerns such as transportation, storage and mixing of hazardous concentrated chlorine

Ozone had advantages including:

- reduced costs in materials and energy requirements
- it does not persist in the environment
- minimal health risks unless inhaled in large concentrations and quantities

The general conclusion of the published literature was that ozone is an effective sanitisation method for food processing equipment. There is potential for use of ozone as an alternative disinfectant to chlorine in the meat processing industry. Further research is needed, however, to determine optimal ozone contact time and the effect of ozone on different materials of construction. Health and safety data on the effect of inhalation of fine aerosols of ozonated water should be investigated further before this method could be recommended.

4.3 PRODUCT DISINFECTION

4.3.1 APPLIED AS A SOLUTION

Gorman et al (1995) and Reagan et al (1996) conducted similar studies to compare procedures and interventions for removal of physical and bacterial contamination from beef tissue and carcases respectively. Several treatments were compared for their ability to reduce faecal material and bacterial contamination. Both investigated the effects of hydrogen peroxide, ozonated water, hot water (at various temperatures), spray washing and hand-trimming/spray washing treatments. Gorman et al (1995) included trisodium phosphate, acetic acid and commercial sanitiser. Ozone concentrations ranged from 0.3-2.3ppm (Reagan et at, 1996) to 0.5% (or 6000ppm added to the water – unknown residual ozone concentration) (Gorman et al, 1995).

Of the treatments applied, hot water washing (at approx 74°C – 85°C) was found to be the most effective in reducing bacterial numbers on beef carcases, especially in producing more consistently low bacterial populations by reducing carcase-to-carcase variation. Reductions in bacterial numbers exceeding 3 log (Gorman et al, 1995) and 2 log (Reagan et al, 1996) were achieved as opposed to reductions of approximately 2 log and 1.8 log respectively by trimming and spray-washing.

Ozone treatment reduced total bacterial numbers on carcases by approximately 2.8 log (Gorman et al, 1995) and 1.3 log (Reagan et al, 1996) and both ozone and hydrogen peroxide were more effective than other chemical interventions applied such as trisodium phosphate, acetic acid and commercial sanitiser (Gorman et al, 1995). However, both ozone and hydrogen peroxide were approximately equivalent to the conventional method of trimming followed by washing. A further reduction in bacterial numbers by approximately 0.5 log was achieved when trimming/spray-washing was followed with ozone treatment (Gorman et al, 1995). *E. coli* numbers were reduced by approximately the same magnitude for each treatment in the study by Reagan et al (1996), however, Gorman et al (1995) did not investigate reductions of this organism.

Neither of these studies made mention of the effects of ozone at the rates applied on the colour or appearance of the meat or on equipment surfaces.

Both studies concluded that the conventional industry practice of trimming and washing of beef carcases consistently resulted in low bacterial populations and visual scores for faecal contamination and that hot water rinsing was the most effective intervention treatment for reducing bacterial numbers on carcases. Chemical interventions of ozone and hydrogen peroxide should be considered as an alternative to hot water for decontamination of carcase surfaces, especially when applied after conventional trimming and spray washing treatments.

4.3.2 APPLIED AS A GAS

Kaess and Weidemann (1968) and Greer and Jones (1989) investigated the effects of continuous ozone treatment on meat spoilage organisms on muscle slices and carcase sides respectively and any associated muscle changes accompanying the ozone treatment. The effect on pathogenic microorganisms was not investigated during either study.

Kaess and Weidemann (1968) applied ozone at concentrations of between 0.07 and 2.5ppm at 0.3°C. They found that in the presence of ozone, lag phases of bacteria were increased but growth rate of surviving organisms was not affected. At ozone concentrations of 0.07ppm, there was practically no bactericidal effect for the organisms tested. Ozone was mainly effective on organisms directly exposed to concentrations greater than 0.3ppm. At 0.3ppm, inhibitory effects were not always significant between different species. Small but significant inhibitory effects on *Pseudomonas spp*, yeasts and moulds were obtained with ozone concentrations of 1ppm and growth was strongly reduced on muscle tissue exposed to 2.5ppm ozone.

Kaess and Weidemann (1968) found that the application of high concentrations of ozone to obtain a reduction of the microbial population on the surface of meat is restricted by the high sensitivity of meat pigments to oxidation. The study found that myoglobin and haemoglobin quickly oxidised to brown heme compounds when ozone was applied continuously at concentrations of 1ppm and greater but did not differ from that of controls when the ozone concentration was <0.3ppm. They did not state how many days after commencement of treatment discolouration was apparent. It was found that daily ozonisation with 5ppm for 3 hrs extended the lag phase of bacteria but it could be applied only for about 3 days if discolouration of the meat was to be avoided (Kaess and Weidemann, 1968).

Greer and Jones (1989) investigated the effects of ozone (0.03ppm) upon beef carcase shrinkage, carcass characteristics, muscle quality and total mesophilic and psychrotrophic bacteria. Paired sides were either continuously ozonated using a commercial ozone generator or subjected to conventional air chilling under identical conditions of humidity (95%) and temperature (1.6°C) for up to nine days of aging. After aging, control and ozone treated sides were processed and bacterial growth and retail case life determined for steaks in simulated, retail display.

Growth of psychrotrophic and mesophilic bacteria were significantly retarded on ozone treated carcasses ($P<0.05$). Over 9 days of aging, bacterial populations essentially remained the same when treated continuously with 0.03ppm ozone. Conversely, however, Kaess and Weidemann (1968) found that the bactericidal effect of ozone practically disappeared with a concentration of 0.07ppm and less.

Greer and Jones (1989) found that although ozone retarded bacterial growth on the surface of aged carcase meat, bacterial numbers on steaks derived from ozone treated or control carcases were not significantly different during retail display. Fat colour and muscle shear values were not influenced by treatment, however, ozone was found to have other deleterious effects on the meat. Carcase shrinkage over nine days was significantly higher in ozone treated sides compared to control sides and this difference between treatments increased following trimming of discoloured and dry muscle tissue. After aging, loin eye area was reduced and muscle colour was significantly darker in ozone treated sides compared to control sides.

However, Greer and Jones (1989) quote another report by Anonymous (1986) as stating that ozone can reduce evaporative losses from beef carcases by 0.9 – 1.7% during 3 and 7 day storage respectively (ozone concentration not stated). Kaess and Weidemann (1968) stated that colour of treated muscle did not differ from control muscle in ozone concentrations of less than 0.3ppm.

Greer and Jones (1989) concludes that although ozonisation prevented bacterial growth on carcass surfaces during 9 days of aging, it did not improve the bacterial condition, nor the keeping quality of retail beef steaks derived from ozone treated carcases. However, the published data appear contradictory as to the ozone concentration that produces deleterious effects on muscle tissue (*cf* Kaess and Weideman, 1968 and Anonymous, 1986). It should be kept in mind that Kaess and Weidemann's study was a laboratory scale exercise where steaks were treated with ozone in small containers whereas the study by Greer and Jones was conducted in meat processing premises in the chillers. Also, in practice, it is unlikely that carcases will be left to age for 9 days in Australian meat processing premises.

Dr John Welsh of Wild Game Resources Pty Ltd, Brisbane, investigated the use of ozone for decontamination of product in the chillers. During and in-house trial, ozone was applied continuously at 5-6ppm at approximately 8°C. At this concentration and time, they reached similar conclusions to Kaess and Weidemann (1968) in that lag phase of bacteria was increased but growth rate of surviving organisms was not retarded. Lag phase extensions of 5 – 7 days were reported (*pers. comm.* John Welsh, Wild Game Resources). After 5 days at 5ppm, the carcases were discoloured and dried, however, Dr Welsh stated that it would be very unlikely for carcase product to be held for longer than one day in most instances. They found that where carcases were pushed together and touching each other, ozone had no effect on bacterial growth. He stated that as standard practice, carcases would often touch each other in the chiller and transport vehicles, and therefore ozone would not be of great benefit to their situation. Monitoring of the ozone concentration was also a problem in that an employee had to enter the chiller and read the ozone concentration off a hand held meter. At concentrations of 5ppm, pungent odours were noticed and irritation of the respiratory passages and eyes occurred. Although ozone was found not to suit their applications, it was stated that ozone did work well when carcases were spaced apart in the chiller. Discolouration should not be a problem because carcases were not held for long periods of time. Reductions in mould growth in the chillers were observed and no corrosive effect on surfaces in the chillers was observed.

4.3.3 SUMMARY

It appears that the use of ozone as a product decontaminant is not of great benefit. Although ozone was found to significantly reduce bacterial numbers when applied to meat both as a gas and in solution, the benefits were outweighed by other factors. When applied as a solution, it was found that ozone had equivalent effects on reduction of bacterial numbers as current industry methods and that decontamination of carcases with hot water was more effective than ozone. When applied as a gas, the concentration must be limited due to the undesirable darkening of the meat and possible increases in carcase shrinkage. In cases where carcases touched each other, ozone appeared to have no effect.

4.4 AIR DISINFECTION

Bacterial and fungal contamination of air leads to cross contamination of product and growth on chiller walls and ceilings. In studies by Whistler and Sheldon (1988) and Masaoka et al (1982), the use of ozone for the disinfection of air in a poultry hatchery and hospital bioclean room respectively was investigated. Both studies compared the effects of ozone against formaldehyde. Formaldehyde is a noxious gas that is an irritant to the eyes and the nose, has a lingering noxious odour and presents a problem in venting of the vapours.

Whistler and Sheldon (1988) found that both ozone and formaldehyde resulted in similar population reductions (approx 6 log reduction) of *E. coli*, *Pseudomonas fluorescens*, *Salmonella typhimurium* and *Proteus sp* after 2 minutes exposure to 1.52% to 1.65% by weight (18,000 to 20,000ppm) ozone. By eight minutes, approximately 7 log reduction of microorganisms occurred. The study concluded that ozone was an effective alternative disinfectant against important poultry pathogens and hatchery isolates. However, the effect of inhalation of high concentrations of ozone by the hatched chicks (or hatchery workers) was not considered in the study.

In the study by Masaoka et al (1982), a comparison between formaldehyde gas and ozone for the decontamination of bioclean rooms in hospitals was carried out. Ozone was applied at 40ppm for 72 hours. The ozone dissipated after 60 minutes as opposed to formaldehyde gas, which persisted and produced eye irritant symptoms 7 days after treatment. Bacterial numbers decreased from approximately 100 CFU/cm^2 to 0.05 CFU/cm^2 and 0.02 CFU/cm^2 after ozone and formaldehyde treatments respectively. The study found that ozone was a good decontaminant of test organisms, but did not penetrate materials as thoroughly as formaldehyde gas. It also found that ozone caused marked corrosion of rubber at these concentrations and contact times. However, no change was observed in painted metals, stainless steel, vinyl, or melamine laminates. Iron nails, however, rusted when in contact with both ozone and formaldehyde. Ozone was superior to formaldehyde with regard to convenience, dissipation after treatment, and inhalation effects on hospital staff.

Mr Ross Cridland of Glengor Pastoral Company, Gosford, owns and operates 9 ozone-generating machines in total. Five of these machines operate in the boning room for 24 hours continuously and they also operate in the carcase chillers for 24 hours continuously. The machines are set to run at an ozone concentration of 0.1ppm, which is set by a dial on the machine. The accuracy of the dial is occasionally checked by the supplier of the machines. They are currently happy with the performance of the machines and report that they have noticed an improvement in the microbiological condition of the boning room surfaces, which are checked pre-operationally 3 times weekly. They do not perform microbiological testing of product surfaces so it is not known whether any reductions in counts are observed there. They assume that if the air in the chiller is cleaner, then the microbiological quality of the meat should be better. They have however done some preliminary work as to the shelf life they can obtain with ozone treated carcases. In non ozone treated chillers, a shelf life of 7 days was achieved before carcases became "sticky", but in ozonated chillers, a total of 14 days shelf life was achieved before the carcases became "sticky". This agrees with observations made by Dr John Welsh of Wild Game Resources Pty Ltd. Glengor Pastoral Company have observed no detrimental carcase characteristics such as discolouration or excessive shrinkage (i.e. any difference with non-ozonated carcases). An ozone generator is also used in the carton storage room where it has reduced dust in the air. They have had no health related complaints from employees regarding the use of ozone. However one employee has expressed concern over use of the machine while he is working, so an agreement was reached that the employee in question turns the machine off before he starts work and then on again as he finishes work.

4.4.1 SUMMARY

It appears that benefits from ozone can be achieved when used as an air disinfectant. In interviews with meat processing premises operating ozone generators and in the literature, reductions in bacterial numbers on surfaces and/or in air were achieved. The optimal dose rate could not be concluded from any source. An assumption made is that if the air in a room or transport vehicle is clean, the microbiological condition of product and surfaces within that room or vehicle will be better.

Where employees are present, concentrations of 0.1ppm may not be exceeded, however, concentrations may be increased during overnight decontamination of air and cleaned surfaces. The ozone generator can be set on a timer to switch on after completion of the days operations and switch off at least 1 hour prior to commencement of the following days operations to allow dissipation of the ozone.

5. CONCLUSIONS AND RECOMMENDATIONS

The literature reviewed the use of ozone as a disinfectant for water, air, surfaces and equipment and product. References as to the use of ozone as a deodouriser in rendering plants were not found in the reviewed literature.

Although ozone use as a disinfectant is not new (European wastewater has been treated with ozone since 1906) its use as a disinfectant in the food industry has not been widely embraced.

Contact time, temperature, relative humidity, pH value and presence of inorganic and organic materials influence the germicidal effects of ozone.

From the literature, it appears that ozone has many potential applications to the meat industry. These include disinfection of carcases, sanitising air in closed chillers, boning rooms, slaughter floors and transport vehicles and sanitising water used for washing equipment and surfaces during clean down, disinfecting slaughterhouse effluent and deodourising rendering plants.

The main disadvantage to the use of ozone is that it is toxic to humans. The NIOSH (1996) have stated the IDLH (immediate danger to life and health) level for ozone is 5ppm. Worksafe Australia has adopted 0.1ppm as the maximum exposure standard allowed for Australian workers. AQIS have limited ozone exposure of employees in meat processing plants to 0.1ppm at any time. This limits the concentration of ozone that can be used during working hours when employees are present. It can be corrosive to rubber, iron and some plastics in high concentrations, however it does not seem to affect painted metals, vinyl, melamine laminates, concrete or coated aluminium.

It appears that the most beneficial uses for ozone are for air decontamination in closed boning rooms, slaughter floors, chillers and transport vehicles at concentrations of between 1 and 5ppm (for overnight decontamination). The ozone generator could be set by timer to switch on at the end of cleandown and then to switch off at least 1 hour before work commences the next day. This would allow the ozone to dissipate before employees arrived.

Ozone was reported to be an effective sanitation method for food processing equipment. There is potential for use of ozone as an alternative disinfectant to chlorine in the meat processing industry. Further research is needed, however, to determine optimal ozone contact time and the effect of ozone on different materials of construction and safety to workers.

Another potential use for ozone is in the decontamination of slaughterhouse effluent. However, Assoc. Professor Mike Johns of the University of Queensland believes that disinfection of effluent with any chemical would be a cost burden that industry could not meet.

It appears that ozone application to fresh meat as a gas or in solution has benefits regarding retardation of bacterial growth, however, the concentrations required for optimal destruction of microorganisms may cause undesirable qualities such as discolouration of exposed tissue and carcase shrinkage. Where carcases touch each other in the chiller, ozone appears to have no effect, and it has been reported that there was no increase in shelf life of steaks from ozonated carcases after processing. Current industry practices of trimming and washing or washing with hot water were found to have just as good if not better ability to reduce microbiological contamination as ozone on freshly dressed carcases.

Many different ozone concentrations and contact times were investigated for all the above situations. No conclusion can be drawn as to the optimal ozone concentration or contact time from the literature in the above situations. It appears that this may need to be validated for each situation.

Our recommendations are therefore:

1. A trial should be conducted to assess ozone as an air and surface decontaminant in closed chillers, boning rooms, slaughter floors and transport vehicles. Optimal ozone concentration and contact times should be established in such a trial.

2. A trial should be conducted to compare ozonated water and chlorinated sanitisers for plant disinfection at cleandown. Optimal ozone dosage rates would need to be determined along with any corrosive effects on surface materials examined. A cost analysis to determine the overall benefits of ozone usage would also need to be considered. Health and safety data regarding the effects of inhalation of fine aerosols of ozonated water would also need to be investigated, however, an experiment of this type may be out of the scope of projects funded by MRC.

3. As ozone is a known deodouriser, and no published literature was found evaluating its use in rendering plants, research to determine the effectiveness of ozone in this capacity should be considered.

4. Although ozone was found to have potential for the chemical disinfection of effluent, practicalities of using this technology in industry is such that benefits may be outweighed by other factors (particularly cost). It is recommended that a project of this nature be given low priority.

5. No further research is justified as to the use of ozone as a decontaminant of carcase surfaces, unless combined with the trial in Recommendation 1.

6. No further research is justified to determine the effectiveness of ozone as an alternative treatment for elimination of vermin.

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