The Development, Implementation and Validation of a Cleaning System within Dairy Processing Plant

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Abstract

Food manufacturing companies are under increased pressure to ensure food safety and compliance with legislation. Constant media and press coverage has increased consumer awareness of the quality and microbiological safety of the foods they consume. Control of the bacterial genus *Listeria* and *Listeria monocytogenes* in particular is of paramount importance to producers of ready to eat foods.

The dairy industry is recognised as a high risk product due the nature of the pH of some cheese being able to potentially sustain microbiological growth and also due to some bacteria having the ability to live under chilled conditions.

The purpose of the work reported in this thesis was to develop and validate a cleaning system which would be validated under laboratory conditions then *in situ* to ensure it had the same effect in a working environment, and then the assess the effectiveness of ozone within a food manufacturing environment.

To carry out this final section of work and ozone machine was installed and a regime was put over a period of time where the ozone machine would be on for assessment and then turned off to assess the effect no ozone would have. The laboratory work carried out showed that the cleaning chemicals and methods in place were sufficient to reduced residual contamination after each of the cleaning stages. Once the validation work was carried out *in situ*, it showed that the combination of a good cleaning system using the dedicated Holchem chemicals followed by ozone did work within the dairy
environment. The results showed that there was a positive downward trend of the counts obtained after each of the cleaning stages, proving that each cleaning stage does have it’s own importance in a successful cleaning system.

The results obtained from this research shows the benefits of using ozone as an additional disinfection stage within a cleaning programme within the dairy manufacturer. The work carried out has opened up the opportunity for further research in this area to look into further benefits of ozone and its applications in other manufacturing situations.
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hit him! However, he stood by me throughout and it is to my parents, sister and David that I owe the most, and to whom I dedicate this thesis.
Quotation

The early bird gets the worm, the second mouse gets the cheese.

Jon Hammond
Chapter 1

1.1 Introduction and Review of Literature

In recent years there has been an increasing demand by retailers and consumers for higher quality foods. With the increased awareness of the many issues associated with a microbiologically poor quality product and food poisoning, higher standards and compliance with legislation is becoming increasingly important. Prevention of microbiological contamination is a pre-requisite and a requirement for food safety control within food manufacturing companies. There are a number of routes for contamination and effective cleaning systems are a key control measure.

The dairy industry in particular has high risk foods associated with it. The dairy industry has many processes within the manufacture of the final product which has the potential of contaminating the food. The dairy industry often has many processes from the incoming raw material to the finished product many of which may involve the direct handling of the product which also has its own potential of causing contamination of the products.

The quality of the product is paramount for achieving customer requirements and for maintaining the company standards. By producing products which have low bacterial, yeast and mould counts will ensure products reach their required shelf-life and ensure product quality.

Within cleaning systems it is necessary to monitor the hygiene standards, which is often done by looking for indicator organisms such as *Enterobacteriaceae*. Carrying out monitoring such as environmental swabbing and Adenosine Tri-Phosphate (ATP) swabbing allows poor hygiene standards to be highlighted. From this the appropriate
actions can be taken if any indicator organisms are identified to highlight the source. Also
the presence of pathogens must be monitored to ensure that all hand washing procedures
are effective and being adhered to in order to ensure that no cross contamination is
occurring from personnel working within the food production unit. The presence of
\textit{Listeria monocytogenes} can be a concern within food processing factories as \textit{Listeria
monocytogenes} is ubiquitously distributed within the environment.

Therefore the control of factory hygiene and contamination is a must to ensure
consumer safety, adherence to commercial pressure, product quality, shelf life and
adherence to specification. This can be achieved by correct cleaning schedules for both
high and low risk areas, segregated personnel facilities, correct facilities and correct and
adequate training which is considered commensurate with the roles of the staff.

\section*{1.2 Food Safety Legislation}

The Food Safety Act 1990 provides legislation to ensure that food manufacturers
and retailers provide food which is safe and wholesome. More recently, the European
Union has issued the Safe Production of Food (EC178) and Hygiene of Foodstuffs
(EC852). This legislation has come into affect since the application and risk assessment
of the food business operative and ensuring food safety. In addition to the Food Safety
Legislation, Commission Regulation (EC) No 2073/2005 of 15\textsuperscript{th} November 2005 on
Microbiological criteria for foodstuffs introduces us to microbiological standards for
foods and food premises. The regulation states that environmental sampling of a food
manufacturing environment can be an affective tool for identifying and monitoring
pathogenic organisms in foods. The regulation brought in two criteria, Food safety
criteria and Process hygiene criteria. The regulation also states the need for sampling plans within the environmental testing procedure along with the use of trend analysis of the results as this method is ideal for highlighting any changes, good or bad, within the environmental monitoring. Despite the increased understanding of the possible routes of product contamination and hygiene management the number of reported food poisoning outbreaks is still on the increase as reported by the Steering Group on the Microbiological Safety of Food.

1.3 Cleaning stages

Within a successful cleaning system is a cleaning phase which is carried out prior to the disinfectant stage. The cleaning phase is the initial stage during which any remaining organic debris is removed from the area. By having the initial cleaning phase it ensures that the debris is removed. The initial cleaning stage of an effective cleaning system is thought to be the most important stage for the removal of attached microorganisms and for minimising microbial colonisations (Carpenteir & Cerf 1993), (Dunsmore 1981) and (Gibson et al 1999). After the cleaning phase the disinfection stage is the crucial part in which the microbiological numbers are reduced and the viability of the microorganisms is decreased (Holah 1995). The effectiveness of disinfection can vary greatly depending on the conditions they are exposed to in practice. Not only can this efficacy of disinfectants be significantly affected by the practical conditions but also in the case of quaternary ammonium compounds the efficacy can also vary greatly against different bacterial species depending on the degree of water hardness and the specific chemical structure of the compound being evaluated. Studies have been carried out
looking into the effect the practical conditions have on the effectiveness of the chemicals kill rate to certain bacteria. Comparative studies have been carried out looking at the effect a high protein load has on the kill rate of a quaternary ammonium compound and hypochlorite. Results to such studies have shown that a high protein load has little effect on the rate of kill of both quaternary ammonium compounds and hypochlorite against gram negative bacteria, although studies have also shown that a high protein load does not significantly reduce the kill rate of gram positive bacteria for both quaternary ammonium compounds and hypochlorite. The same study has been carried out looking at the kill rate of yeasts by hypochlorite which was shown to significantly reduce, however, the same study has not been carried out on quaternary ammonium compounds (Bessems 1998).

1.4 Listeriosis

Between 1983 and 1988 there were at least 150,000 cases of Listeriosis in the United States of which 54 cases resulted in death due to the consumption of pasteurised milk and Mexican style cheeses, which were contaminated with *Listeria monocytogenes*. Due to the number of outbreaks of listeriosis associated with the dairy industry, there was an increase in the surveillance of the dairy industry by the Food and Drugs Administration. In addition there was an increase in the information available to pregnant women during 1988 and 1989 (HPA Website). As a result of the increase in the information given to pregnant women the annual number of pregnancy associated and non pregnancy associated cases fell during in the 4 years following the increase in awareness however, there were then a few years where the number of cases increased
from 2001 to 2004. As a result of the increased surveillance, *Listeria monocytogenes* was isolated from a number of cheeses including, Liederkranz, Mexican style, Ricotta, Cheddar, Brie, semi-soft cheese and soft ripened cheese imported from France (Ryser *et al* 1988). At least four well documented outbreaks and a number of sporadic cases of listeriosis involving different types of cheeses have been reported (Farber & Peterkin 1991). Products often become contaminated with *Listeria* post-processing for example heat treated deli meats, pate or cheese. *Listeria* is considered to be relatively difficult to control within food processing plants as it is ubiquitously distributed in the environment, it is relatively resistant to heat, high salt concentrations and other adverse environmental conditions. Work has been carried out on the occurrence of *Listeria monocytogenes* in or on soft cheese. The work showed contamination rates of 0.5% (Farber *et al* 1987). Research has also shown that 43% of soft and semi-soft cheeses made from raw milk were contaminated with *L. monocytogenes* and 2% of soft and semi-soft cheese made from heat treated milk contained *L. monocytogenes* (Loncarevic *et al* 1995). Research involved with the characterising of *Listeria* strains have shown that from 19 soft and semi-soft cheese two hundred strains of *L. monocytogenes* have been isolated (Loncarevic *et al* 1995). It has also been reported that *Listeria monocytogenes* can form biofilms which then provide a unique niche for the extended survival of the bacteria (Blackman & Frank 1996), (Ronner & Wong 1993). From these findings it is evident that *Listeria monocytogenes* poses a serious problem to the dairy industry.

After considering the frequency of which *Listeria* is detected within dairy processing, the consequences from an infection by *Listeria monocytogenes* and the probable low oral infectious dose required by susceptible individuals such as newborn
infants, pregnant women and immunocompromised adults it may be beneficial to consider the addition of preservatives, especially sorbic acid together with an increase the acidity of the product to pH 5.0 by the addition of small amounts of lactic and/or acetic acid both of which would decrease the survival chances of *Listeria monocytogenes*.

1.5 Biofilms

Research has shown that bacteria can attach to surfaces and grow to form a biofilm. Food processing plants have an abundance of areas which allow the attachment of bacteria and allow them to attach, grow, develop into microcolonies and possibly form a surface covering in excess of $10^7$ cells/cm which constitutes a biofilm (Holah & Kaerney 1992). Biofilms tend to occur on solid surfaces which are in contact with one or more kinds of liquids, such as water, milk, oil and sea water (Zottola & Sasahara 1994). Many bacteria in their natural environment survive change to the environment by adhering to a surface and living under a polysaccharide layer. With time this becomes a biofilm and where many different kinds of organism can live together. Biofilms not only provide a protective environment, they are also a way of trapping nutrients (Poulsen 1999).

Within the food processing environment the conditions favour bacterial attachment and biofilm formation such as, flowing water, suitable attachment surfaces and ample nutrients (Taylor & Holah 1996). Biofilms not only include bacteria but also the extracellular material produced at the surface and any material trapped within the resulting matrix. It is considered that a true biofilm takes hours or days to form. As the biofilm grows, pieces will detach which will allow more matrices to develop. Within the
food industry biofilm can be of great concern as if the biofilm was to dislodge it could cause great negative problems if it were to contaminate a product making it potentially microbiologically unsafe for consumption.

The time available for biofilm attachment is dependant on the frequency of cleaning for each particular area. Product contact surfaces may be cleaned several times throughout a shift whereas environmental surfaces may be cleaned only once per week. Due to the time lapse between cleaning of the environmental areas there is more time for the bacterial attachment and biofilm formation. It has been shown that bacterial attachment to a variety of surfaces in the food industry does readily occur although extensive colonization and biofilm formation only occurs on environmental surfaces (Mafu et al 1990). It is the environmental surfaces such as the floors, walls and drains which cause indirect contamination of the products due to vectors such as air, production operatives and cleaning systems. Surfaces that appear visually clean however can still be contaminated with large numbers of viable microorganisms that could contaminate food. Biofilm formation and the production of extracellular materials can be influenced by the type of surface, nutrient level and type of organism. It has been shown that bacterial biofilm populations present on stainless steel surface reduced by 3-5 log by the range of detergents and non-detergent sanitisers used in the study. In contrast planktonic (suspended) cells were reduced by 7-8 log by the sanitisers. It was concluded that chlorine and anionic sanitisers generally removed extracellular material from biofilms better than iodine and quaternary ammonium detergent sanitisers. It was also shown during the study that biofilm cells and extracellular material may remain on the surface which had been sanitised but no viable cells recovered (Ronner & Wong 1993).
Therefore the hygiene of environmental surfaces affects the quality and safety of finished products. (Gibson et al 1999).

1.6 Factory Design

The design of a production environment, to allow implementation of an effective cleaning system, is the major method of bacterial control within a production plant. The design of a factory must take into consideration, process flow, raw ingredient intake and preparation, finished product transfer to storage, designated cleaning areas, equipment specification and material finish and environmental surfaces and drainage.

The factory design must ensure that there is a systematic flow from the start of a production process through to finished product storage in order that there is no risk of cross contamination between the processes.

If as in the case of Abergavenny Fine Foods, raw milk was handled on site then it must be in a segregated area to any other process due to the risks associated with raw milk. Again in such a case it is important that the process flow for raw milk intake is such that the raw milk section is segregated from the post-pasteurised milk.

A dedicated cleaning area for cleaning items such as small pieces of equipment, buckets and plastic pallets is also useful in order to keep any potential aerosol contained away from the production environment.

The environmental factors to be taken into account are the drainage within the production areas, they should be easy to clean, self flowing and be located away from production lines. The floor and walls should be easy to clean and non permeable. The
floors should be constructed of material such as epoxy resin which will withstand heavy traffic flow and resist chemicals which will be used in the areas.

As part of factory design it is also important that the equipment and production utensils used within the factory are of food grade standard and suitable for the purpose of which they are intended to be used. The ease of cleaning, visual inspection and swabbing should be taken into account.

Factory design should also take into account changing areas for production staff. This area should have segregations between outdoor clothes and production clothes, adequate washing facilities and storage for outdoor clothes.

1.7 Hygiene and Cleaning Systems.

If an effective cleaning system is not in place then microorganisms and food residues may remain at a level where the quality and safety of foods is at risk. There are two main stages to an effective cleaning system, the cleaning stage which involves the breakdown of the soil and to reduce the attachment strength of the microorganism to facilitate the removal of them from the surface. The disinfection stage involves the chemicals reducing the viability of the microbes that may remain after the cleaning stage. The effectiveness of a good cleaning system must however be supported by a well documented cleaning schedule and record system. A regular and effective cleaning system is a pre-requisite usually used in relation to HACCP for all food handling sites (Rothe 2000).

In food manufacturing companies, hygiene is concerned with the prevention of cross contamination of food products by direct or indirect methods. Products may come into contact with a contaminated area and therefore become directly contaminated
whereas a product may become indirectly contaminated by, a production operator, via air or water droplets during cleaning. There are a number of particular areas within a food processing plant which are considered to be areas which can potentially harbor environmental pathogens such as, drains and conveyor systems for example:

From the conveyor system which transfers product raw materials to the ingredient preparation area may enter the high risk production environment and be the cause of cross contamination (Cotton & White 1992).

With the majority of organisms forming biofilms on floors and walls rather than food contact areas, there is a possibility of indirect cross contamination during the cleaning of these areas. This is mainly due to the cleaning methods used to clean such areas of production which may involve the spraying of walls. Due to the spraying of such areas these there is then the potential of producing aerosols that contain viable microorganisms. It has been shown that high and low pressure hoses can move water droplets in excess of 2m high and 7m distance (Rothe 2000).

Cleaning and sanitation are undertaken to remove undesirable material such as food debris, foreign bodies and to reduce if not remove microorganisms from surfaces to an acceptable level where, any remaining residues are of minimal risk to the quality of or the safety of the product.
Effective cleaning systems are becoming increasingly important as the demand for higher standards of hygiene within the manufacture of short shelf life chilled foods grow and with the increased awareness from customer, consumers and legislation for higher standards of hygiene within high risk manufacturing plants.

When cleaning programmes are undertaken correctly, they can be cost effective, easy to manage and effective against microbiological and foreign body contamination (Holah & Kaerney 1992).

An effective cleaning system will have the following aims:

1. Removal of product debris: By removing debris from previous production runs the visual appearance of the production line and end product may be improved.

2. The removal of microorganisms: By removing microorganisms it will reduce the chances of product contamination by pathogens and spoilage organisms thereby maybe extend the life of the product and reduce customer complaints.

3. Removal of material used in previous production runs: Removal of used utensils, machine parts and other material used on previous production runs may prevent a foreign body contamination.

4. Health and Safety; providing a clean safe environment for all employees therefore increasing productivity and morale.

5. Increase process performance.

6. Giving a good image to the customers and public.

There are a number of requirements to which a cleaning system must satisfy:

1. Effective

2. Suitability for a food processing plant
3. Must be non-toxic to the product
4. Safe for all cleaning and food production operators
5. Non-tainting

The application of disinfectant is usually carried out by manual means e.g. brush, mist or spray. One method which is used throughout the food industry is the “Bucket and brush” method. The buckets and brush method is one which is commonly referred to within the food industry which refers to the method of cleaning when carried out using a manual method using buckets of detergents and dedicated brushes or cloths. Factors which must be taken into account with methods of cleaning are, the contact time the chemical has on the equipment and the temperature at which the chemical is most effective. A disadvantage with the “bucket and brush” method is the relatively short contact time it will allow the chemicals to have on the horizontal surfaces. Contact time can be increased by implementing a system which involves a gel or foam. But for this to be practical the chemicals being used must be compatible with either foam or gel technology.

It has been shown that bacteria attached to a surface are more resistant to a biocide than when in suspension (Holah 1995). This leads to the question as to whether the concentration of disinfectant used in the food processing plant is of a sufficient concentration to have a biocidal effect on organisms attached to surfaces, as the initial “effectiveness tests” of the chemicals are usually carried out in bacterial suspension.

In addition to this there are also other factors within the food processing environment which may also affect the performance of disinfectants such as the temperature of the production environment, pH, mechanical stress and attachment.
As surface attached bacteria having an increased resistance to biocides it is important to note that disinfectant testing which takes place in suspension does not necessarily give a true representation of the effectiveness of the disinfectant when in place within a food processing plant.

There appears to be a gap in research within this area, of the validation of “approved” cleaning chemicals. There is a requirement that all biocides give a >5 log reduction in suspension tests under laboratory validation suspension tests (Anon 1998), this can be followed through by the validation of the cleaning chemicals within industry. This method of research would have resulted in the log reductions achievable under laboratory conditions forming a basis to which the validation work of the cleaning chemicals within industry could be compared. This would highlight the true effectiveness of cleaning chemicals within industry. Studies have also taken place to study the attachment of Salmonella species and *Listeria monocytogenes* on three different surfaces: stainless steel, rubber and polytetrafluoroethylene. The study shows that bacteria attached in higher numbers to the surface which is more hydrophobic material. The cleaning of materials with commercial sanitisers resulted in a decrease of in the contact angle of the bacteria and also a reduction in the number of bacteria in comparison with the standard conditions. The level of reduction of the bacterial adhesion varied with the species of bacteria. The substrate material and the sanitiser tested, quaternary ammonium compounds were proven to be more effective against salmonella attachment than *Listeria monocytogenes* attachment. Diethylentriamine showed a similar efficacy against both bacteria. Polytetrafluurethynne showed the greatest reduction in bacterial attachment after being washed with commercial sanitiser. From this study it has been concluded that
stainless steel is less adhesive than rubber or polytetrafluorethyne, although polytetrafluorethyne has been shown to be more easily sanitised (Sinde & Carballo 2000). Studies have taken place looking into the efficacy of commercial disinfectants such as potassium persulphate, isopropanol, hydrogen peroxide, perocetic acid, quaternary ammonium compounds (QAC), hypochlorite and many more. The efficacies of all disinfectants were tested against 8 strains *Listeria monocytogenes* species representing three different ribotypes. The efficacy tests were also carried out in soiled conditions to replicate a food manufacturing environment. From these tests 5 of the 9 were considered effective. All disinfectants were proven to be effective when tested by means of a suspension test with an exposure time of 30 seconds at the lowest recommended concentration. On clean surfaces all the cleaning agents were effective when the exposure time was 5 minutes and the cleaning concentration was the average as recommended by the supplier. (Aarnisalo 2000). Research has also been carried out looking at the efficacy of 4 commonly used dairy sanitisers, 2 x QAC, acid anionic sanitisers and a chlorine containing sanitisers. Efficacy tests carried out on all four sanitisers showed a >5 log reduction, 99.999% efficacy, regardless of the type of sanitisers, concentration or exposure time. The extent of cell death, injury and repair was found to be dependant on the concentration of cleaning sanitisers, exposure time, bacterial strain and the enrichment procedure. QAC were the most effective while the acid anionic sanitisers was the least effective. The lethal effect of the sanitisers is found to be dependant on the concentration and exposure time. *Listeria monocytogenes* F5027 was found to be the most resistant of the strains to QAC and acid anionic sanitisers (Sallam & Donnelly 1992).
1.8 Ozone.

There has been increased publicity in recent years on the effectiveness of ozone as a terminal disinfectant. Ozone is a molecule composed of 3 oxygen atoms. Ozone acts on substances such as microorganisms by the third oxygen atom detaching from the ozone molecule and reattaching to the microorganism. This reattachment alters the chemicals composition of the microorganism which results in the death of the microorganism. This can be seen in Figure 1.1:

Fig 1.1 Computer simulation of the effect of ozone on a bacterial cell membrane.

1. Computer generated image of a bacterial cell
2. Close up of an ozone molecule coming into contact with the bacterial membrane
3. Ozone penetrating and creating a hole in the bacterial membrane
4. Close up of ozone on the cell membrane
5. Bacterial cell after a few ozone molecules come into contact
6. Destruction of the bacterial cell after ozone contact (Cell lysing)

In food manufacturing environments the food processing and handling equipment become contaminated by the air and by the products being processed. Within such an environment the growth of bacteria is encouraged by the inevitable presence of food debris and residual moisture, not only on food contact surfaces but also within crevices, on surfaces or at joints within walls, floors or drains and equipment (Bott 1991).
Where ozone has been used for the disinfection of water, there has been little research carried out in the use of gaseous ozone within production plants. Ozone is a more powerful oxidiser than chlorine and reacts with organic materials. Ozone in solution is relatively unstable and therefore reverts back to oxygen in a relatively short period of time. The half life of ozone is approximately 20 minutes which indicates that ozone does not persist in the environment and doesn’t remain a potential hazard for long after its application.

There are however disadvantages to the use of ozone. Because ozone has a relatively short half life by when it reverts back to oxygen, ozone cannot be stored and therefore has to be generated against ozone requirements. Due to this the production of ozone requires the installation of an ozone generator. The second disadvantage of the use of ozone in food manufacturing plants as a disinfectant is, the high chemical reactivity ozone has, it is corrosive to many materials such as some plastics and some rubbers often used in the seals of machinery.

Research has been carried out looking into the efficacy of ozone as a terminal disinfectant under laboratory conditions. This work involved the inoculation of stainless steel coupons with microorganisms which are associated with the food industry. Survival of these microorganisms from these controls were compared to an identical set of coupons which were exposed to ozone. The level of ozone exposure was 2ppm for a period of 4 hours. The organisms used in this study *Escherichia coli* ATCC 25922, *Staphylococcus aureus* (food isolate), *Serratia liquefaciens* (food isolate), *Listeria innocua* (environmental isolate) and *Rhodotorula rubra* (food isolate) (Moore et al 2000).
The results of this investigation showed that when exposed to ozone for a period of 4 hours and at 2ppm the log reduction achieved for these microorganisms was at least a 5 log reduction in the viability of 4 of the 5 organisms. To achieve a 5 log reduction on *S. aureus* it was necessary for the concentration of ozone to be increased to 5ppm. The results obtained from this work suggests that Gram-negative organisms are more susceptible to gaseous ozone that Gram-positive organisms. This has also been reported in other work (Whiteside & Hassan 1987). Gram-negative bacteria are thought to be more susceptible to natural drying time therefore to take into consideration the loss in viability due to the drying. Therefore the loss in viability due to natural drying time was taken from the total reduction to give a true bactericidal effect of ozone. It is thought however that yeasts are less sensitive to the bactericidal effects of ozone application. This was shown in the study where the log reduction was 1.30 log units after four hours exposure to ozone. It is thought that one reason why yeasts may have extra protection against ozone is the 100–200nm cell wall, which acts as a barrier and retards the penetration of ozone to the active sites at the plasma membrane (Ishizaki *et al* 1986). The work also compared the efficacy of ozone on bacteria in the presence of organic matter. For this work the bacterial species were in the presence of UHT milk or meat broth. From this work the log reductions in viability ranged from 3.30 to 0.0 and from 3.01 to 0.03 log units respectively. This compares to 4.28 to 1.30 log units in the absence of residual organic matter. From the log reductions obtained from both experimental methods in the presence of organic matter and without the presence of organic matter indicates that ozone is effective at reducing bacterial numbers in the absence and presence of organic matter, but, its efficacy is compromised in the presence of organic matter (Broadwater *et
al 1973). It is thought that this compromise is due to the demand of the organic material within for example food debris (Kim and Frank 1995). The demand for ozone by the organic matter will result in less ozone being available for bactericidal activities on the microorganisms. Another possibility for the reason in which there is a lower log reduction in the presence of organic matter is the physical protection the organic matter gives the bacterial cell or the organic matter is limiting the accessibility of ozone to the bacterial cells (Kim et al 1999).

As previously discussed, ozone has potent bactericidal properties. Its use within the food processing environment is becoming more widely accepted following research to prove its use as an effective terminal disinfectant. There are a number of emerging food processing technologies such as pulse electric field, oscillating magnetic field pulses, intense pulsed light, UV light, ultrasound, x-rays and microwave processing are all emerging technologies. From studying papers during this research it has been noted that there appears to be a gap in the research ozone. There has been research carried out under laboratory conditions looking into the effectiveness of ozone as a terminal disinfectant when applied as a gas, however there is no evident literature which shows that ozone has actually been implemented into a food processing environment and validated within industry. This is an important gap within research on the uses and potential uses of ozone. If validated within industry the research would confirm its effectiveness as a potential terminal disinfectant within a food processing environment which will take into account the environmental conditions associated with food manufacturing companies such as the humidity, temperature, amount of organic matter, air flow and moisture levels of the environment. The findings from research such as this
would give a true representation of the log reduction achievable through ozone application under industrial conditions.

Studies looking at the future uses of ozone has examined its potential uses in hospitals against resistant strains such as methicillin resistant *Staphylococcus aureus* (MRSA) (Yamayoshi & Tatsumi 1993).

Another factor which must be taken into account especially if setting up a new factory, food processing equipment must be able to be effectively cleaned and disinfected (Klob 2000). Although research in the laboratory has shown the effectiveness of ozone as a terminal disinfectant, ozone is however unlikely to ever be used directly in foods for the following reasons:

1. The organic constituents of food compete with microorganisms for ozone therefore reducing the effectiveness of ozone and as a result would require a higher dose of ozone to be applied.

2. Due to the possible requirement for an increased dose of ozone, the increased dose may alter the sensory attributes of the product and as a result adversely affect the acceptability of the product.

Studies have shown that attached microorganisms are more resistant to disinfection than free living cells (planktonic). This also appears to be true with ozone application. During this work the survival of *S. aureus* appeared to be prolonged. It is not however clear if this prolonged survival is due to the allowance of a drying time of 4 hours until ozone exposure or due to the cells being suspended in UHT milk and
therefore encouraged to attach to the steel surface by being left on a continually wet surface for 4 hours, and therefore allow the formation of a biofilm prior to exposure to ozone. Even though the true reason for the prolonged survival of ozone cannot be identified this does indicate the need for an effective cleaning programme to take place prior to any ozone exposure the removal of organic matter and as a result giving ozone the best conditions possible for the maximum effectiveness to be achieved.

There are certain conditions which are thought to amplify the antibacterial effect of ozone. The presence of moisture is thought to be one factor which has the ability to amplify the activity due to increased formation of radicals (Kowalski et al 1998). Ozone did appear significantly more effective at reducing the viability of E.coli when the bacterium was present in a continuously wet environment rather than a dry environment. Defence mechanisms against cellular damage caused by both hydroxyl and superoxide radicals can involve superoxide dismutases, peroxidases and catalases. Studies have shown that these enzymes may be induced in response to acute ozone exposure. Superoxide dismutases and catalases are highly susceptible to ozone (Whiteside & Hassan 1987). It is assumed that the thick peptidoglycan cell wall of S. aureus offers this bacterium more protection as ozone targets the cell wall.

On the basis of the results obtained in this work it can now be suggested that ozone could be an effective part of a full cleaning system within production environments and form an effective terminal disinfectant stage (Moore et al 2000).

Laboratory work has been carried out looking into the sensitivity of various bacterial species to ozone. The normal method of practice for providing a microbiologically safe, aesthetic, potable end product was low level chlorination. Low
level chlorination is not always an effective bactericide, but instead a bacteriostatic, and, as a result a re-growth of the organisms may occur. Another issue with the method of chlorination is the ability to enhance the capacity of certain organic compounds to cause taints and odours in finished water supplies (Carpenteir and Cerf 1993). Due to the potential issues which may arise with the use of chlorine, ozone has been suggested as the best possible alternative for the treatment of water to replace chlorination methods.

Research has been carried out looking at the minimal dosage of ozone required to kill the vegetative cells of *E.coli*, *B. cereus* and *B. megaterium* and also the spores of two Bacillus species. The data obtained was examined to determine if the response of each bacterial spp to ozone could be described as “all or none” response. The “all or none” response is a phenomenon in which a threshold dose of a bacteriocide must be attained before any cells die at which point a total population is killed (Fetner & Ingols 1956). Within this research there were two experiments carried out. One series involved washing the cells twice with physiological saline to remove any organic matter and in the other series the cells were not washed, this was to demonstrate the protective effect organic matter has on cells subjected to ozone. Concentrations of the cells in water subjected to ozone were approx. $10^6$/ml. These cells were exposed to ozone for 5 minutes with a specific dose based on specific data presented by research carried out (Meddows-Taylor 1996). This research suggests that ozone has a bacteriocidal effect as opposed to a bacteriostatic effect. The results of this research has shown that the vegetative cells of all three bacterial species were extremely sensitive to low concentrations of ozone. The threshold of toxicity for *Bacillus cereus* was approx. 0.12mg/liter, *Bacillus magaterium* was 0.19mg/liter and *E.coli* was 0.19mg/liter. All three organisms showed the all-or-non
effect. When the same research was carried out using the bacterial spores of \textit{Bacillus cereus} and \textit{Bacillus magaterium} it was found that the spores were 10 – 15 times more resistant than the vegetative cells with a threshold of 2.03mg/liter to 2.29mg/liter. The greater resistance shown by the spores as opposed to the vegetative cells is probably due to the structural difference between the vegetative cells and the spores of the bacterial cells. The protoplasm of the vegetative cells is protected by a cell wall, whereas the protoplasm of a spore is protected by a thick cortex, multi-layered spore coat and an exosporangium. The chemical and physical compositions are similar in both the \textit{Bacillus cereus} and the \textit{Bacillus magaterium} spores which, is probably why there were equally resistant to ozone in this experiment. The study concluded that in relatively low concentrations, ozone is an effective bactericidal of both vegetative cells and spores. However, it was highlighted that when used in water, if there was a presence of organic matter then this matter would exert an ozone demand and therefore prevent the full utilisation of the applied dose to be used as a disinfectant.

\section*{1.9 Hygiene Monitoring Systems}

The effectiveness of a cleaning system within food processing plants can be monitored by two main methods:

1. Traditional environmental swabs
2. ATP Bioluminescence techniques

The advantage of traditional microbiological swabbing is that the result of each swab allows you to know the actual organism with which that area is contaminated. The disadvantage with such swabbing is the length of time the analysis of environmental
swabbing takes before a result is obtained, a minimum of 2 days, but usually between 3 and 7 days depending on what analysis is requested and the assay and the nutrients used (Ogden 1993). Within food processing plants it is often necessary for rapid results of hygiene monitoring due to the frequent requirement for process equipment to be tested for hygiene standards prior to production start up, and if necessary for a clean down to be repeated if the process equipment is not to the required hygiene standard. This is not possible with traditional environmental swabbing.

The food industry is increasingly adopting food safety and management systems which are proactive and preventative, opposed to those that are reactive and rely on end product testing. This has lead to the requirement for a rapid hygiene test and the development of ATP Bioluminescence. The advantage of ATP Bioluminescence is that ATP (Adenosine triphosphate) is present in all living things which is the universal energy donor for metabolic reactions. An enzyme substrate complex, luciferase-luciferin, which is present in the tails of firefly converts the chemical energy associated with ATP to light by stoichiometric reaction. The amount of light emitted from this reaction is proportional to the concentration of ATP present and can be quantified using a luminometer. It can be assumed that the level of ATP in a cell remains relatively constant, therefore the light produced during a reaction is directly proportional to the number of metabolically active cells present in the assay (Griffith & Mansel 1996).

ATP has been used in a number of industries such as breweries, dairy plants and fruit juice plants. It has also been used for identifying inadequately cleaned milk transporters. The traditional way of testing the hygiene of milk transporters was by using microbiological swabs to sample the inside of the tankers. This method took 72 hours for
a result, and even after this length of time non-microbiological residues were not detected. One of the main issues with the traditional microbiological technique is that it cannot detect non-microbial residues. Therefore even though the swab may pass the microbiological test for which it was tested, it does not indicate the overall hygiene standard of that tank. Therefore the tanker may contain non microbiological residues such as milk which can provide nutrients for any organisms to survive and grow. The use of rapid hygiene methods would be beneficial for such environments in order to re-clean and re-test (Bell et al 1994)

Generally there is about 75% agreement between surfaces that are either passed or failed by both the ATP and plate count (microbiological) method. Of the remaining 30% the majority are failed by the ATP method and passed by the microbiological method, due to the inability of the microbiological method to detect food residues. 5% of the remaining 30% is where high readings are gained on the microbiological methods and low reading are found on the ATP method. This occurs when there is a presence of spores on the areas being swabbed, as spores do not contain ATP, or it can be due to contamination of the surface by sublethaly damaged organisms with depleted intracellular pools of ATP (Griffith & Mansel 1996).

2.0 Past Research within the Dairy Industry

In 1999 a study took place by UWIC (Elvers & Peters 1999) looking into the potential for biofilm formation at a high risk dairy processing plant. The study involved initially selecting sites within the plant that may support the growth of biofilms. Areas selected for the study included: Stainless steel surface under a conveyor, Floor, Cheese press, Top
surface of screw in mixer, Underside of a mixer lid, plastic cheese mould and the bottom of a mixer. These areas included both environmental and food contact surfaces.

These sites were swabbed using the environmental swabbing technique with a pre-moistened cotton tipped swab and the clean-trace biotrace swabs for ATP analysis. The sample area for each swab was an approximate 10cm² using the standard swabbing technique to gain maximum coverage per site. For each site 3 environmental and 3 biotrace swabs were used. Once swabbed, depending on the analysis and the organism the swabs were placed in 10 or 20ml of buffer/pre-enrichment medium and the organisms were resuspended by vortexing for 30 seconds.

The environmental swabs were examined for the following organisms:

- Aerobic plate count
- *Enterobacteriaceae*
- *Staphylococcus Spp.*
- *Pseudomonas spp.*
- *Bacillus spp.*
- *Salmonella spp.*
- *E.coli 0157*
- *Listeria spp.*

From a total of 11 sites swabbed, 9 areas gave a result which could indicate the presence of bacterial attachment to the surface as a biofilm. There was no isolation of specific pathogens from the environmental swabbing although the presence of *Listeria spp* at most sites did indicate the potential for *Listeria monocytogenes* (Elvers & Peters 1999).
The overall opinion concluded from this study was that the cleaning systems in place within the high risk dairy did not seem effective. Results obtained from some areas indicated recontamination of some areas and proliferation of surface areas. Recommendations made from the outcome of this study was to assess the cleaning schedules for effectiveness and the results form a basis for a validation of cleaning systems and chemicals within the high risk unit of the cheese manufacturing company (Elvers & Peters 1999).

The work carried out for this project highlighted that there was an issue with the cleaning schedules in place at the site and that the hygiene standard required a vast improvement to bring the high risk areas to an acceptable level where bacterial numbers are reduced to an acceptable level after cleaning. In food manufacturing company’s hygiene is concerned with the prevention of cross contamination of food products by direct or indirect methods.

The Food Safety Act 1990 provides legislation to ensure that food manufacturers and retailers provide food which is safe and wholesome. More recently there has been a new regulation brought into force, Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. The regulation states that the safety of foodstuffs is mainly ensured by a preventative approach, and that microbiological criteria can also give guidance on the acceptability of foodstuffs and their manufacturing, handling and distribution. It also discusses the environmental sampling of production and process areas which can be used to identify and prevent the presence of pathogenic organisms. Despite the increased understanding of routes of product contamination and hygiene management the number of reported food poisoning
outbreaks is still on the increase as reported by the Steering Group on the microbiological safety of food (Steering group).

2.1 The Work Presented in this Thesis

The concept behind carrying out the research presented in this thesis was to develop, implement and validate an effective cleaning system to be applied to a high risk area of a cheese manufacturing company.

Previous research carried out in conjunction with the host cheese processing company highlighted that the cleaning systems which were in place were not suitable and were not effective for such a production process (Elvers & Peters 1999).

Areas researched in the field of hygiene and cleaning within the food industry has been widely discussed but as yet no known research has taken place looking into the validation of cleaning systems and the use of ozone as a terminal disinfectant within industry. Research has however been conducted under laboratory conditions on the potential use of ozone as a terminal disinfectant within the food industry (Moore et al 2000).

The apparent lack of published research in this field to have been conducted within industry, suggests that the development, implementation and validation of effective cleaning systems within industry would be a step forward in research and also be of particular value to the host company in giving them a leading edge within the sector in that the effectiveness of the cleaning systems in place have been validated, are proven to be effective under industrial conditions and within the working environment and will
be an effective tool for the company to present customers with during audits as a pro-active reference material.

The work was carried out during the tenure of a Teaching Company Scheme between Abergavenny Fine Foods and UWIC.

2.2 Aims and Objectives

The aim of this research project was to develop and evaluate an effective cleaning system which is suitable and effective for use within the dairy environment. The objectives of this work were:

1. To validate the cleaning chemicals under laboratory conditions

2. To validate the cleaning chemicals in situ, and,

3. To validate the effectiveness of ozone as part of a complete cleaning system.
Chapter 2

Validation of cleaning chemicals under laboratory conditions

2.1 Introduction.
In order to compare the effectiveness of the chemicals in reducing bacterial logs research was carried out within laboratory conditions. Laboratory validation is a well proven method which has been tried and tested throughout the industry. Laboratory validation is often considered to be the initial step in proving a system or method works. This was to assess the effectiveness of the chemicals within laboratory conditions eliminating any variables which may affect the true results. To enable the work to be carried out to be of relevance to a dairy environment, samples of dairy product were obtained to allow the conditions to be replicated as much as possible.

2.2 Aims and objectives
The aim of this validation work was to,

1. Develop and validate a cleaning protocol under laboratory conditions

The objectives of this work were to

1. Review cleaning procedures in a high risk dairy plant and develop new cleaning protocols.
2. Develop surface contamination protocol
3. Develop a lab-based cleaning protocol
4. Replicate the conditions and procedures used in the dairy processing unit within laboratory conditions in order to validate the system.
5. Validate the results obtained from the work in order to prove the cleaning system works.

2.3 Review of Cleaning Procedures

A review of the existing cleaning procedures was carried out to look at the cleaning methods, chemicals and protocols used and to see where improvements could be made. The cleaning method used within the dairy was the traditional “Bucket and Brush” method whereby brushes and J-cloths were used in conjunction with a portable bucket of cleaning chemicals. The problem with this method which was highlighted during the review was that there appeared to be a lack of staff training with regards to clean as you go, cross contamination and good housekeeping. It was evident from the observation exercises that there were a number of issues which raised concern and confirmed that the cleaning methods were not sufficient:

1. The cleaning of the production line took place at the end of a shift which meant that there was no designated cleaning crew or time allowance. Once the production run had finished the cleaning took place. This was a management issue in the way in which cleaning was not considered to be of that great importance. The main priority was the production of saleable items.

2. The J-cloths used were not always disposed of after cleaning each piece of equipment but instead were used on various pieces of equipment throughout the cleaning shift. This meant that it was possible for cross contamination to occur between the various pieces of equipment.
3. The cleaning would start with a dose of Dairy Detergent in a bucket however this solution would not be refreshed during the clean, therefore the equipment being cleaned later on during the shift would be cleaned using chemicals contaminated with food debris and any bacteria which may have been present on previous equipment. It was also noted that when the cleaning solutions were made up from the neat concentration to the required diluted concentration that there was no accurate way of dosing the chemicals. The chemicals were seen to be poured into a bucket and diluted without any precise measuring.

4. The dairy had colour coded equipment, Red for food contact areas and Blue for drains and floors. It was observed that some members of staff were using the incorrect colour brushes for the food contact areas and floors and drains. This meant that the possibility of cross contamination was increased and that there was an obvious lack of training and monitoring of the cleaning methods used and hygiene standards being achieved.

5. During the cleaning there was a lack of contact time given to the chemicals. This was again a result of lack of training and a lack of time being allocated to the cleaning stage of the shift. The cleaning took place by the production operators at the end of the production shift. Part of the problem was that the opinion of the production staff was that they shouldn’t be responsible for cleaning. This highlighted the need for training and re-education of staff to see the importance of cleaning and the correct way to clean.

6. The rinsing of the chemicals took place using a hose pipe which had often been left on the floor. The hose pipe was used to rinse down machinery and
any debris from the floor. This meant that there was potential for cross contamination via aerosols to be sprayed from the floor along with any foreign body contamination there may be on the floor or surrounding environment.

The chemicals used within the dairy were Dairy Detergent, a non-ionic detergent at 2% purchased from R.M. Jones, Agricultural supplies, Abergavenny and Sodium Hypochlorite (14%) used at 1% also purchased from R.M. Jones. These chemicals were not specifically for food manufacture premises but mainly used within the farming industry for the cleaning of milking parlours. The cleaning protocols were written up to include the chemicals to be used, the concentration and bucket and brush cleaning method. The protocols did not include the colour coding system, the need to use a clean J-cloth for each stage or contact time required for each chemical. There was a lack of training amongst the staff along with a lack of information and guidance on the cleaning protocols.

Overall the cleaning system in place was not sufficient to maintain a satisfactory microbiological standard within the dairy. This was confirmed by a study carried out by the Food Safety Research Group of UWIC. The microbiological results obtained both from finished product and environmental and food contact swabbing indicated that there were issues within the dairy with high coliform counts and the presence of *Listeria* on swab results and finished product. Some results also suggested re-contamination of surfaces after cleaning and proliferation of surface contamination. (Elvers & Peters 1999).
2.4 Development of the Cleaning Process

The existing cleaning processes were reviewed and a major cleaning chemical company was commissioned to help establish an appropriate suite of cleaning protocols within the dairy. The objective of this was to find cleaning chemicals that would be effective on both food contact areas and environmental surfaces. Holchem were contacted to discuss the chemicals they were able to offer and the service they could provide to the dairy industry. The important factor in choosing to work with Holchem to supply the cleaning chemicals was that they were able to provide:

1. Chemicals which were suitable for Bucket and Brush cleaning method, within the dairy industry and effective on both food contact and environmental surfaces,
2. Cleaning protocols for individual pieces of equipment in specific areas,
3. Auditing service on a monthly basis,
4. Safety signs and chemical information packs
5. Accurate dosing system for the chemicals,
6. Staff training on chemical handling.

The chemicals chosen to work with was Holsolve and Terminol. Holsolve is a highly effective low alkaline based detergent which may be used for light/medium duty cleaning. Terminol is a highly effective amphoteric and QAC based disinfectant which has passed the European standard test method for disinfectant efficiency and contact taint test. It is also an approved disinfectant by Marks and Spencer. This was an important factor as Abergavenny Fine Foods were suppliers to Marks and Spencer. Both the
Holsolve and Terminol were suitable for use with the bucket and brush method and effective on food contact areas and environmental areas.

Cleaning protocols were written for every piece of equipment and its associated parts for all areas within the dairy. Within the protocol all aspects of the cleaning were detailed:

1. Area of dairy
2. Item of equipment
3. Health and safety advise if applicable
4. Any engineering requirements
5. Cleaning equipment required including colour coded items
6. Chemicals required, the concentration and contact time
7. Rinsing instructions
8. Key inspection points and swabbing areas

Holchem were also able to support Abergavenny Fine Foods by carrying out a monthly audit to support the company, ensure all chemical awareness signs and protocols were in place and to discuss the results being obtained along with any issues or queries there may have been. Staff training was organised for all Production, Warehouse, New Product Development staff and Quality Assurance staff. The training covered all aspects of chemical handling including:

1. Introduction to Holsolve and Terminol
2. Health and safety
3. The importance of cleaning
4. How to clean
2.5 Methods

2.5.1 Preparation of Innoculum

To resemble the working environment samples of the following dairy products which are produced on the site were taken: 10g of Pant-ys-gawn soft goats cheese and 10g of mature cheddar; were obtained from Abergavenny Fine Foods and homogenised with 70ml of deionised water. Added to this homogenate was then 10ml of overnight culture of *Listeria* and *Enterobacteriaceae* which were at the concentration of $10^7$ CFU/g. The final concentration was approximately $10^6$ CFU/g per organism. The *Listeria* sample was isolated from a food manufacturing plant obtained from the departmental culture collection through personal communication with Peters and Elvers. The *Enterobacteriaceae* had been previously inoculated in nutrient broth (NB, Oxoid, Basingstoke, Hants) using the orbital incubator for 24hrs at 24°C. The *Listeria* had been cultured using Buffered Listeria Enrichment Broth (BLEB, Oxoid, Basingstoke, Hants) and was incubated at 24°C for 24 hours in the orbital incubator. After overnight incubation the *Listeria* and *Enterobacteriaceae* cultures were mixed prior to their addition to the cheese and buffer homogenate.
2.5.2 Preparation of the sampling table

A stainless steel table measuring 200cm x 90cm was marked with a grid reference on the top of the table top. Each of the squares measured 10cm x 10cm square. The grid references were identified by lettering and numbering system. The table was cleaned prior to any inoculation with the inoculated homogenised cheese sample. The cleaning method followed was that validated at UWIC (Moore et al 2000). This cleaning method was validated for the purpose of ensuring that laboratory validation was successful and that cleaning techniques were suitable in order to get a clean surface prior to laboratory work taking place.

2.5.3 Inoculating the sampling table

The inoculated cheese sample (100ml in total) was then spread over the grid reference table. The table was allowed to dry for 5 minutes to represent the factory environment.

2.5.4 Cleaning Process

The cleaning process involved the chemicals being accurately dosed into cold tap water to the required concentrations. The first cleaning stage required 2% of Holsolve. The cleaning was carried out manually using a clean J-cloth. The contact time of 10-15 minutes was allowed during the cleaning prior to swabbing. No rinse was required after the use of Holsolve.

The disinfectant stage was carried out with 1% of Terminol. The cleaning was again carried out manually with a clean J-cloth to replicate factory methods and a contact time of 10-15 minutes was allowed. No rinse was required after the use of Terminol.
2.5.5 Swabbing process

The cotton swabs were premoistened in sterile Difco de-neutralising broth (DENB, Oxoid). 5 squares were chosen at random by pulling out grid references from a box and swabbed as a control measure. The swabbing method used was swabbing a 100cm² first in one direction then at 90°. The swabs were rotated while in contact with the table. These swabs were then released into 10ml of DENB contained in a universal bottle. The swabs were then vortexed and serially diluted to 10⁴ in MRD (Maximum Recovery Diluent). Duplicate pour plates were prepared for each dilution using nutrient agar and incubated for 24 hours at 24°C. The method of plating was done using both, the pour plate method and the spread plate method. Duplicates of each plating method and dilution were carried out. This was repeated after each of the cleaning stages:

1. Control

2. Detergent cleaning stage. Holsolve was used at a concentration of 2%, diluted in cold tap water and allowed contact time of 10-15 minutes.

3. Disinfectant stage. Terminol was used at a concentration of 1%, diluted in cold tap water and allowed a contact time of 10-15 minutes. A rinse is not required with Terminol as it is non-tainting.

4. 1 hour of air drying and

5. 2 hours of air drying.

This was done to replicate the factory environment and cleaning methods. This experiment was repeated 3 times.
2.6 Data Analysis

The plates were read for the number of colonies, both *Listeria* and coliforms present on each plate. The ISO plate counting method was used. These results were recorded and the log reduction was calculated for each plate at each dilution rate and at each stage of cleaning. This was done by using the following formula

\[
\log_{10} \text{mean control count} = \text{CFU/ml-log}_{10} \text{mean count}
\]

To overcome the minimum detection limit (MDL) also known as limit of detection (LOD) issue, the formula below was used in order that a log value is available for further analysis.

\[
\text{LOD} = \frac{\text{Limit of Detection}}{\sqrt{2}}
\]

For example where the LOD = 10:

A count of <10 is recorded as \(10 = 7.1\) then this is logged to give 0.85

\[
\text{LOD} = \frac{10}{\sqrt{2}}
\]

(CCFRA 2002).

Plate counts were used to calculate log reductions achieved at each cleaning stage. This can be seen in Table 2.4.1 The significance of differences in log reductions were assessed using one way analysis of variance, ANOVA (Minitab Vs 11).
Table 2.6.1 The mean log numbers recovered at each stage of the cleaning protocol carried out under laboratory conditions.

<table>
<thead>
<tr>
<th>Control</th>
<th>Holsolve Stage</th>
<th>Terminol Stage</th>
<th>1 Hr Dry</th>
<th>2 Hr Dry</th>
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<tr>
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</table>

2.7 Results

From the one way analysis of variance it shows that there is a significant difference between stage 1 and 2 and 2 and 3, but not a significant difference between stages 3 and 4. This indicates that drying does not have a significant affect on reducing the number of colonies on a surface. However the real value of drying is the prevention of re-growth. This result has provided a further area of research which could look into the re-growth of organisms following cleaning. This research could be carried out during industry where the colony counts are monitored after cleaning during a late shift and the following morning when a drying time has been allowed.
Figure 2.7.1 The mean log_{10} CFU/100cm obtained from the laboratory validation of the cleaning chemicals.

Figure 2.7.1 The mean log_{10} CFU/100cm^2 obtained from laboratory validation of cleaning chemicals. Cleaning Stage 1 = control, Stage 2 = After the Detergent stage, Stage 3 = After Disinfectant stage, Stage 4 = After 1 hour drying time and Stage 5 = After 2 hour drying time. Error bars indicate the pooled standard deviation.

The results show that the disinfectant stage (Stage 3) had the highest log reduction of all of the cleaning stages with a 0.9 log reduction in numbers. This indicates that the detergent stage is very important for the removal of food debris and reduction in microbiological numbers and that the disinfectant stage also has a positive role within the cleaning protocol.
Stage 3, the disinfectant stage complemented the detergent stage by again having a positive effect on the reduction of microbial numbers, this stage achieved approximately 0.7 log reduction in numbers.

Stages 4 and 5 did not show a significant log reduction in the numbers obtained but does show that drying time is essential for preventing re-growth of bacterial numbers.

One way analysis of variance demonstrated a significant (P< 0.05%) log reduction during the cleaning process. Visual inspection of the ANOVA data suggested that the significant difference occurred between stages 1 and 2 and 2 and 3.

2.8 Discussion

The results obtained from this work confirmed that the Holsolve detergent and Terminol disinfectant stage of cleaning systems do have a significant effect on the reduction of microbiological numbers. The work showed that there was a total of approximately 1.6 log reduction after the detergent and disinfectant stage. This is due to the 0.7 log reduction in numbers after the detergent stage and a 0.9 log reduction in numbers after the detergent stage. A study carried out by (Gram et al 2007) showed that a 2-3 log reduction of the number of *Listeria monocytogenes* was achieved when immersed in fish emulsions whereas a 1-2 log reduction of *L. monocytogenes* was achieved immersed in meat emulsion. The study also confirmed that the food matrix present during the clean has a strong influence on the efficiency of the cleaning and disinfectant stages.

Stages 3 and 4 did not have a significant log reduction in the microbial numbers but the counts did remain reduced and did not increase. This confirms that the drying time allocated to stainless steel after cleaning does have a positive effect in that the re-
growth of microbial contamination is controlled. This was an important find as prior to
the cleaning protocols being reviewed it was often found that production equipment
would be soaked overnight. On investigating this it was discovered that it was thought to
be beneficial by the production managers as the equipment would be soaking and
therefore remain as clean as they were when put into soak. However this was not the case
as the dosing system in place prior to the review was not sufficient to ensure the correct
concentration of chemicals were being used. Also the renewal of the chemicals was not
carried out at regular intervals as recommended.

2.9 Conclusion

The laboratory validation indicated that the use of Holsolve and Terminol to clean
contaminated dairy waste from stainless steel would achieve an acceptable microbial
population. The results show that a successful cleaning system was achieved under
laboratory conditions. The implementation and in-situ validation would be critical.
3.1 Introduction

Much work has been carried out in testing the efficacy of disinfectants. The most common test for such efficacy tests are suspension tests. These tests allow for the quantitative estimate of the log reduction factors of the disinfectant on test microorganisms which are in suspension of the disinfectants on test.

Work has also taken place within industry looking into the techniques of cleaning and cleaning systems. Research was carried out by means of environmental swabbing and for coliforms and TVC at the various stages of cleaning, before cleaning, after the detergent stage and after disinfection (Gibson et al 1999). It has also been identified that more research is required in microbial inactivation by the use of ozone and to optimize its use in food applications. Work carried out under laboratory conditions indicate that ozone could be used as an effective terminal disinfectant as part of an adequate cleaning system (Moore et al 2000). Ozone has been proven to effectively inactivate *Listeria innocua* at concentrations of 50 and 100nl l (-1) during short exposure times at both 5°C and 20°C (Fan et al 2007). It is from these studies that the validation of cleaning chemicals and ozone as part of a complete cleaning system was developed. It was considered that such work had been carried out to the point of the cleaning and disinfection stage but no evidence of including the use of ozone as a terminal disinfectant has been found which has been carried out within industry. From the literature reviews into the past research...
there appeared to be a gap in the research which leads to the following experiments being designed.

### 3.2 Aims and objectives

The aims of this chapter were to:

- Implement and evaluate the effectiveness of a cleaning process
- Determine the effectiveness of each cleaning stage of the full cleaning system
- Determine the effectiveness of ozone as a terminal disinfectant

The objectives of this work were to

- To implement a cleaning process and install ozone gas as a terminal disinfectant process
- Design an experiment which will allow the monitoring of each stage of the cleaning system including, pre-clean, detergent, disinfectant and ozone application.
- To analyse the results to determine the effectiveness of each of the cleaning stages and the additional terminal disinfectant of ozone.

### 3.3 Methods

To have a fair comparison of food contact and environmental surfaces the names of every area which was available to swabbing was documented. To avoid any additional cleaning no members of staff or production supervisors or management were made aware of the sites which were to be tested on those days.
3.3.1 Implementation of Cleaning processes

To carry out a successful validation of a cleaning system the process started by having fully documented cleaning protocols which covered all aspects of the cleaning. The initial stage was to carry out a full audit of the production and packing areas of Abergavenny fine foods and document each piece of equipment and the cleaning process which best suited that piece of machinery or equipment. The audit took into account any assistance which may be required from the Engineering department in order to carry out a successful clean. A colour coding cleaning utensil system was in place in each department of the dairy, these details were included on the cleaning schedules along with the chemicals and their concentrations to be used for each cleaning stage. The cleaning protocols were written in conjunction with Holchem (Lancashire). The cleaning methods implemented was the “Bucket and brush” method using clean J-cloths or the dedicated colour coded brushes, the required concentrations and contact times were also included in the protocols.

3.3.2 Installation of Ozone system

The ozone generator was supplied by Ozone Industries (Corona 10000, Ozone Industries, Hampshire, UK). The unit produced 10g/hr of ozone gas at a flow rate of 4l/min using 95% oxygen feed gas from an oxygen concentrator.

The ozone generator was set up in the roof void of the production unit, from this transfer pipes were located through the roof void to two areas of the production facility to allow an even distribution of ozone throughout the area. To ensure an even flow of ozone to all areas, 2 fans were put in place to distribute the ozone. It was discovered that ozone
can have a detrimental effect on rubber and latex. Due to this an audit was carried out to assess if any machinery or pieces of equipment could be affected by this. As a result it was decided that all rubber seals found on the machinery would be cleaned after use, then sealed in a plastic bag and locked in the equipment store which would prevent exposing them to ozone.

As part of health and safety requirements, steps had to be put in place to inform all employees when ozone was running. This warning was achieved by the installation of blue flashing lights at each entrance into the factory which operated 15 minutes prior to ozone being generated, during ozone generation and then for 45 minutes after ozone generation.

The ozone generator was set up to administer a maximum of 2ppm. This was monitored by Ozone Industries. The Ozone system was programmed to work during the night when the factory had closed. Therefore once the generator had finished producing ozone there was adequate time for the ozone to degenerate and for the level to drop prior to the next production shift.

3.3.3 Selection of testing areas

Ten areas, 5 environmental areas and 5 food contact areas, were chosen at random to swab at each cleaning stage, the pre-clean, detergent stage, disinfectant stage and the ozone application. This was done by picking references out of a container.
3.3.4 Swabbing process

At each stage environmental swabs were taken and tested for TVC and coliforms. The swabs were taken on a 10cm x 10cm square. Each swab was taken from this size area to ensure consistency. The swab was rotated whilst in contact with the surface in one direction then turned 90° and again rotated to ensure accurate pick up. 10 swabs were taken for each area. The swabs were sent to South Wales Food Laboratories (Newport, South Wales) for analysis.

3.3.5 Cleaning stages

The cleaning stages followed the routine cleaning system as used daily within the dairy. The cleaning schedules followed were those supplied to the dairy by the supplier of the chemicals. The chemicals were supplied by Holchem. All cleaning schedules were written in conjunction with Holchem, Lancashire and Abergavenny Fine foods following on from auditing the equipment and set up of the factory in order to ensure the cleaning schedules would allow a clean to the highest standard.

The pre-clean is the stage during which the residual debris is removed. The debris is removed by means of scraping excess cheese from the surface of the equipment in order to remove the excess debris to allow an easier clean.

The detergent stage is the main clean in which the Holsolve detergent is used (2% concentration). The detergent stage is carried manually using j-cloths or dedicated brushes. The detergent has a contact time of 10 minutes. The food debris can be difficult to remove during cleaning and can prevent weak detergent from getting under the surface
of the food debris and therefore making the detergent stage difficult. Following on from this stage is the disinfection stage.

The disinfection stage is carried out with the use of the Terminol disinfectant (1% concentration from Holchem, Lancashire). The disinfection stage is carried out manually using a J-cloth. As part of the disinfection stage there is a rinse to remove any residual chemicals. Each of the initial 3 stages is carried out manually. This is otherwise known as the bucket and brush method. The final stage of the cleaning system was the terminal disinfection stage of the ozone (2ppm). The monitoring of the different stages of the cleaning system was carried out over a 3 week period.

3.4 Data Analysis.

The swabs were taken and sent to South Wales Food Laboratories (UKAS Accreditation) for analysis. The swabs were each tested for Total Viable Count (TVC) and coliforms.

A comparison of the results obtained from the swabbing at the various cleaning stages was carried out and a reduction in the colony counts was noticed after the different stages of the cleaning system. The results were analysed to see the log reduction in the TVC and coliform cfu/g obtained after each of the cleaning stages. This was carried out using the same formula as quoted in Chapter 2. One way analysis of variance was carried out using Minitab Version 12. The one way analysis of variance was carried out on both the TVC and coliform results for the environmental areas and food contact areas to see if there was a significant difference between each cleaning stage.
3.5 Results

It was evident from the results obtained that each of the cleaning stages had a significant effect on the reduction of the microorganisms detected from the area however, the addition of the ozone application also made a significant difference after application following on from the disinfection stage. The results of the swabbing were then logged in order that the results could be shown graphically. For those results which were below the limit of detection (<10) the formula shown in Chapter 2 was used to obtain a figure which was then applied to log format for graphical display.

From the one way analysis of variance it was shown that there is a significant difference between the pre-clean and detergent stage. One way analysis of variance also showed that there was no significant difference between the detergent and disinfectant stage or the disinfectant stage and ozone.
Figure 3.5.1 Average Log reduction of TVC on Food Contact areas at each stage in the validation of ozone. Stage 1 = Pre-clean, Stage 2 = Detergent Stage, Stage 3 = Disinfectant Stage, Stage 4 = Ozone.

As the one-way analysis of variance calculated, and as can be seen in figure 3.5.1, there was a significant difference between cleaning stage 1 and 2. However there was not a significant difference between cleaning stages 2 and 3 or 3 and 4. However there is still a downward trend as shown in the graph which indicates that stages 2, 3 and 4 are all having an effect on the reduction of the bacteria present on the food contact areas.
Figure 3.5.2

Average Log reduction of TVC on Food Environmental areas at each stage in the validation of ozone. Stage 1 = Pre-clean, Stage 2 = Detergent Stage, Stage 3 = Disinfectant Stage, Stage 4 = Ozone.

Figure 3.5.2 shows that in agreement with the one way analysis of variance there is a significant difference between cleaning stages 1 and 2 but not between 2 and 3 or 3 and 4. However as displayed in the graph, it shows that there is a definite reduction in the average log reduction after each cleaning stage. Therefore the reduction may not be considered as a significant difference between the cleaning stages but there is still a log reduction between them which proves the importance of having a cleaning system with multiple cleaning stages.
Figure 3.5.3  Average Log reduction of Coliforms on Environmental areas at each stage in the validation of ozone. Stage 1 = Pre-clean, Stage 2 = Detergent Stage, Stage 3 = Disinfectant Stage, Stage 4 = Ozone.

The one way analysis of variance showed that there was a significant difference between cleaning stages 1 and 2 but not significant between 2 and 3 or 3 and 4. However the graph shows that there was still a continuing decrease in the colony counts obtained after each cleaning stage.

The average log reduction of the coliforms on environmental surfaces was shown to have a significant difference between stage 1 and 2 but no significant difference between stages 2 and 3 and 3 and 4.
The graph shows the average log reduction obtained for coliforms from food contact areas. As the graph shows and as the one way analysis of variance showed there is no significant difference between the counts obtained from each of the cleaning stages, however this could be due to the number of outliers which were due to the fact that the numbers were generally low. Therefore the statistics on this data are unreliable. From looking at the data in a graphical form it does still however appear to be a reduction in the average log obtained at each stage which indicates that each cleaning stage does have a positive effect in reducing colony counts on food contact areas.
3.6 Discussion

From looking at the results obtained from this work it is clear that each stage of the cleaning system has a positive effect on the reduction of microbial counts on both food contact areas and environmental areas. Even though the difference between each of the stages is not significant in terms of the one way analysis of variance it is clear from the graphical displays of the Log reductions achieved that each stage has an important role to play within a complete cleaning system.

This has been shown in previous studies where there was no significant difference found between ozone and chlorine inactivation of the bacteria with the exception of Pseudomonas putida, where as results indicated that there was a significant difference achieved with ozone and chlorine when exposed to biofilm bacteria (Dosti et al 2005).

From the results obtained from the work carried out at Abergavenny Fine Foods (Elvers & Peters 1999), it is evident that the cleaning systems developed and implemented within this chapter have improved the hygiene conditions within the plant. This is due to the comparison of the results obtained from each set of work. The study carried out by Elvers and Peters showed that many of the sites returned counts which could indicate the presence of bacterial biofilms. The purpose of the study was to highlight that the cleaning process which was in place was not effective at controlling or reducing the bacterial counts within the dairy.

3.7 Conclusion

It is clear that a positive impact has been achieved by the implementation of the new cleaning system. There is a reduction in the number of microbial numbers recovered after each cleaning stage for both food contact and environmental areas and for TVC and
Coliforms which indicates that the system is working well and is effective in a dairy processing environment such as Abergavenny Fine Foods. The next stage of research which would follow on from this chapter would be to look at the effects of ozone over a longer period of time. This would then show how ozone performs within industry over a period of time.
Chapter 4

The monitoring of environmental cleanliness of food contact and non-food contact areas over a period of time with and without ozone application.

4.1 Introduction.

The next stage in the research into the use of ozone was to monitor the hygiene within the dairy environment with and without the use of ozone. Previous work has been carried out looking into the effectiveness of ozone as a terminal disinfectant (Moore et al. 2000) however in situ validation of the use of ozone as a terminal disinfectant is missing in the work carried out to date. The research would also look into the effect of ozone use over a period of time.

It was decided to use dip slides for this work as the sampling needed to take place after the cleaning stages carried out after the afternoon shift and the following morning after the ozone application. By using dip slides they could be incubated in house following the sampling and would not have to be held until the following morning for collection by the laboratory.

Research has been carried out under laboratory conditions assessing the effectiveness of ozone on reducing bacterial numbers (Moore et al. 2000). This research suggests that if ozone is applied after an adequate cleaning system that gaseous ozone could be used as an effective terminal disinfectant in-situ. It is important that an effective cleaning system is in place as inadequate cleaning favour the attachment of bacteria which can lead to biofilm formation which are more resistant to disinfectants than free living cells (Andrade et al. 1998; Hood & Zottola 1995; Norwood & Gilmour 1999). Due
to this is if an adequate cleaning system is not in place for both food contact areas and environmental areas then effect of ozone may prove less effective. There appears to be a lack of research which has taken place in-situ within industry to assess how effective ozone is as a terminal disinfectant within food manufacturing plants.

### 4.2 Aims and objectives

The aim of this work was to:

- Assess the effectiveness of the overall cleanliness of the dairy over time using the implemented cleaning system alone and alone and in combination with the use of ozone as a terminal disinfectant.
- The aim of this validation work was to demonstrate the long term effectiveness of ozone as part of a cleaning system within industry.

The objective of this work is to

- Validate the effectiveness of ozone when used in situ in conjunction with a proven cleaning system.

### 4.3 Method

#### 4.3.1 Setting up the testing regime

To enable this validation work to be carried out in situ it was necessary that all production operators, supervisors and managers were not informed when ozone was running or not in order that the cleaning remained constant and that there was no changing factor as far as the staff were concerned. Therefore only QA were aware of when the ozone was on or not.
4.3.2 Health and Safety

As part of health and safety requirements, steps had to be put in place to inform all employees when ozone was running. This warning was achieved by the installation of blue flashing lights at each entrance into the factory which operated 15 minutes prior to ozone being generated, during ozone generation and then for 45 minutes after ozone generation. The system installed at Abergavenny was a Corona 10000. This unit produces 10g/h of ozone gas at a flow rate of 4 L/min using a 95% oxygen feed gas from an oxygen concentrator. The ozone generator worked at a concentration of 2ppm.

4.3.3 Sampling plan.

To validate the effectiveness of each of the cleaning stages in situ, dip slides (Supplied by UWIC, Cardiff) had to be taken after each cleaning stage and ozone application in order to provide a full analysis. Dip slides were used as it felt that it was a good method to use as there were a number of samples to be taken throughout the afternoon shift after each cleaning stage and that the dip slides could then the incubated in house using a portable incubator. Dip slides were taken prior to cleaning, after the detergent stages (Holsolve), after the disinfectant stage (Terminol) and the following morning after ozone application. This way the counts and log reductions obtained after each stage will be validated and the effectiveness proven. The dip slide monitoring was carried out on food contact and environmental areas. This was to ensure that the validation took into account both types of surfaces and areas found within the food industry. The areas for the dip slide monitoring to take place were chosen at random. 5 random environmental areas were
sampled and 5 random food contact areas were sampled. The dip slides used were those to monitor APC (aerobic plate count/TVC) and Enterobacteriaceae. Once the dip slides had been taken the outer pots were labeled up to ensure traceability. The dip slides were then incubated at 24°C for 24 hours until they were read. The portable incubator used was from Biotrace of Bridgend, 280v. The results of the dip slides were converted to a scaling system to allow the results to be presented in a graphical format.

4.4 Analysis of results

As dip slides are scored on a system of $10^2$, $10^3$, $10^4$ growth etc in order to put these results into a format where they could be displayed graphically the following system was employed:

1 = $10^2$

2 = $10^3$

3 = $10^4$

4 = $10^5$

This was done due to the scoring system employed on the dip slides. The results were graphically displayed and a trend line applied to show the effect of ozone. The results were plotted on four different graphs.

Figure 4.4.1 shows the average Enterobacteriaceae results obtained from food contact surfaces before and after ozone application.
Figure 4.4.1

As the graph shows there is a definite downward trend with time and with the application of ozone. Even though the results were not suitable to be analysed statistically the results clearly show that ozone is having a positive effect on reducing the counts of Enterobacteriaceae. The results were taken over a period of three weeks and as the graph shows the results the application of ozone has a positive effect on food contact areas throughout the dairy. The advantage of ozone application is that due to it being in a gaseous state that it is able to have a positive effect on all surfaces not just on horizontal surfaces as found with fogging. From the graph it is clear to see that during the whole trial there were ten days which showed a decrease in the microbial numbers after the ozone exposure to the production areas, 5 days showed no difference in the number of counts before and after ozone exposure and 1 day showed an increase after ozone
exposure. By applying the trend line to the graph it is evident that the overall effect of ozone application over a period of time does have a positive effect on the number of Enterobacteriaceae recovered from food contact areas.

Figure 4.4.2

![Graph showing average Enterobacteriaceae counts before and after ozone application.]

Figure 4.4.2  Average Enterobacteriaceae on Environmental areas before and After Ozone

As figure 4.4.2 shows, on environmental surfaces the ozone again has a very positive effect on reducing the number of Enterobacteriaceae obtained from environmental areas. The trend lines applied to the graph show the continuing reduction of the counts obtained over time on environmental areas. With time the results show that even before the ozone application the results being obtained are lower.

The results show that during the research there were 13 days where there was a reduction in the Enterbacteriaceae obtained after ozone application, 2 days where the counts remained the same and 1 day where there was an increase in counts after the
ozone application. These results indicate that ozone has a positive effect on reducing the number of Enterobacteriaceae on environmental surfaces.

The analysis was also carried out looking at the average aerobic plate count colonies located from food contact and environmental areas. Figure 4.4.3 shows the average APC obtained from food contact areas before and after ozone.

Figure 4.4.3

![Average APC on Food Contact areas before and after ozone](image)

The trend lines on the graph shows that the results obtained from the dip slides before and after ozone are not reducing as significantly as the previous results but the results after ozone application are remaining constant whereas the results before ozone are increasing. This indicates that ozone is having a significant effect at controlling the counts obtained. Therefore ozone is having an effective control on the aerobic colonies within the dairy on food contact areas. The graph shows that 12 days of the trial showed a
reduction in the number of counts obtained after ozone application, 2 days which remained the same and 1 day where there was a rise in the number of counts recovered.

Figure 4.4.4

Figure 4.4.4  Average APC results obtained from Environmental areas.

Again the results show that ozone is having a positive effect on the reduction of APC on environmental surfaces. The trend line for the results obtained after the ozone application shows a definite downward trend whereas the trend line for the results obtained from the environmental areas before ozone application indicates an upward trend. Over this period of time there were 13 days where there was a reduction of counts after the ozone application and 2 days where there was an increase in the counts after ozone application.

Following on from the research to see the effect ozone has over time on the microbial counts obtained after the cleaning phase and ozone application, the second part of the work was to look into what effect no ozone application would have on the counts
obtained. The following graph shows the counts obtained of APC results obtained from food contact areas after cleaning and the following morning after no ozone application.

Figure 4.4.5 Average APC count obtained from food contact areas after cleaning and the following morning after no ozone exposure.

This graph shows the average APC count obtained from food contact areas during the time when ozone was switched off. The trend lines show that during the time when ozone was switched off there was a dramatic increase in the average number of counts obtained in particular the following morning after the cleaning process. This indicates that even over the space of a few days the lack of ozone application does appear to have a significant effect on the counts obtained. The graph shows that over a 2 week period there were 3 days where the counts obtained did reduce in the morning after the cleaning process, 1 day had the same result immediately after cleaning and the following morning.
and 10 days had an increase in the count from immediately after the cleaning process to the following morning.

Figure 4.4.6

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Figure 4.4.6  Average APC count obtained from Environmental surfaces after cleaning and the following morning after no ozone application.

The trend lines applied to the graph show the increase in counts obtained as time progressed without the application of ozone. During the 2 weeks 2 days had a reduction in the counts obtained between those samples taken immediately after the cleaning stage and the following morning. 4 days had the same result obtained from both the samples after the cleaning process and the following morning and 8 days had an increase in counts on the following morning after no ozone application.
Figure 4.4.7  Average count of Enterobacteriaceae on food contact areas after cleaning and the following morning after no ozone application.

The trend lines show an increase in the number of counts obtained over time without the application of ozone. During the 2 week period 1 day showed a decrease in the number of counts obtained between the cleaning process and the following morning, 4 days gave the same counts between the samples taken immediately after the clean to the following morning and 9 days showed an increase in the number of counts achieved between the samples take immediately after the cleaning in comparison to the following morning without the application of ozone.

The final graph in this section shows the results obtained from the average Enterobacteriaceae on environmental areas with no ozone application.
Figure 4.4.8

Average count of Enterobacteriaceae on Environmental surfaces after cleaning and after no ozone application.

The trend lines applied to this graph show the increase in Enterobacteriaceae counts obtained from environmental surfaces immediately after the cleaning process and the following morning. 1 day showed a reduction in the number of counts obtained from immediately after the clean to the following morning, 4 days had the same result and 9 days had an increase in counts from after the clean to the following morning.

This research confirms that ozone does have a positive effect as part of an effective cleaning system within dairy processing in reducing and controlling the number of microbial counts found on both food contact and environmental areas.
The following graph shows the overall effect on the presence of Listeria the implementation of the cleaning system and ozone as a terminal disinfectant. It also indicates when the major stages occurred with regards to ozone installation, Staff training was carried out by Abergavenny Fine Foods and Holchem and when the ozone was switched off.

The graph shows that during the months of September to December when he monitoring was taking place without any changes to the cleaning system that the incidence of positive *Listeria* results from swabs was high with monthly numbers ranging from 70 to 100 positive *Listeria* swabs per month. This was an extremely high number of positive *Listeria* swabs to be having within a dairy plant and highlights just how vital it was for the development, validation and implementation of an effective cleaning system.

The graph highlights when the ozone was installed in the manufacturing plant, it clearly

**Figure 4.4.9** Graph showing the percentage of positive *Listeria* swabs recovered from environmental areas over a 17 month period. Highlighted on the graph are the major events which took place throughout the research.
shows that the months following the installation of ozone there is a significant decline in the number of positive Listeria results being obtained. As part of the implementation of the cleaning system, staff training took place in May, as shown on the graph, this was carried out by Holchem in conjunction with Abergavenny Fine Foods. By looking at the number of positive Listeria results obtained in the months following the training it is clear that the staff training had a positive effect on the results as the number of positive swabs obtained. To see the effect ozone was having it was decided that it would be switched off; this was not common knowledge to any production staff. This action was initially planned for 2 months but due to the dramatic increase in positive Listeria swabs in the first month the trial was cut short and the ozone generator was switched on as it was decided that the ozone stage of the cleaning system was too important in the control and elimination of microbial contamination not to have running. The final stage of the graph shows the results being obtained when ozone was back on, with the results of the final month being monitored, February having no positive Listeria swabs from the production area. This proves the effectiveness of ozone within dairy manufacturing.

4.5 Discussion

It was important to carry out research into the effectiveness of ozone over a period of time, in-situ to determine how effective ozone would be once applied to the manufacturing environment. These results indicate that ozone is having a significant effect on the counts obtained as it is keeping under control and reducing the colonies associated with the environmental and food contact areas. This indicates that ozone has a definite advantage over other systems such as fogging as ozone works on horizontal and
vertical surfaces. The section of research which was carried out without ozone application had a negative effect on the number of microbial counts recovered from the sites sampled. When the research into the effect of ozone being switched off started it was evident in a short amount of time that ozone quickly needed to be switched back on within the dairy due to the bacterial counts increasing. Also by the last month of monitoring having no positive *Listeria* swabs it indicates just how important ozone can be at a terminal disinfectant within dairy processing.

### 4.6 Conclusion

From this research it is evident that ozone application as a terminal disinfectant to an effective cleaning system does have a positive effect in controlling and reducing the colonies found within food contact and environmental areas of a dairy processing unit. This work demonstrated that ozone does have the desired effect over time as part of an effective cleaning system.
Chapter 5

Discussion

Prior to the work carried out in this thesis there appeared to be little work carried out in situ of a food manufacture company within production time to validate the effectiveness of ozone *in-situ*. It was evident from the work that has been documented that there has been work carried out into the uses of ozone but it has not been carried out looking into its use in a food factory environment and that the validation of ozone has not been carried out within a working factory (Moore *et al* 2000). Work has been carried out looking into the uses of ozone however the main area’s of work has been the use in hospitals and treatment of water. There has been work published within these sectors but little has been published within the food industry. The work carried out was done to cover the whole aspect of cleaning within the food industry and to monitor the effectiveness of the chemicals within the laboratory environment and then to see the effectiveness within the working environment to gain a true representation of how the chemicals perform in industry when exposed to organic debris and to see the true effectiveness of ozone within industry.

The validation of cleaning chemicals within laboratory conditions was carried out to assess the effectiveness of the chemicals used within the factory and as a starting point to see is they have the same benefit when used within a working factory environment. The results indicated that there was a significant difference between stage 1, control swab and stage 2 which was the swabbing which took place after the detergent stage. There was not a significant difference between the detergent and disinfectant stage or the
disinfectant stage and the drying time stage, although there was however a downward trend in the number of colonies obtained from the swabs. Therefore even though there wasn’t statistically a significant difference there was a benefit from each of the cleaning stages. The drying stage isn’t so concerned with the removal of bacteria but more beneficial in preventing re-growth of any of the remaining bacteria which may be there.

Chapter 3 looked at the validation of cleaning chemicals and ozone within dairy processing. This section took section two to a further stage in validating the effectiveness of each of the cleaning chemicals and also the addition of ozone to the cleaning chemicals. This work was carried out in four sections looking at two bacterial groups, APC and Entrobacteriaceae and two areas of the factory, environmental and food contact. Looking at the results obtained for the APC obtained from the food contact areas there was a significant difference between the pre-clean and the detergent stage, which shows that the detergent stage does play an important role within the cleaning process. There was no significant difference between the other stages of the cleaning process however each of the stages showed a decrease in the number of colonies obtained. The results of the APC on the environmental surfaces again did not show a significant difference statistically however there was still a log reduction within the stages. This again shows the importance of each cleaning stage including ozone.

Enterobacteriaceae was monitored on environmental surfaces and statistically proved to have a significant difference between the pre-clean and the detergent stage but not a significant difference between stages two and three or three and four. Even though there was not a significant difference statistically, there was still a log reduction between all four stages.
When the counts were monitored on the food contact areas for Enterobacteriaceae there was no significant difference between any cleaning stage statistically however there was still a downward trend. Even though statistical analysis was carried out using one-way analysis of variance and didn’t show a significant difference there was still a downward trend shown from the log reductions obtained. Therefore it is clear that each of the cleaning stages plays an important role within a complete cleaning system.

Chapter 4 was carried out to investigate the effect of the cleanliness of food contact areas and environmental areas with and without the use of ozone. Due to the method used for the swabbing of this work statistical analysis could not be carried out due to the random swabbing. The results have shown that for the average Enterobacteriaceae on food contact areas that there is a downwards trend line for the results obtained with time. It also shows that with time and without the exposure of ozone that the trend line starts to have an upward trend, which indicates that with time and without the exposure of ozone that the average Enterobacteriaceae is increasing. The trend line on the counts with ozone exposure indicates that ozone does have a positive effect on all surfaces within the factory as the areas swabbed included horizontal surfaces, vertical surfaces and the undersides of equipment. The effectiveness of ozone on such surfaces is extremely beneficial in reducing the counts of Enterobacteriaceae within food environments as ozone is gaseous and is able to get to all surfaces, even those which can be difficult to clean.

The results for the average Enterobacteriaceae on environmental areas also show a downward trend with time and an increase when the ozone is not on. It was clear from
the results obtained that the addition of ozone overnight had a clear benefit on the counts obtained the following morning.

The monitoring of the APC on the environmental and food contact surfaces also show that with the exposure of ozone there is a downward trend in the number of colonies obtained during the time when ozone was on during the night compared to an upward trend when there was no ozone present. When the APC was monitored on environmental areas there was again a downward trend after the exposure of ozone and an upward trend of counts when the ozone was not present. This result was also seen in a study by Fielding et al (2007) where ozone was proved to be a successful cleaner within beer lines and potentially other drink lines and dispensers within the food and drink industry.

Overall the work presented in this thesis confirmed the positive effect ozone has on decreasing the bacterial count. Even though statistically there was not a significant difference this could have been due to the random sampling methods employed which resulted in the results not being suitable for statistical analysis. However from the trend analysis carried out it clearly shows that there is a positive effect in having ozone present within a food manufacturing environment in order that there is an additional disinfection stage. The trend analysis carried out ties in with the Commission Regulation (EC) No 2073/2005 of November 15th 2005 on Microbiological criteria for foodstuffs. The regulation quotes that “Trends in test results should be analysed, as they are able to reveal unwanted developments in the manufacturing process enabling the food business operator to take corrective actions before the process is out of control”. The benefit with trend
analysis is that it can quickly show up good and bad differences which may be as a result of any changes within a manufacturing process, cleaning schedule or staff team.
Chapter 6

Conclusion and Recommendations

Overall the whole investigation confirmed the positive effect a suitable cleaning system has on decreasing the bacterial count. Even though statistically there was not a significant difference this could have been due to the random sampling methods employed which resulted in the results not being that suitable to statistical analysis. However from the trend analysis carried out it clearly shows that there is a positive effect in having ozone present within a food manufacturing environment in order that there is an additional disinfection stage. From this work the next stage would be to repeat the principle of this work in a larger field in order that even more data could be obtained and as a result compare various food and drink manufacturers and carry out further statistical analysis. This work has confirmed the benefits of ozone and also set a good base from which further work could be carried out and published as prior to this work, there was no work found to look into the benefits of ozone in a working food environment and has lead the way to future work being carried out in situ.

To date, the cleaning system implemented within Abergavenny Fine Foods as a result of this work is still being used within the new processing plant of Abergavenny Fine Foods along with ozone application running during the night. This supports the results in that the system is continuing to run within the dairy 7 years on from the development and research into a successful cleaning system for the dairy. Abergavenny Fine Foods are now a major supplier to all the major multiples of goats cheese, deli cheeses, breaded party foods and blended cheeses.
The work within this thesis represents a detailed case study in a small to medium size dairy, developing and evaluating a cleaning process that includes a novel application of gaseous ozone as a terminal disinfectant. The work was undertaken as part of a Teaching Company Scheme programme.
Chapter 7

References


CCFRA. (2002). The use of chlorine in fresh produce washing. CCFRA Guideline No 38. Campden and Chorleywood Food Research Association, UK.


